period some 10 to 30 minutes following administration of rutin. On this account, in the first group of rabbits, (Table I, No. 1, 2, 5; Table II, No. 1) the time interval between injections of rutin and histamine varied between 15 and 30 minutes. Under these circumstances no protection was observed, with up to 2000 mg of rutin. To exclude the possibility that the antihistamine effect of rutin in the rabbit is characterized by a long latent period (as is seen clinically in the treatment of abnormal capillary fragility states) an unstable emulsion of rutin was injected in addition to the solution and these were given repeatedly. The persistence of rutin deposits in 3 of 4 rabbits so treated, would appear to indicate that an extended absorption had taken place. Even with this procedure no antihistamine power could be found.

The complete results are presented in tabular form. In addition, 2 rabbits given 50 mg of rutin daily orally for 24 days, failed to survive a 0.5 mg per lb dose of intravenous histamine.

Summary and Conclusions. From the tables it can be seen that pretreatment with large doses of rutin, up to 2000 mg failed to protect rabbits against a minimum lethal dose of histamine. It therefore appears that in the normal rabbit, rutin is devoid of noteworthy antihistamine properties.

16556

Mouse Leukemia. XIII. A Maternal Influence that Lowers the Incidence of Spontaneous Cases.

E. C. MAC DOWELL AND M. J. TAYLOR.*

From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.

The discovery[1] of the milk factor as the cause of the difference in reciprocal hybrids between mouse strains of high and low incidence mammary cancer has naturally led to the search[2] for a similar agent to account for parallel differences between reciprocal hybrids between strains of high and low incidence of leukemia.[3] But evidence of a specific maternal factor inducing leukemia was not found. The question remains, what mechanism is responsible for the differences in reciprocal F_1 hybrids?

In the following experiment a high-leukemic strain C58, gens. 59-61, brother x sister, was crossed with a low-leukemic strain StoLi, gens. 54-57, brother x sister. (In a cross between such highly inbred strains, all first generation hybrids have the same genetic constitution.) The incidence of spontaneous leukemia was observed in 6 classes of F_1 females: namely, from reciprocal matings, fostered by nurses of strain C58, StoLi, or Balb. Each litter was divided among foster nurses of the 3 strains without receiving a drop of milk from its own mother. Each parturition was witnessed by one of a team of 4 observers† who maintained an uninterrupted, night and day vigil of each pregnant

* Diagnoses of cases doubtful in gross were contributed by Dr. M. N. Richter and Dr. R. A. Miller with technical assistance from Miss L. Lewis.

1 Little, C. C., *et al., Science,* 1933, **78**, 465; Bittner, J. J., *Am. J. Cancer,* 1940, **39**, 104.

2 Barnes, W. A., and Cole, R. K., *Cancer Res.,* 1941, **2**, 99; Furth, J., Cole, R. K., and Boon, M. E., *Cancer Res.,* 1942, **2**, 280; Kirschbaum, A., and Strong, L. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 404.

3 MacDowell, E. C., in *Year Book,* Carnegie Inst. of Wash., 1934, **33**, 42; MacDowell, E. C., and Richter, M. N., *Arch. Path.,* 1935, **20**, 709.

† The authors, with the highly appreciated cooperation of Dr. Vernon Bryson and Miss Elsie Ward.

female from the 18th day after the observed mating plug. Each new-born mouse was removed from the box after the mother had cleaned it up as much as she would before the next one appeared. When the interval between successive births was prolonged, as occasionally happened, the new-born mouse was replaced by one or more from the Balb strain (impossible to confuse with the black-eyed hybrids), to focus the mother's activity until the next birth. The litter was not distributed to the nurses until the last one was born. Even though the last birth might not occur for several hours after the first, no difficulty was encountered, provided the new-borns were thoroughly warmed up when offered to the nurses and all traces of blood carefully washed off by the observer, if not by the mother. Every nurse had been suckled by other young for 4-24 hours before the experimental animals were presented, so that none of the experimental animals received the first milk. StoLi and C58 mothers to be used as nurses were temporarily given Balb new-borns to draw off the first milk and to maintain nursing behavior until needed. Each nurse raised 5, or, in a few cases, 6 young, the number being increased to 5 when necessary by Balb new-borns. On the 28th day after birth each litter was weaned and distributed to permanent boxes, each box housing one mouse from each of the 6 classes.

Results. The occurrence of leukemia in each of the 6 classes is given in Table I. The curves in Fig. 1 represent the total leukemic deaths that had accumulated at successive 50-day periods as per cents of the total number in the class. The 3 deaths between weaning and 6 months have been omitted. As in the figure, the 6 classes will be identified by the initial of the mother's strain followed by that of the nurse's strain.

TABLE I.

Incidence of Leukemia and Average Length of Life of F_1 Females According to the Strain of the Mother and of the Nurse. Parentheses = Total Number of Mice.

Mother-nurse strain	Leukemics, %	Length of life, avg—days	
		Leuk.	Neg.
C-C	85.3 (34)	575.7	519.0
C-S	55.9 (34)	733.4	740.6
C-B	93.7 (32)	568.9	——
S-C	80.0 (30)	771.2	747.1
S-S	54.8 (31)	855.6	850.1
S-B	50.0 (28)	765.6	799.0

The role of B nurses provides the key to the interpretation. This experiment was actually undertaken to verify the seemingly direct influence of nurses of this strain in raising the incidence of leukemia unexpectedly observed in an experiment designed as a test for genetic segregation.[4] But long before the present experiment was concluded, further analysis of the previous data showed that the B nurses had no effect upon leukemia as such, but they had increased the resistance to an entirely unrelated disease to which males alone were susceptible. As a result, the longer lives of males with B nurses gave more opportunity for leukemia to appear. Thus, a seeming sex influence on leukemia was eliminated. The present results confirm the conclusion that nurses of this strain have no influence upon leukemia. B nurses give virtually the same results as the nurses of the mother's strain. That is, with C mothers, B and C nurses give the same result, and with S mothers B and S nurses give the same result. In the first case the B nurses are actually higher and the second actually lower than the nurses of the mother's strain, but such inconsistent deviations appear to have no significance. If B nurses are neutral, C-B, and S-B indicate the full effects of the purely maternal factor, which thus is responsible for highly significant differences in the proportion of leukemics and in their longevity. It follows, also, that nurses of the mother's strain do not augment this influence from the mother herself. This does not mean that C and S nurses are neutral, for with mothers of the opposite strain (C-S and S-C) they completely reverse the maternal influence upon leukemia and less fully modify the maternal influence upon longevity. Accordingly, within each of these 2 nurse strains, the mother's strain would be supposed to have no influence upon leukemia, but only upon longevity.

C nurses move the whole longevity curve for S mothers (S-S) nearly half-way towards the C mothers' curve (C-C). S nurses move the curve for C mothers more than 2/3 of the way toward that for S mothers. The fact that the nurse can entirely reverse the maternal influence upon the proportion of leukemics and only partially reverse this influence on longevity suggests that the association between longer lives and fewer leukemics may not be directly dependent. Indeed, the longevity of non-leukemics follows that of leukemics so closely (Table I) that this longevity factor appears to be non-specific, resisting all causes of death, and thus unable to account for the modification of the proportion of leukemics.

The foregoing analysis indicates a strain differential in the maternal contribution of a non-specific influence upon longevity and a specific influence upon the incidence of leukemia. Further, it may safely be supposed that the mechanisms responsible for these maternal influences are basically the same as those responsible for corresponding influences transmitted by nurses. But it does not necessarily follow that the higher incidence of leukemia from C mothers is dependent upon a positive leukemia-favoring factor, parallel to the virus-like milk factor in mammary cancer of mice. The action of the S nurses with C mothers gives evidence that the S effect is positive resistance and not merely the absence of a possible pro-leukemia influence from a C nurse, for C-B shows that the absence of a C nurse gives no such reduction in the proportion of leukemics.

Further evidence that the S mother influence is positive resistance to leukemia appeared in the experiment previously cited.[4] In this experiment the heredity from strain C58 was carried exclusively by males. The critical 2nd back-cross, included 50 families of about 50 mice each, all born to inbred S mothers and a different 1st backcross father for each family. These families varied in the incidence of leukemia from 0% to 41%. Considering the quarter of these families with the highest total leukemia, the highest incidence appeared in the mice with youngest mothers; with increasing age of the mother at parturition the per cent of leukemics was increasingly reduced until it was almost eliminated. The next lower quarter of the families showed the same declining incidence with increasing age of mother, but the maximum incidence from the youngest mothers was necessarily lower. As this maximum was further lowered in the next quarter of the families the effect of mothers' increasing age was less obvious and in the quarter of the families with only 0%-10% leukemics no change with mother's age could be recognized. A maternal factor becoming increasingly potent with increasing age of the mother at parturition was responsible for the highly effective suppression of inherited tendencies to leukemia.

This progressive reduction in the frequency of leukemics was accompanied by increasing length of life. Since the longevity of non-leukemics in all the 50 families taken together showed no correlation with mother's age, the declining incidence of leukemia was

TABLE II.
Distribution of Leukemic and Non-leukemic Deaths of F_1 Females from Young and Old S Mothers Which Nursed Their Own Offspring. 50-Day Classes Represented by Low Limit.

Length of life, days	Mothers: Young Leuk.	Young Neg.	Old Leuk.	Old Neg.
300	1	1		
350	4	1		
400	5	—		
450	5	2		
500	8	0		1
550	9	4	2	2
600	10	1	1	2
650	7	1	5	3
700	4	1	6	8
750	4	—	4	4
800	1	1	4	6
850	4	1	7	6
900			13	4
950			3	1
1000			3	—
1050			1	1
1100			1	
% L	82.6	(75)	56.8	(88)

Diff. 25.8 ± 2.17. $\chi^2 = 13.8$. $P = .0002$.

[4] MacDowell, E. C., Potter, J. S., and Taylor, M. J., *Cancer Res.*, 1945, **5**, 65.

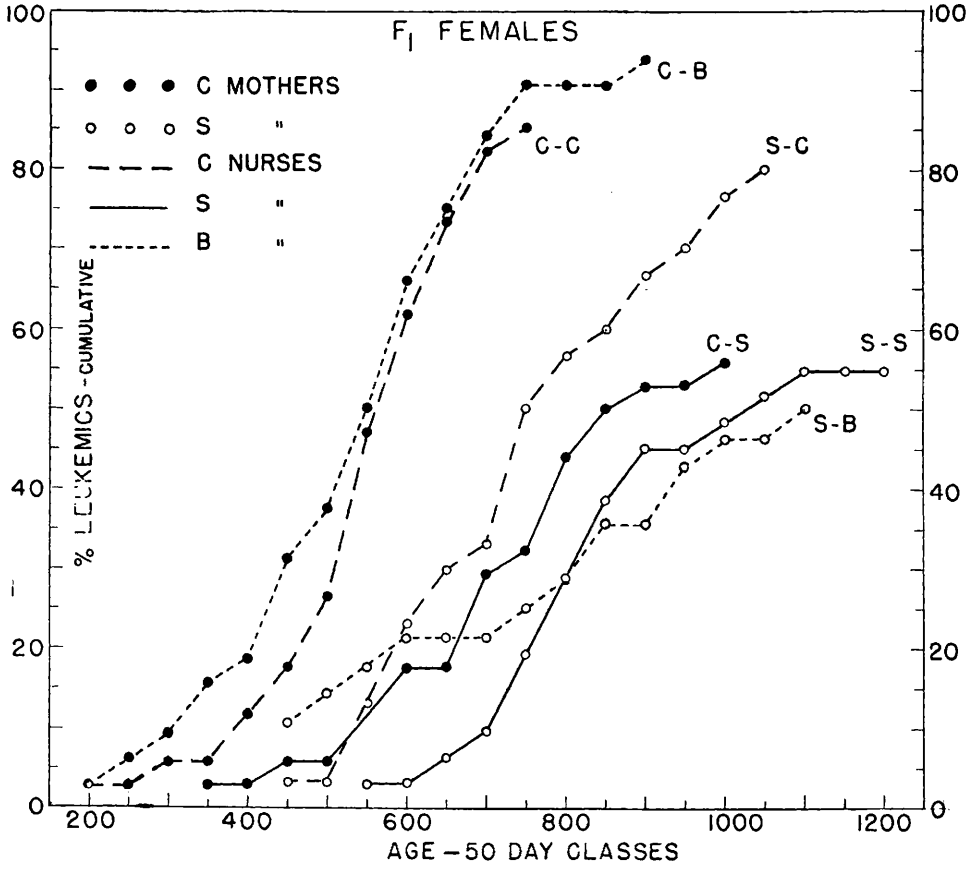

FIG. 1.

Strain of mother vs. strain of nurse. Leukemic deaths of F_1 females accumulated at successive age periods, given as % of total number of mice, according to the strain of the mother (first initial) and of the nurse (second initial): C = C58, S = Storrs-Little (StoLi), B = CSH Bagg albino (Balb); age classes designated by low limit.

at that time interpreted as a secondary result of the maternal factor specifically delaying the appearance of leukemia. Considering the evidence of non-specific maternal influence upon longevity given above and to follow, this interpretation appears now to be untenable. In the 2nd back-cross families an extremely complex situation was created by the simultaneous segregation of genetic factors for leukemia and for non-specific longevity. These probably confounded what now appears, under far more simple conditions, to be a non-specific maternal influence on longevity.

A special experiment was set up to determine the influence of older S mothers under more simple conditions. As in the nursing experiment the test animals were F1 females; one group from young and one from old StoLi mothers. Each mother nursed her own young. In order to obtain reliable reproduction with old mice it is necessary to start breeding them while young. The S females to serve as old mothers were continually mated with their sibs. At the appropriate time their daughters were saved to serve as young mothers. When the old females were 29 weeks old and the young females 7 weeks old they were mated to C58 males with 3 young and 2 old females in each mating. The offspring were housed, 5 of the same class per box, boxes from young and old mothers alternating on the shelves.

Table II, Fig. 2 give the primary results.

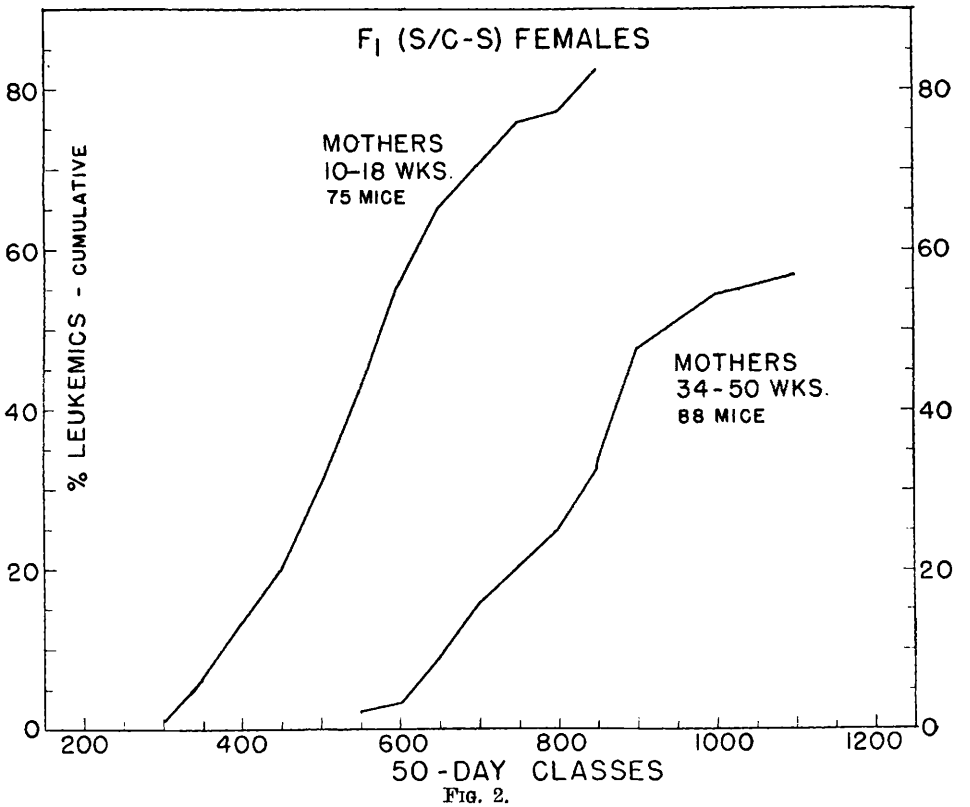

FIG. 2.

Young vs. old S mothers. Leukemic deaths of F_1 females nursed by own mothers. Presentation as in Fig. 1.

Mothers 34 weeks and older unquestionably reduce the incidence of leukemia and lengthen the lives of leukemics. The first leukemic death of mice from young mothers occurred about 250 days before the first leukemic death from old mothers (550-day class) at which time 42% of all the young from young mothers had died with leukemia. At 850 days when the last deaths of mice from young mothers gave a final 82% leukemics, 33% of the mice from old mothers had died with leukemia. Here again the close association of less leukemia and longer lives is evident, but here also the longevity of non-leukemics as well is influenced. Non-specific factors delaying all causes of death will not account for modification of the incidence of leukemia. Fig. 3 shows the cumulative %-distribution of deaths of negatives and of leukemics. The negatives from young mothers coincide with the leukemics. The negatives from old mothers are actually slightly earlier than the leukemics, but in face of the difference for the negatives from young mothers this difference is probably insignificant. A curve for the distribution of leukemic deaths among 40 inbred C58 females (mothers from nursing exp.) is added to show another break in the longevity-leukemia association, for the F_1 females even with young S mothers live considerably longer than inbred C58s, although the incidence of leukemics is virtually the same. To check the reliability of the difference between young and old mothers and to find possible minor effects within the young and old mother classes, each class has been subdivided (Fig. 4). The two curves from young mothers give the same incidence of leukemia, but those from the 15-18 wk.-females lived slightly longer. The two curves from old mothers show the same longevity, but the final incidence of leukemia is slightly

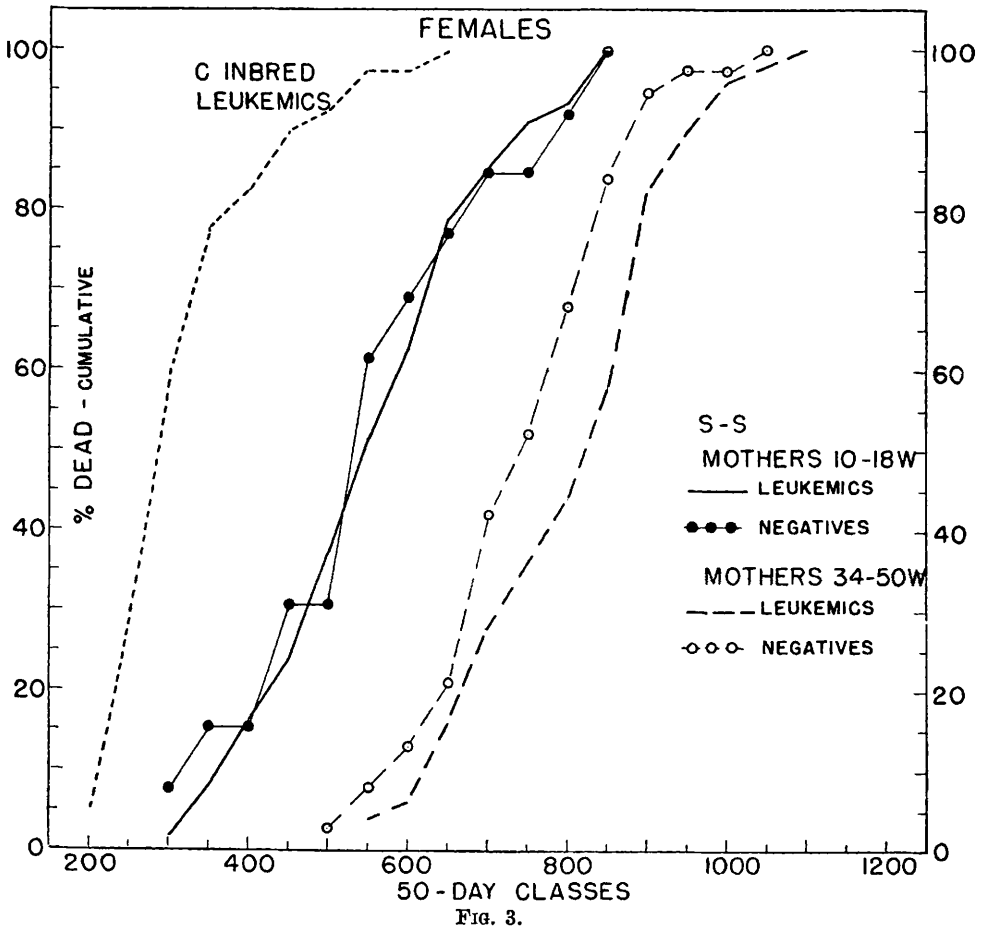

FIG. 3.

Young vs. old mothers. Age distribution of leukemic and of non-leukemic deaths of F_1 females as cumulative % of the final number with each diagnosis. Also, corresponding age distributions of leukemic deaths of inbred C females.

lower for the older mothers. These differences between sub-classes may be suggestive but not significant; each half of the data gives essentially the same result.

The bearing of this experiment upon the nursing experiment is made clear by superimposing the nursing experiment curves for S-S (open circles) and C-C (solid dots) upon Fig. 4. The virtual identity of the S-S curves in the two experiments indicates that the S females in the nursing experiment were "old", (as the records show them to have been) and justifies the interpolation of the term 'S mother's age factor' in the interpretation proposed for the results of the nursing experiment. From that experiment it becomes evident that the S mother's age influence may be transmitted before birth, and it may also be transmitted through the nursing (milk) alone.

The agreement between the young mothers' curves and the C-C curves indicates that the difference between reciprocal F_1 hybrids between strains C58 and StoLi disappears when the resisting influence of old S mothers is not present. C58 males contribute as strong a leukemic tendency to F_1 hybrids as C58 females and in these experiments this appears to be virtually as strong as in inbred C58, in other words full dominance.

Conclusion. The maternal influence responsible for a difference in the incidence of leukemia in reciprocal F_1 hybrids between a high—(C58) and a low leukemic strain

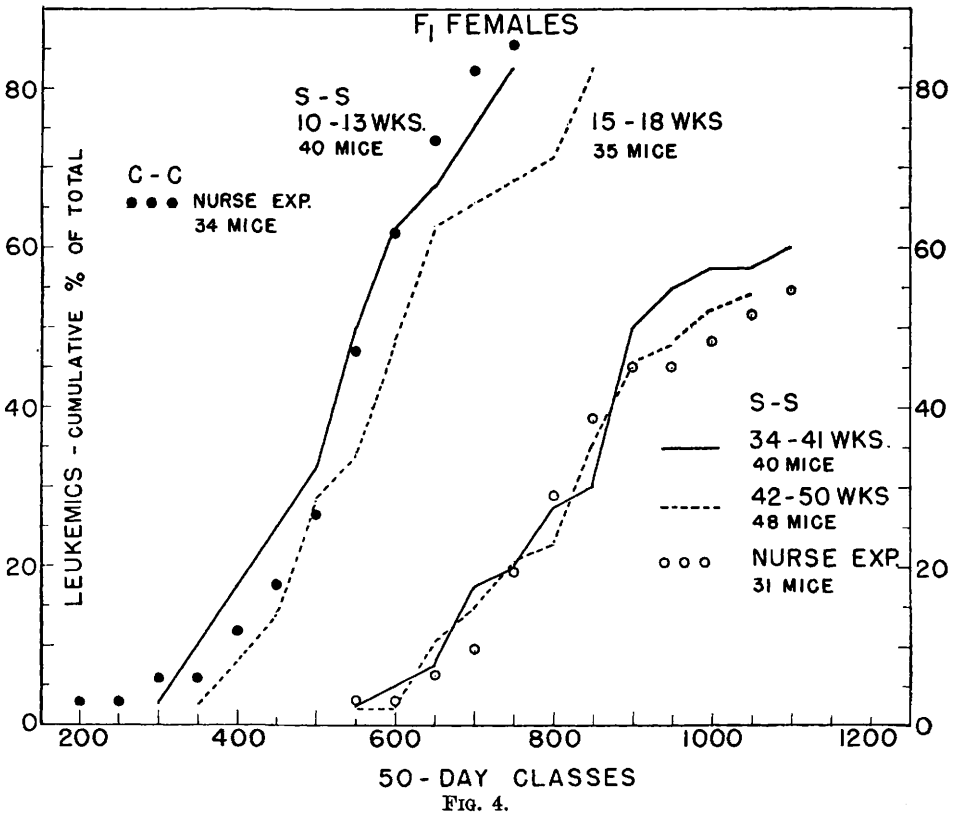

FIG. 4.

Young vs. old S mothers. Data of Fig. 2 with young and old mothers' groups subdivided. Also, curves from Fig. 1 for C-C and S-S represented by unconnected points.

(StoLi) consists of a definite resistance to leukemia contributed by the low strain mother. This maternal resistance may be contributed before birth or through nursing alone. It is not found at earliest sexual maturity, but becomes increasingly potent with advancing age. When this resistance is absent the leukemic heredity of C58 shows full dominance, whether introduced by a father or mother. An influence resisting all causes of death non-specifically is similarly transmitted by StoLi mothers as their age increases.