of yeast isolated from patients complaining of moniliasis following antibiotic therapy established a definite inhibitory effect by the methyl and propyl esters of paraben. Oral, vaginal, and rectal studies involving 186 patients demonstrated that the addition of these agents to aureomycin was of value in preventing the development of moniliasis. This was especially obvious in the oral studies. When aureomycin alone was administered, 63% of those patients whose stools were initially free of *C. albicans* subsequently revealed these organisms. In contrast, when aureomycin containing paraben was employed, only 13% disclosed yeast. While the number of patients involved in the rectal studies was limited, results were consistent with those observed in the oral and vaginal cases. With 1 exception, *C. albicans* infections became more severe with aureomycin therapy; however, no alteration was observed when aureomycin containing paraben was administered. The combination of aureomycin and paraben caused the disappearance of *C. albicans* in only 2 cases. In 3 patients having monilial vaginitis the intravaginal insertion of 200 mg of paraben daily for 3 weeks resulted in an amelioration of symptoms but did not eradicate the yeast. It appears from these studies that methyl and propyl paraben exert only a weak or moderate inhibitory action on *C. albicans in vitro* and *in vivo* but are capable of preventing its overgrowth during aureomycin treatment.

Little variation was noted in the occurrence of nausea, vomiting and diarrhea in patients receiving aureomycin alone and in those given aureomycin containing paraben esters. No toxic effects to the esters of paraben were observed.

Summary. (1) *In vitro* sensitivity studies established a definite inhibitory effect by methyl and propyl paraben (para-hydroxybenzoic acid) on 5 strains of yeast. (2) Methyl and propyl paraben are essentially non-toxic when administered orally, vaginally or rectally. (3) Methyl and propyl paraben are of value in preventing the overgrowth of *C. albicans* associated with aureomycin therapy.

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Comparison of Normal Blood Picture of Young Adults from 18 Inbred Strains of Mice. (19210)

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In this study of the formed elements of the blood of normal mice, a total of 18 inbred lines were investigated, including sublines of 10 major strains, a large proportion of those widely used in biological research(1-3). All are maintained together in the "inbred nucleus" of the Jackson Laboratory, where certain of the lines (marked with †) form the background of all mice sold to other institutions. Experimental mice were virgins, 2 to 3

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TABLE I. Mean Values for Erythrocyte Characteristics of Each Strain Tested. All means are based on 10 animals, except those marked (*) based on 20 animals (see text). Those strains marked (†) are major strains available to other laboratories.

Strain	RBC/mm³ Mean ×10⁶	S.E.	Haematocrit Mean	S.E.	Mean cell vol, mu³ Mean	S.E.	Haemoglobin, g/100 cc blood Mean	S.E.	Haemoglobin, g/cc cells	Reticulocyte, %
A/Jax†	9.42	± .28	42.5	± .4	45.1	± 1.4	12.9	± .2	.30	3.5
A/HeJax†	9.48	± .18	42.5	± .5	44.8	± 1	12.7	± .2	.30	2.9
AKR/Jax†	9.38	± .24	45.6	± 1	48.5	± 1.6	13.9	± .2	.30	2.3
BALB/cAnJax	10.14	± .15	46.5	± .8	45.9	± 1.1	14.5	± .2	.31	3.3
BALB/cJax†	10.51	± .16	48	± .7	45.7	± 1	15	± .2	.31	2.9
CBA/Jax	10.04	± .27	45	± 1.3	44.8	± 1.8	13.5	± .2	.31	2.6
C3H/Jax†	8.79	± .24	39.5	± .7	44.9	± 1.4	12.2	± .4	.31	2.8
C3H/SeJax	9.63	± .26	43	± 1	44.7	± 1.6	13.2	± .3	.30	2.2
C57BL/6pJax	9.70	± .15	43.4	± .8	44.7	± 1	13	± .3	.30	2.5
C57BL/6Jax†	9.66	± .09	44	± .4	45.5	± .6	13.3	± .2	.30	2.6
C57BR/cdJax†	10.54	± .17	50	± .5	47.4	± .9	14.6	± .2	.29	2.4
C57L/HeJax†	9.82*	± .20	50.6*	± .4	51.5*	± 1.1	14.9	± .2	.29	2.6
DBA/1Jax†	10.52*	± .27	43.8*	± .6	41.6*	± 1.2	13.2	± .2	.30	1.5
DBA/WaJax	9.93	± .27	43	± .6	43.3	± 1.1	12.5	± .2	.29	2.6
DBA/2Jax†	10.30	± .25	42.6	± .5	41.4	± 1.1	12.7	± .1	.30	3.1
I/Jax	10.27	± .27	46.8	± .7	45.6	± 1.5	13.5	± .1	.29	2.4
RIII/Jax	9.63	± .25	44.5	± .6	46.2	± 1.3	13.7	± .2	.31	2.8
ST/Jax	9.88*	± .19	44.1*	± 1.1	44.6*	± 1.4	12.1	± .2	.31	2.1
Mean	9.92	± .07			45.3	± 1.3	13.4		.30	2.6

months old. Preliminary experiments had indicated that before this age the rapid growth of the animal and correlated changes in blood counts made determinations too variable. Five females and 5 males of each strain were used for each type of determination. It should be stressed that in no case has there been any direct selection by any investigator toward high or low levels of any type of blood cell (with the possible exception of high leukocyte counts in older animals of leukemic strains). Whatever values now characterize these strains have been attained either by natural selection or by chance genetic fixation. Other experiments of this type, studying the blood of certain inbred strains of mice, have been reported in the literature(3-14) but the breadth and lack of selection in this survey should make its results approach more closely a complete characterization of the normal level of formed elements of the blood of the young adult house mouse as a species than has previously been possible.

Technical methods. Standard methods were used for erythrocyte count, hematocrit determination, hemoglobin content, reticulocyte percentage, total leukocyte count and differential count. Special adaptations for the small samples available from single mice were the use of the Van-Allen hematocrit tube and the cyanomethemoglobin micromethod of Turner(15) for hemoglobin content. 1.3% sodium oxalate is isotonic for mice. The hemoglobin results were checked by duplicate determinations on samples pooled from several mice by the Turner method and the Sendroy(16) modification of the Van-Slyke-Neill(17) oxygen capacity method. Where two independent samples were taken from the same animals, accuracy of the results can be judged from the ratio of mean difference between samples to the grand mean for all animals combined (RBC/mm³, 5%; Ht, 2.3%, Hb, 2.8%, WBC/mm³, 13%). For all determinations tail blood was used, obtained after heating the tail in hot water. For erythrocyte counts, hematocrit and hemoglobin determinations, and total leukocyte counts, nembutal anesthesia was used since preliminary experiments gave lower counts and lower relative variability in animals so treated. In determinations of reticulocyte percentage, 500 cells were counted in smears prepared with alcohol solution of brilliant cresyl blue, counterstained with Wright's. A single differential leukocyte count from each animal (smear stained with Wright's) included 300 cells scattered at random over the slide. Although cells were re-

TABLE II. Mean Values for Leukocyte Characteristics of Each Strain Tested (Total No. and Proportion of Granulocytes). All means are based on 10 animals.

Strain	Total leukocytes/mm³ Mean 10^3	S.E.	% granulocytes ♀♀ Mean	S.E.	S.E./mean	♂♂ Mean	S.E.	S.E./mean
A/Jaxt	8.71	± 1.37	10.7	± 1.1	.10	14.5	± 5	.35
A/HeJaxt	6.14	± .68	19.8	± 2.3	.12	37.2	± 7.5	.20
AKR/Jaxt	6.80	± .65	21.6	± 4.5	.21	23.8	± 5.2	.22
BALB/cAnJax	8.70	± 1.03	15.4	± 2.3	.15	29.6	± 5	.17
BALB/cJaxt	8.54	± 1.04	14.9	± 2.1	.14	15.3	± 3.8	.25
CBA/Jax	6.44	± .33	26.8	± 4.5	.17	24.2	± 2.5	.10
C3H/Jaxt	7.34	± .80	21.4	± 4.3	.20	27.5	± 5.1	.19
C3H/ScJax	5.07	± .30	19	± 3.9	.21	22	± 2.2	.10
C57BL/6pJax	10.61	± .64	12.1	± 3.8	.31	15.3	± 5.1	.33
C57BL/6Jaxt	11.43	± 1.04	8.2	± 1.2	.15	10.4	± 3.5	.34
C57BR/cdJaxt	9.43	± .78	10.6	± 1.1	.10	11	± 2.4	.22
C57L/HeJaxt	10.82	± 1.13	8.5	± 1.3	.15	6.7	± 1.4	.21
DBA/1Jaxt	8.60	± 1.60	16.5	± 1.5	.09	20.4	± 4.2	.21
DBA/WaJax	8.32	± .96	19.6	± .9	.05	17.2	± 1.5	.09
DBA/2Jaxt	9.28	± .29	16.7	± 2.3	.14	18.1	± 1.3	.07
I/Jax	11.62	± 1.41	13.2	± 3.2	.24	18.1	± 2.9	.16
RIII/Jax	5.87	± .64	26.2	± 2.8	.11	18.6	± 3.7	.20
ST/Jax	7.74	± 1.23	14.9	± 2.4	.16	19.4	± 3.7	.19
Grand mean	8.41	± .26	16.5			19.4		
Mean, sexes combined					18			

† Major strains available to other laboratories.

TABLE III. Results of Analyses of Variance for Each of the Formed Element Determinations Tested.

Measurement	Source of variation	df	Mean square	F value	Level of significance, %
Erythrocyte No.	Total	179			
	Lines	17	5.37	4.93	.1
	Sex	1	0	—	—
	Line × sex	17	1.77	1.62	—
	Error	144	1.09		
Haematocrit determination	Total	179			
	Lines	17	201.50	22.74	.1
	Sex	1	6.95	.78	—
	Line × sex	17	16.72	1.88	5
	Error	144	8.86		
Total leukocytes	Total	179			
	Lines	17	75.11	3.80	.2
	Sex	1	11.77	.60	—
	Line × sex	17	14.56	.74	—
	Error	144	19.76		
% granulocytes	Total	179			
	Lines	17	338.9	6.99	.1
	Sex	1	382.5	7.89	1
	Line × sex	17	82.8	1.71	5
	Error	144	48.5		

corded as monocytes, lymphocytes, and neutrophilic or eosinophilic granulocytes for statistical purposes, only the two most distinct groups, granulocytes (almost entirely neutrophils) and agranulocytes (almost entirely lymphocytes) were classified. Smudged cells were omitted from the total, which could possibly lead to distortion of results. It is felt that the erythrocyte counts and hematocrit and hemoglobin determinations are sufficiently reliable to be accepted as standards for these strains and ages. The other determinations, due either to lack of precision in method or known variability with the state of the animal,

are useful only for determining relative status of strains and for approximating their absolute level.

Statistical methods. The mean and standard error was determined for each strain for each type of measurement, but in general the differences betwen strains are small enough, and there is sufficient overlap among the measurements, so that significant strain differences are not apparent by this method. However, using analysis of variance and comparing the proportion of the total variance attributable to the particular factors which can be separated out in this experiment (strain, sex, and factors involving an interaction of strain and sex) with the variance due to unanalyzed "errors" (experimental errors in method or sampling; environmental differences such as nutrition, temperature, exposure to pathogenic organisms, time of day, season of year, stress, etc.) it is possible to determine definitely whether or not the isolated factors contribute significantly to the total variation. In certain cases, Chi-square tests for non-random distributions were also used. Neither of these methods tells what the genetic factors are, nor in which strains they reside, but proves their reality.

Results. Tables I and II summarize the results by strain and Table III indicates the significance of contributions of tested sources to total variations observed for each variation. Genetic differences among the strains contributed very significantly to variations in all tested determinations. Sex, independent of strain, contributed significantly to only one determination, percent granulocytes. In addition, there is a possibility, significant at the 5% level only, that the strains differ in their tendency to favor expression of sex differences in hematocrit value and percent granulocytes. Since hemoglobin values correlated completely with hematocrit determinations on the same animal (Table I) no strain differences were found in hemoglobin concentration within cells, and conclusions for total hemoglobin content coincide with those for hematocrit level. Fig. 1 indicates that in the great majority of strains, variations in hematocrit mean can be explained by variations in cell number, as the ratio of Ht mean to RB mean falls

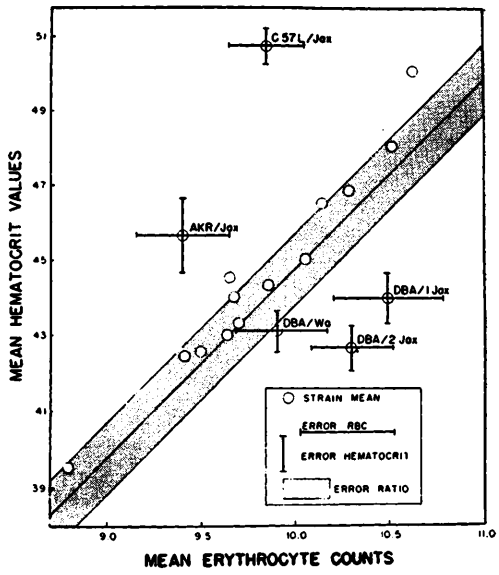

FIG. 1.

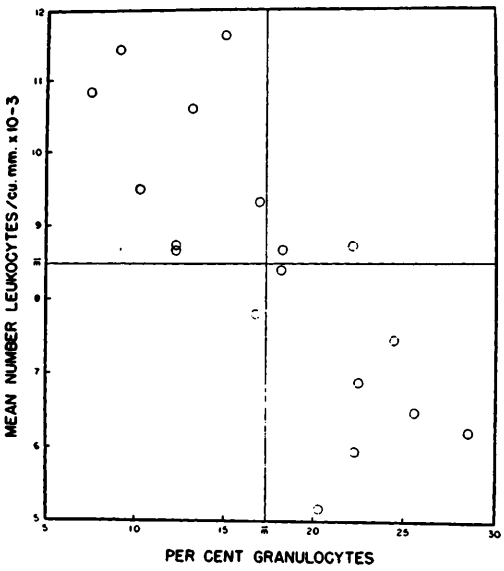

FIG. 2.

within the range of error of a straight line whose slope, 45.3 ± 1.3, is the mean cell volume of all strains combined. In a few strains (DBA/1Jax, C57L/HeJax, and ST/Jax) the recorded values are based on 20 rather than 10 individuals to test more critically for differences in cell size. One strain only, C57L/HeJax, showed a mean cell volume (51.5) significantly higher than that for all strains combined. The narrow low range of reticulo-

cyte percentage makes statistical analysis by strains impractical, so this measurement is given simply to indicate the typical level in 2-3 month old mice. Fig. 2 shows an inverse relationship between mean granulocyte percentage of a strain and the total leukocyte count for the same strain, indicating great strain variability in absolute number of lymphocytes combined with small or insignificant strain difference in absolute granulocyte number. Another interesting finding is a highly significant difference in proportion of granulocytes between 2 sublines of a single major strain, separated in 1927 after the lines had been more than 20 generations inbred. The A/HeJax had the highest percentage of any tested strain, while A/Jax is considerably below the grand mean. For two determinations (RBC/mm^3, A-strain subline difference in % granulocytes) Chi-square tests for possible significant differences due to portions of the year or related factors were negative.

Discussion. A study of the formed elements of the blood in young adults of 18 different inbred strains has resulted in a characterization of the normal level for the laboratory mouse for each type of determination. It was possible by statistical methods, particularly the analysis of variance, to demonstrate that a very significant contribution to the observed variations in erythrocyte number, mean cell volume (one strain only), total leukocyte count, and proportion of granulocytes to agranulocytes came from genetic differences among the inbred strains used. Related inbred strains in many cases seem to have been fixed for some of the same genes during their common history. The similarity of total and differential leukocyte counts of the entire C57 group offers a good example of this phenomenon. On the other hand, related strains or sublines occasionally differ by genes unfixed at the time of separation or by new mutation. In one case (the differential counts of A/Jax and A/HeJax) there is a highly significant difference between sublines separated after more than 20 common generations of inbreeding. Few of the differences in this data are due to sex alone. The animals are sexually mature, and would have had litters if mated, but hormones or other factors associated with sex have a strong effect on only one of the determinations, the proportion of granulocytes in the differential (Tables II and III). However, sex may also interact with genetic difference among strains to alter granulocyte percentage and hematocrit level (Table III). The inverse correlation between total leukocyte count and proportion of granulocytes (Fig. 2) suggests that differences in genetic factors affecting production or release of lymphocytes are more prevalent or more effective than are genes affecting granulocytes.

In each of these inbred strains, the observed values must fall within the limits of normality for the line to persist. The inbreeding process would eliminate quickly any genes severely deleterious to individuals before the height of the reproductive period. The results indicate that the strains differ from each other in genes with beneficial, neutral, or only slightly deleterious effects. Inbred strains may differ from each other in patterns of physiological balance, and blood characteristics may be correlated with differences in other characters. Physiological attributes to which erythrocyte level might be related are degree of bodily activity, basal metabolism, or, following the suggestion of Strong(4), tendency to develop mammary cancer. Hemoglobin content shows no clear-cut relation to activity level, as judged by this laboratory's investigators handling the mice. The most active DBA group ranged from 12.5 to 13.2 mean g Hb/100 cc, and the least active C3H group ranged from 12.2-13.2 mean g Hb/100 cc blood. BALB/c and C57BL/6 strains, with intermediate activity range from 14.5-15.0 g Hb/100 cc blood. No data are available on basal metabolism. Strong's hypothesis that age drop in hemoglobin of females with tendency to develop mammary cancer cannot be tested here. At the tested ages of 2-3 months, the mean hemoglobin content of mammary tumor strains (A/HeJax, C3H, CBA, all DBAs, RIII, and ST) ranged from 12.1 to 13.7 g Hb/100 cc blood, while that of tumor-free strains (BALB/c, all C57s, and I) ranged from 13.0 to 15.0 g Hb/100 cc blood. It is doubtful but not impossible that this slight range difference has meaning in relation to prospective tumor incidence. Many environ-

mental factors have been shown to influence total leukocyte and differential counts(18,19), but the only genetic ones for which we have definite knowledge of these particular strains are antibody-building response and tendency to develop leukemia. The antibody production of five of these strains (DBA/1, BALB/c, C3H, C57BL/6, and A) has been tested against pneumococcus polysaccharide and egg albumin(20) with significant strain differences completely unrelated to total or differential leukocyte count. Mice which later develop lymphatic leukemia might tend to have high lymphocyte counts. However, AK/RJax, in which 80% of the mice die with lymphatic leukemia before one year, gave a low mean of 6.80 leukocytes/mm^3. Older mice of DBA/2Jax and older males of C3H strains occasionally develop leukemia, and these also have low leukocyte counts at 2-3 months. It has thus not been shown that an observed strain difference in formed elements is one facet of a difference in a particular known pattern of physiological balance.

Another explanation for the observed strain differences might be chance fixation of a particular genotype. Environmental variations may have been so great as to prevent effective natural selection in each strain of the same best type. Or it may equally well be that there actually is no selective advantage within the total observed range. If this is so, this survey should not only prove the existence in normal mice of genes affecting the formed elements of the blood, and their differential distribution among the tested inbred strains, but should come close to establishing the range of values normal for the species as a whole.

Summary. (1) Erythrocyte count, hematocrit determination, hemoglobin content, reticulocyte percentage, total leukocyte count, and proportion of granulocytes were determined in the blood of 2 to 3 month old virgin males and females of 18 different inbred strains. The means and standard errors for each determination are given for each strain. (2) Although there was considerable overlap among the strains, it was possible by analysis of variance to show that there were significant genetic differences among the strains in factors affecting erythrocyte number (as shown both in count and hematocrit), and mean erythrocyte volume (one case only), total leukocyte count, and proportion of granulocytes. (3) Mean total leukocyte count for a strain was negatively correlated with proportion of granulocytes. (4) In this data, the observed strain differences do not appear to be related to differences in other known physiological characteristics.

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