THE HEMOCYTOLOGICAL CONSTITUTION OF ADULT MALE RABBITS FROM FIFTEEN STANDARD BREEDS

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A study of the blood cytology of normal rabbits has been undertaken in this laboratory for some years for the purpose of obtaining precise data which could be used in our investigations on the susceptibility and resistance of the animal host to disease agents (1). The work has included repeated examinations of groups of rabbits over periods of 2 weeks to 2 years, and in addition a large number of animals derived from various sources have been examined one or more times. The information obtained has shown that wide variations in the blood cell formulae of normal rabbits are found (2). Some of the differences could be related to seasonal conditions while others were apparently due to technical causes or to intercurrent disease. It was also found that certain differences in the blood formulae of individual animals could be definitely correlated with the individual reaction to experimental syphilis (3) and to a transplantable neoplasm (4).

This study on blood cytology has been extended in various directions and the influence of such factors as disease (5), breed (6), sex (7), age, certain physiological states, and diet has been investigated. The present paper is the report of observations made on laboratory bred strains of standard varieties of rabbits. The results are not to be interpreted as representing necessarily so called normal or standard blood cell values for these particular breeds but rather are to be considered from the standpoint of genetically related and genetically unrelated individuals. A preliminary report on certain of the results has already been published (6).

Materials and Methods

The results to be reported are based upon an analysis of the blood cytology of 180 rabbits representing fifteen standard breeds (Table I). A detailed description of these breeds may be found in the Catalogue of the American Rabbit and Cavy Breeders Association (8).[1] These fifteen varieties had been propagated in pure line in this laboratory from 3 to 8 years under uniform dietary and housing conditions.

TABLE I

Hemocytological Constitution of Standard Breeds of Rabbits
Data on Material and Methods

Breed	No. of animals	Age		Bleedings		Determinations			
		Mean	Variance of mean			RBC, Hb, and platelets	Total WBC	Total smears	Total white cells counted in smears
		mos.	*mos.*	*days*	*wks.*				
Havana	24	7.6	1.27	3.6	2.2	3.8	5.2	6.5	700
Himalayan	19	5.1	0.07	1.6	1.3	4.0	4.0	5.9	937
Belgian	14	9.8	4.80	3.6	2.4	4.2	6.1	6.6	814
English	24	8.4	2.47	3.3	1.8	4.2	5.0	6.4	700
Polish	16	8.3	2.48	4.1	2.4	4.1	6.4	6.8	713
Dutch	18	9.5	3.55	3.0	2.0	3.5	5.0	6.5	833
Beveren	18	8.9	3.28	4.3	3.3	4.7	5.0	6.8	739
Rex	13	7.7	1.65	4.0	2.5	4.0	6.1	6.5	715
Chinchilla	10	9.6	4.55	4.0	2.0	4.0	5.5	6.0	600
French Silver	8	6.3	—	4.0	2.0	4.0	5.5	6.0	600
American Blue	6	10.0	—	3.0	1.7	4.0	5.0	5.7	733
New Zealand	4	4.7	—	1.0	1.0	4.0	4.0	5.0	1000
Flemish	3	10.7	—	1.0	1.0	4.0	4.0	5.0	1000
Gouda	2	5.0	—	1.0	1.0	4.0	4.0	5.0	1000
Tan	1	4.7	—	3.0	1.0	3.0	6.0	6.0	600
Total	180	8.1		3.3	2.1	4.0	5.4	6.3	759

Sex.—Only male rabbits were employed.

Age.—The exact age of each rabbit was known. When the counts extended over more than 1 week the age was recorded as being that of the middle of the

[1] The animals were all derived from the large rabbit breeding colony of Dr. Wade H. Brown which has been maintained at The Rockefeller Institute for some years for the study of constitutional problems.

period; this period was in no instance longer than 1 month. The majority of the animals were from 4 to 10 months old with extremes of 3.4 and 36.0 months, and a mean age for the 180 animals of 8.1 months. With one exception the mean ages of the various breeds were not significantly different. The Himalayan breed was the exception with a mean age of 5.1 months. This breed had no animal younger than 4.2 months or older than 8.0 months at the time of examination (Table I).

Diet.—The diet which was constant throughout the entire period of observation consisted of hay, oats, and a commercial food pellet, with a free access to water. The pellet was rich in vitamins and mineral salts.

Period of Observation.—The observations were begun on Mar. 25, 1931, and were terminated on Nov. 5, 1932. The 180 animals were examined as follows: 14 in March, 1931; 25 in March, 1932; 1 in April, 1931; 41 in April, 1932; 11 in May and June, 1931; 10 in September, 1931; 20 in September, 1932; 44 in October, 1932; 7 in November, 1931; and 7 in November, 1932 (Table II). Three-fourths of the animals were examined in five groups containing 14 to 41 animals per group, and each representing a number of breeds. Of the total number of rabbits, 87 were examined in the spring and 88 in the fall. Only 5 of the 180 rabbits were examined in the summer and winter (June, July, August, December, January, and February). Of the eleven breeds represented by 6 or more animals, six were equally, and five were unequally (Himalayan, English, Beveren, Dutch, and Polish) distributed between spring and fall. This point will be discussed later.

Physical Condition and Housing.—The animals were in excellent physical condition and free from intercurrent disease as far as could be determined by frequent inspection. The observations were all made between 1.5 and 21.0 months prior to the occurrence of an epidemic of rabbit pox, the first cases of which appeared in this colony late in December, 1932 (9). During the 60 days preceding the outbreak only 7 animals were examined. These were distributed as follows: 2 Havana, 1 Belgian, 1 English, 1 Beveren, 1 Chinchilla, and 1 Black and Tan. (Table II). The 7 animals were seemingly healthy, were scattered through six breeds and made only 1/26 of the total number examined. Even had rabbit pox been present in several of the 7 animals no appreciable bias in the results could have occurred.

The animals were not subjected to any other tests at the time of counting. Many of the older animals had been used for breeding. Each animal was kept in an individual cage in a well ventilated room with good lighting.

Hematological Technique.—From one to six blood samples (average 3.3) were obtained from each animal during a period of 1 to 4.5 weeks (average 2.1 weeks). The blood samples were taken between 9 and 12 a.m. and between 1.30 and 4 p.m. Approximately the same number of counts were made on each breed during the morning and afternoon periods. Two individuals alternated in taking the blood from the marginal ear vein, and opposite ears were used for succeeding counts. (Alcohol, 50 per cent, was used to wet the ear before shaving.) Two or three persons made the differential counts using duplicate or triplicate smears and one

person made the red blood cell, the hemoglobin, the platelet, and the total white blood cell determinations. For each animal from 3 to 6 red blood cell, platelet, and hemoglobin estimations were made with the general average of 4.0; 4 to 12 total white cell counts were made with a general average of 5.4; from 5 to 8 blood smears with an average of 6.3. The total number of white blood cells counted on all smears for each animal varied from 500 to 1200 with a general mean of 759

TABLE II

Distribution of Animals According to Periods of Observation

Mean of period	Ha*	H	B	E	Bv	D	P	C	R	S	F	A	Z	G	T	Totals
1931																
Mar. 25.....	0	6	2	3	0	0	0	0	0	0	3	0	0	0	0	14
Apr. 27.....	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
May 19.....	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	6
June 11.....	0	0	1	0	2	0	0	0	0	0	0	0	0	2	0	5
Sept. 17.....	0	7	0	0	0	3	0	0	0	0	0	0	0	0	0	10
Nov. 18.....	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	7
1932																
Mar. 25.....	6	5	0	8	0	6	0	0	0	0	0	0	0	0	0	25
Apr. 19.....	7	0	4	7	1	0	6	5	5	4	0	2	0	0	0	41
Sept. 28.....	2	0	2	4	2	2	2	4	0	0	0	2	0	0	0	20
Oct. 18.....	6	0	4	0	2	6	8	0	7	3	0	0	0	0	0	36
Oct. 28.....	1	0	0	1	3	1	0	0	1	1	0	0	0	0	0	8
Nov. 3.....	2	0	1	1	1	0	0	1	0	0	0	0	0	0	1	7
Total (12 periods)..	24	19	14	24	18	18	16	10	13	8	3	6	4	2	1	180
Winter.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spring.....	13	12	6	18	1	6	6	5	5	4	3	4	4	0	0	87
Summer.....	0	0	1	0	2	0	0	0	0	0	0	0	0	2	0	5
Fall.....	11	7	7	6	15	12	10	5	8	4	0	2	0	0	1	88

* Ha, Havana; H, Himalayan; B, Belgian; E, English; Bv, Beveren; D, Dutch; P, Polish; C, Chinchilla; R, Rex; S, French Silver; F, Flemish; A, American Blue; Z, New Zealand; G, Gouda; T, Black and Tan.

(Table I). Standardized Trenner automatic pipettes were used. The red blood cells and the platelets were counted by the method of Casey and Helmer (10). The hemoglobin was determined by the Newcomer method, using a Bausch and Lomb hemoglobinometer. Repeated checks with other instruments and by several observers were made. The neutral red supravital technique was employed for the differential white cell counts. Smears were counted in the open laboratory without the use of the hot box, from 1 to 4 hours after their preparation (11).

Statistical Procedures.—All observations of the 14 blood factors (red blood

cells, hemoglobin, platelets, and total white cells, and neutrophiles, eosinophiles, basophiles, lymphocytes, monocytes, both per cubic millimeter of blood and in per cent) were averaged for each animal and the means of these values together with the variance of the means were determined for each breed (12). The analysis of the variance and other statistical procedures follow the methods outlined by Fisher (13) and Snedecor (12).

RESULTS

The breed means of each blood factor are summarized in Table III, and the variance of these means (the square of the standard error of the mean) for the eight breeds with the largest representation in Table IV. A general mean for the nine breeds represented by 10 or more animals is presented for each blood factor (Table III), and also for the fifteen breeds (averaging 12 animals per breed) (Table III). Means for the 180 animals without regard to breed means are presented at the foot of this table.[2]

The most important results, however, concern an analysis to determine whether for the various blood factors the variance between the eight most represented breeds was greater than the variance within these breeds. The results of this analysis expressed by the distribution of F are to be found in Table V. The significance of F (12) is presented by the probability column in this table.[3] As shown at the

[2] The two tables can be used as follows: To determine whether the red blood cell level in the Havana breed was significantly greater than that in the Rex breed, one would proceed in the following manner. First, the difference between 5,730,000 red blood cells per c.mm. in the 37 Havana rabbits and 4,870,000 red cells per c.mm. for the 13 animals in the Rex breed was 860,000 red blood cells. The significance of this difference is calculated by adding the variance of the mean for red cells in the Havana breed of 104 (Table IV) to the variance of 147 for the mean red cell level in the Rex breed giving a value of 251. This latter value is the variance of the difference. The square root of 251 equals 15.8. This is the standard error of the difference, and the difference between the two mean red cell values is then expressed as 860,000 $\pm$ 158,000 red blood cells. As the difference is more than 5 times the standard error of the difference the Havana breed can be said to have had a significantly higher red blood cell level than the Rex breed. The differences between other blood cell factors can be determined in a similar manner by the use of Tables III and IV.

[3] Significance is taken to mean a value which should not be expected to occur by random association of variables more frequently than once in 100 opportunities, and probable significance a value which would not be expected to occur more often than once in 20 opportunities or less often than once in 100 opportunities.

TABLE III

The Hemocytological Constitution of Standard Breeds of Rabbits (180 Healthy Young Adult Males)

Breed	No. of animals	Age	Means of individual means													
			RBC	Hb	P	WBC	N	B	E	L	M	N%	B%	E%	L%	M%
		mos.		*per cent*								*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Havana	24	7.6	573	73.4	562	653	349	36	7	203	57	53.7	5.6	1.1	31.0	8.5
Himalayan	19	5.1	565	67.8	476	707	325	44	12	263	65	46.2	6.6	1.7	36.5	9.3
Belgian	14	9.8	532	68.6	527	776	376	68	14	239	77	48.9	9.0	1.9	30.6	9.7
English	24	8.4	534	68.1	561	680	372	55	11	181	60	54.1	8.5	1.7	26.7	8.9
Polish	16	8.3	562	73.9	577	865	375	32	9	378	71	43.5	3.8	1.1	43.5	8.1
Dutch	18	9.5	526	71.8	635	733	398	37	11	214	71	53.6	5.3	1.6	29.9	9.6
Beveren	18	8.9	520	68.1	531	858	374	69	13	310	84	43.8	8.5	1.7	35.6	10.1
Chinchilla	10	9.6	497	62.3	548	905	429	53	16	325	79	47.6	6.2	1.7	36.1	8.4
Rex	13	7.7	487	63.6	595	782	377	62	7	262	74	48.1	7.9	0.9	33.9	9.2
Mean breeds			533	68.6	557	773	375	51	11.1	264	71	48.8	6.8	1.49	33.8	9.1
French Silver	8	6.3	498	64.9	607	816	389	37	5	267	118	47.8	4.6	0.7	32.3	14.6
American Blue	6	10.0	520	67.3	713	841	463	66	12	200	101	55.1	8.0	1.5	24.0	11.6
New Zealand	4	4.7	562	69.8	798	989	436	64	11	366	113	43.4	6.4	1.2	37.5	11.5
Flemish	3	10.7	555	69.1	388	802	374	47	26	270	85	46.5	5.9	3.4	33.6	10.6
Gouda	2	5.0	598	74.8	462	936	565	48	37	181	108	59.3	5.2	4.3	19.8	11.6
Tan	1	4.7	555	76.3	690	648	281	30	10	296	31	43.5	4.5	1.6	45.7	4.8
Mean breeds	12.0	7.8	539	69.3	578	799	392	50	13.4	264	80	49.0	6.4	1.74	33.1	9.8
Mean individuals	180	8.1	537	69.1	566	769	378	50	11.1	255	74	49.4	6.7	1.51	32.9	9.5

RBC, red blood cells (0,000 omitted); Hb, hemoglobin (Newcomer); P, blood platelets (000 omitted); WBC, N, B, E, L, M, total white blood cells, neutrophiles, basophiles, eosinophiles, and monocytes (0 omitted); N%, B%, E%, L%, M%, relative numbers of the white blood cells.

TABLE IV

The Hemocytological Constitution of Standard Breeds of Rabbits

Breed	No. of animals	Age	Variance of the mean (the square of the standard error of the mean)													
			RBC	Hb	P	WBC	N	B	E	L	M	N%	B%	E%	L%	M%
		mos.		*per cent*								*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Havana	24	1.27	104	1.49	322	853	316	13	0.5	140	30	2.21	0.22	0.01	1.57	0.30
Himalayan	19	0.07	50	1.03	296	1950	606	15	2.0	526	21	3.59	0.35	0.02	2.93	0.23
Belgian	14	4.80	144	0.43	519	2180	663	33	5.5	420	81	2.83	0.50	0.08	2.74	0.45
English	24	2.47	104	1.37	251	886	521	17	0.9	174	19	2.65	0.50	0.02	2.32	0.18
Polish	16	2.48	111	1.78	635	1040	479	11	1.4	596	82	3.07	0.19	0.02	5.08	0.80
Dutch	18	3.55	122	1.32	1835	1237	1039	12	1.1	286	54	5.71	0.20	0.04	3.94	0.81
Beveren	18	3.28	163	2.06	329	2497	659	60	6.8	995	41	5.11	0.99	0.09	4.76	0.59
Rex	13	1.65	147	3.91	503	981	576	72	0.7	189	51	3.85	0.50	0.01	1.92	0.44
Total	180	0.04	17	0.23	77	179	67	3	0.5	59	6	0.45	0.06	0.01	0.44	0.06

TABLE V

Hemocytological Constitution of Standard Breeds of Rabbits
*Analysis of the Variance**

Standard deviation	No. of animals	Age	RBC	Hb	P	WBC	N	B	E	L	M	N%	B%	E%	L%	M%
		mos.		*per cent*								*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Total	145	6.5	52	6.1	108	174	104	25	6.6	102	28	9.0	3.3	0.8	8.8	2.8
Within breeds	138	6.5	46	5.4	101	161	105	22	6.2	85	28	8.1	2.8	0.8	7.5	2.8
Between breeds	7	6.3	116	14.0	203	337	95	62	11.6	273	39	19.5	8.6	1.5	22.1	2.6
F		1.01	6.7	6.7	4.0	4.4	1.2	8.0	3.5	10.3	1.9	5.8	9.4	3.5	8.7	1.2
P		—	0.01	0.01	0.01	0.01	—	0.01	0.01	0.01	—	0.01	0.01	0.01	0.01	—
Significant		—	Sig.	Sig.	Sig.	Sig.	—	Sig.	Sig.	Sig.	—	Sig.	Sig.	Sig.	Sig.	—

* Limited to the eight most represented breeds.

bottom of Table IV the breeds were found to differ significantly among themselves with respect to red blood cells, hemoglobin, blood platelets, total white blood cells, to basophiles, eosinophiles, and lymphocytes per cubic millimeter and in per cent, and to neutrophiles in per cent. No significant differences in the monocytes per cubic millimeter or in per cent, or in the neutrophiles per cubic millimeter, or in the age of the various breeds were detected.

DISCUSSION

The present study of the hematological constitution of rabbits carried out on 180 male animals of known age and breed is unique in several respects. Earlier studies of our own (14) as well as those of other workers were not corrected for breed and age (15–17). The present investigation concerns mean blood levels rather than random blood cell determinations, uncorrected for technical error. The extent of the variations are, therefore, much less than that reported by ourselves or others. The animals were well adapted to cage life and were perhaps better nourished and healthier than the mongrels studied earlier. It will be seen that the mean red blood cell values for male rabbits do not significantly differ from the mean red blood cell values for human males as published by Price-Jones (18), Osgood (19), Wintrobe (20), McGeorge (21). The mean value compiled from the four authors for the 323 healthy young men, averaging about 25 years of age, was 5,433,000 red blood cells per c.mm. of blood. This differs from the 5,370,000 red blood cells obtained for rabbits by 63,000 $\pm$ 45,300, and since the difference is less than twice the standard error, it is not significant. It seems quite remarkable that two species so widely different as to size and many other characteristics including hemoglobin should be alike in this respect. Since the samples of both species examined are comparatively large the results are probably fairly accurate.

The present results show significant variation in the blood cell levels between the breeds for red blood cells, hemoglobin, basophiles, lymphocytes, eosinophiles, platelets, and white blood cells. This variation was significantly greater between the breeds than within the breeds. This would seem to indicate that in the rabbit genetically related individuals have similar blood formulae and genetically unre-

lated individuals dissimilar blood formulae. This conclusion is tenable only if it can be shown that no other variables have biased the results. A consideration of such factors as age, sex, season, nutrition, housing, intercurrent disease, hematological technique, and stature which might influence the results will, therefore, be made.

Sex and Age.—Sex played no part because the animals were males. Since the work of Sabin (22) has shown that the blood formula of rabbits changes from infancy to maturity some consideration of age had to be made. All breeds were of comparable mean age with the exception of the Himalayan in which there were no animals older than 8 or younger than 4 months. The age of this breed was probably significantly less than that of most of the other breeds ($P = 0.05$). However, the remaining breeds which show no difference as to age show wide variations in their formulae. In the case of the eight breeds with 13 or more animals (Himalayan included) the variance between the breeds due to age was not different from the variance within the breeds ($F = 1.01$; Table V). Furthermore, the blood formula of the Himalayan breed is that of adults (note the lymphocyte level) and not that of young or immature rabbits. It must be concluded, therefore, that neither age nor sex will explain the breed differences detected. Again an analysis of the material in various age periods revealed no delayed or irregular maturation on the part of any breed to account for its blood formula; nor was the variance between the various age periods significantly greater than the variance within the various age periods for any blood factor. A subsequent publication will discuss the age of the animals in relation to their blood formula (23).

Housing and Nutrition.—All the rabbits were subjected to the same housing and nutritional conditions and each breed had been propagated in pure line in the same laboratory for periods of 3 to 8 years. However, it is conceivable and there is evidence to support the conception that a diet and indoor life which is suitable for one breed may not be suitable for other breeds. Large animals might have had a blood formula which differed from that of small or medium sized animals in cages of similar size. However, there was a wide variation in the blood picture among such small or medium sized animals as the Dutch, Himalayan, Polish, Havana, and English where the confine-

TABLE VI

Hemocytological Constitution of Standard Breeds of Rabbits
Difference between Large and Small Breeds

	No. of animals	RBC	Hb	P	WBC	N	B	E	L	M	N%	B%	E%	L%	M%
Large and Heavy Breeds															
			per cent								*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Mean	53	525	67.8	574	832	391	62	13	274	91	47.1	7.7	1.6	32.5	11.0
Variance of mean		8	0.8	18	26	14	4	1	15	5	1.1	0.5	0.2	1.1	0.5
Small and Light Breeds															
Mean	80	559	71.9	560	733	364	38	10	255	66	49.9	5.4	1.4	34.5	8.9
Variance of mean		5	0.6	14	36	13	2	1	12	3	1.1	0.3	0.1	1.1	0.3
Differences															
Difference	—	34	4.1	14	99	27	24	3	19	25	2.8	2.3	2.0	2.1	2.1
σ difference		±10	±1.0	±23	±25	±19	±4	±2	±19	±6	±1.6	±0.5	±0.2	±1.6	±0.6
t		3.4	4.0	—	3.8	—	5.7	—	—	4.1	—	4.2	—	—	3.3
P		0.01	0.01	—	0.01	—	0.01	—	—	0.01	—	0.01	—	—	0.01

ment was comparable. Each animal was kept in an individual cage. Possible hereditary variations among the breeds, such as peculiar dietary requirements and differences in adaptability to cage life, might have been concomitant with, rather than the cause of the variations in the blood formula. In this connection the material tabulated in Table VI is of interest. The breeds were divided into heavy, intermediate, and light groups according to weight. The Flemish, New Zealand, American Blue, Blue Beveren, Belgian, and French Silver animals, 53 in all, were grouped in the heavy class. The Rex, English, and Chinchilla animals were grouped as intermediate. The Gouda, Havana, Polish, Himalayan, Tan, and Dutch animals, 80 in all, were grouped in the light weight class. It will be seen that the heavy animals had significantly lower red blood cells and hemoglobin, and higher white blood cells, basophiles, and monocytes than the light weight animals. That such differences are not associated entirely with weight or build is indicated by the formula in the Polish breed which had the highest white blood cell level, and in the French Silver which had a low basophile level. The information at hand does not indicate whether this difference in blood formula is due to body build or weight, or whether animals in breeds having similar weights were derived genetically from a similar stock. A third factor or factors might be responsible for both weight and blood formula. Studies of breed differences in blood formulae in other animal species might clear up this point. In any event the results obtained emphasize the genetic constitution of the blood formula. The significant variations among the breeds as to the blood platelets, the eosinophils, and the lymphocytes, and the neutrophils in per cent were not associated with differences in body weight or size.

Intercurrent Disease.—Studies in this laboratory have shown wide variations in susceptibility of the various breeds to experimental and spontaneous diseases. The view that the variations in the blood formulae as here reported were in reality associated with unnoticed intercurrent disease might be tenable if the clinical observations had been desultory and the blood variations of a simple order. However, the former were systematic and the latter so diverse and so often without relation to each other that an explanation based on disease conditions does not seem plausible or likely. The animals were healthy in appearance, showed no loss of weight, and there was no clinical evidence

of such spontaneous conditions as snuffles, ear canker, gastro-enteritis, or other malady. In this connection it may be pointed out that in previous studies no demonstrable lesions were found at postmortem examination to account for the variations in blood formulae in individual animals.

Technical Error.—From studies of the technical error in blood counting[4] it can safely be said that the differences between breeds could not be explained on this basis. The error in each breed mean due to technique alone is negligible. The time of the day was not a factor since the animals were so alternated that approximately the same number of each breed were counted in comparable periods.

Season.—Investigations in this and in other laboratories have shown wide variations in the blood picture for different seasons of the year. The effect of season was considered in planning the present experiment and no observations were made in winter and only five in summer. The animals were equally divided between the two seasons calculated to show the fewest differences, 87 in the spring and 88 in the fall. Although for most of the breeds the same number of animals were counted in one season as in the other, the variance between breeds as to the month of the year in which counts were made was significantly different from the variance within breeds ($z = 0.786$; $P = 0.01-$, significant). This was because the Beveren, Dutch, Polish, and Rex breeds were largely examined in the fall whereas the Himalayan, English, Flemish, American Blue, and New Zealand breeds were largely examined in the spring (Table II). However, the following tabulation shows that for every blood factor except the red blood cells and the blood platelets there was no significant difference between the spring and fall levels.

	Animals	Breeds	R	H	P	W	N	B	E	L	M
Spring	87	13	548	69.6	585	751	377	50	10	242	70
Fall	88	12	524	68.2	550	775	374	49	11	265	75
Difference	—	—	24	1.4	35	24	3	1	1	23	5
σ difference	—	—	±8	±1.0	±18	±27	±17	±4	±1	±16	±5
t	—	—	2.8	—	1.97	—	—	—	—	—	—
P	—	—	0.01	—	0.05	—	—	—	—	—	—

[4] Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L., unpublished material.

For the hemoglobin and the total and individual white blood cells, therefore, there was no bias in the results which could be ascribed to season, and no correction was necessary. In the case of the red blood cells and the blood platelets, it seemed desirable to correct for seasonal variation. This was done by adding 236,000 (the seasonal disparity) to each fall red blood cell value, and 35,300 to each fall blood platelet value. A series of new mean red blood cell and blood platelet values were obtained for each breed which represented the theoretical spring level for all 180 animals. A small correction for the five summer values was also made. The following tabulation shows the corrected red blood cell and blood platelet values, and the direction and amount of change. The variation between the breeds was not diminished by the correction for season, and its significance was not affected either

	Red blood cells	Change	Platelets	Change
Havana	583	+10	578	+16
Himalayan	574	+9	489	+13
Polish	577	+15	599	+22
Belgian	542	+10	550	+23
English	540	+6	569	+8
Beveren	538	+18	568	+37
Dutch	542	+16	659	+24
Rex	501	+24	617	+22
Chinchilla	509	+12	566	+18
Silver	510	+12	624	+17

for the red blood cells ($F = 5.6$, $P = 0.01-$), or for the blood platelets ($F = 4.4$, $P = 0.01-$). Therefore, although the animals in the fifteen breeds were not distributed evenly between spring and fall these seasonal differences played no part in the variance between the breeds for any blood factor. Furthermore, the results on one large group of 40 animals consisting of various breeds studied during the month of April, 1932, are of special interest (Table VII).

The group comprised 7 Havana, 6 Polish, 4 Belgian, 5 Rex, 5 Chinchilla, 7 English, 2 American Blue, and 4 French Silver animals. There was no significant variation in the ages of the 40 animals. These rabbits were counted in 4 different weeks during this month, and an unusually large number of observations made on each animal. The time of the day was held constant by counting each animal once

TABLE VII

Hemocytological Constitution of 40 Young Adult Male Rabbits
Series of April, 1932

Breed	No. of animals	Age	RBC	Hb	P	WBC	N	B	E	L	M
		mos.		*per cent*							
Havana	7	6.6	558	71.2	605	655	359	43	7	184	62
Polish	6	4.7	557	72.8	604	990	419	26	9	443	94
Belgian	4	6.1	524	67.9	527	886	436	75	8	279	89
Rex	5	5.4	480	61.3	596	772	332	79	8	278	75
Chinchilla	5	4.3	506	63.4	606	811	374	55	12	309	60
English	7	5.4	514	64.4	625	808	489	50	12	185	72
American Blue	2	5.3	488	66.3	828	870	509	60	14	215	74
French Silver	4	6.0	544	72.5	625	786	352	52	3	282	98
Mean	(40)	5.5	525	67.6	613	813	404	53	9	271	77
Mean square											
1. Total		556	2649	3723				647	27	12,099	
2. Between breeds		316	4392	10,138				1484	44	42,425	
3. Within breeds		609	2268	2320				464	23	5465	
F		1.9	1.9	4.4				3.2	1.9	7.8	
P		0.05	0.05	0.01				0.01	0.05	0.01	
		—	—	Sig.				Sig.	—	Sig.	

during the period between 9 and 10.30 a.m., 10.30 and 12 a.m., 1.30 and 3 p.m., and 3 and 4.30 p.m. In order to do this, the order of counting was changed every week. Individuals from the various breeds were alternated so that the animals in a given breed were not examined consecutively. The factors of time of day, and of week, month, and year were thus held constant. Nevertheless, with only a few animals represented per breed the variance between the breeds was significantly greater than the variance within the breeds for hemoglobin ($F = 4.4$, $P = 0.01-$), for basophiles ($F = 3.2$, $P = 0.01$), and for lymphocytes ($F = 7.8$, $P = 0.01-$). Hemoglobin, basophiles, and lymphocytes showed the greatest variation between the breeds in the larger series as well. It is possible that significant breed differences for red blood cells, platelets, and white blood cells would also have been found in this group had there been a larger number of animals. The results on this small group offer other conclusive evidence, therefore, that the variance between the breeds was not due to season.

Breed Relationships.—The exact origin of most of the standard breeds of rabbits is unknown or uncertain, and considerable outcrossing has been practiced from time to time to maintain the vigor of the stock. Although such outcrossing had not been carried out in this laboratory among the breed lines reported in this paper, there is no certainty as to how much this was done before the stocks were brought into the laboratory. There is no certainty that the sample animals for any breed have the same hemocytological constitution as other samples of the same breed elsewhere. It is known that the Havana line used is related to the Dutch in body build, size, and disposition; also inbreeding occasionally demonstrates Dutch markings in pure Havana rabbits. It is also known that the English are in some way related to the Belgians, both being unique in having a greyhound type of body build and similar dispositions. It seemed desirable, therefore, to determine whether or not the blood formulae of related breeds are similar. This was approached indirectly by determining whether related breeds had fewer blood differences than unrelated breeds. This was the case. The Havana blood formula resembled the blood formula of the Dutch more closely than that of any of the other breeds adequately represented, that is, there were fewer signifi-

cant differences. The same was true for the English and the Belgian. Two distinctive breeds such as the Polish and the Himalayan which are unlike any of the other breeds or each other had blood formulae which were also distinctive and unlike that of other breeds. A similar comparison for the fur breeds such as the Cinchilla, Rex, and Beveren is unsatisfactory since coat color or texture may be introduced into various breeds. Thus one may have a Dutch animal with a Rex coat. Analogies as to the blood formulae of these breeds are perhaps unjustified. The Beveren, for example, was found to have a blood formula very similar to that of the Belgian, and although both are large animals and of common Flemish origin no knowledge of interbreeding is available. Finally it should be pointed out that the blood factors observed were but a small sample of the constitutional factors which might have been chosen. The fact that two breeds are alike in a few blood factors does not indicate that they are necessarily alike in other constitutional factors. Of significance, therefore, is the fact that breeds known to be related in genetic origin were also found to have related blood pictures. Certain peculiarities of the breed formulae may be discussed in a subsequent paper.

SUMMARY AND CONCLUSION

A study of the red blood cells, hemoglobin, blood platelets, and the total and individual white blood cells was made on 180 male rabbits of known age and representing fifteen standard breeds. An attempt was made to eliminate or hold constant such variables as age, sex, season, time of examination, technical errors, food, housing, and disease. The mean, variance of the mean, and standard deviation were calculated for each breed sample and for the group as a whole. An analysis of the variance showed that the variation between the breed samples was significantly greater than the variation within the breed samples for the red blood cells, hemoglobin, blood platelets, total white blood cells, basophiles, eosinophiles, and lymphocytes per cubic millimeter and in per cent and the neutrophiles in per cent. No significant variations were detected in the monocytes except when the breeds were divided into heavy and light breeds. No variation in the neutrophiles per cubic millimeter was detected; a large number of the breeds had exactly the same mean neutrophile level. Characteristic

blood formulae were found for the various breed samples having an adequate numerical representation. It was concluded that the varying blood formulae could not be explained on any other, except an hereditary (genetic) basis.

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