STUDIES ON THE BLOOD CYTOLOGY OF THE RABBIT

VIII. The Blood of Normal Rabbits as an Index of Their Resistance to a Transplantable Neoplasm

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This paper reports an investigation in connection with the study of animal constitution as a factor in susceptibility and resistance to disease (1). Blood was the constitutional factor studied because of the basis for work furnished by preceding papers which deal with the distribution, the spontaneous variations, and the interrelationships of the blood cells in the normal rabbit (2–7). A malignant neoplasm was chosen as the disease to be followed because of the knowledge already acquired concerning the blood reaction in rabbits inoculated with the tumor (8).

Two fundamental considerations underlie the present statistical investigation of the relationship between the blood cytology of normal rabbits and their susceptibility to this malignant disease. First, the malignancy of the transplantable neoplasm as observed in this laboratory varies from season to season and year to year, both as concerns groups of animals and among the individuals of the groups (9–11). Second, the cellular elements of the blood of normal rabbits vary seasonally and yearly, and appreciable differences in the cell formula occur both between groups (3–7) and individuals (2).

Material and Methods

Data have been obtained from 2 groups of rabbits inoculated with the Brown and Pearce rabbit tumor. The first group comprised 78 rabbits which were the same as those used in the experiments on which the blood reaction to tumor inoculation was reported (8). A description of the animals and of the experimental methods employed has already been given. The animals which were used for working out the methods of correlation were distributed as follows:

TABLE I

Group	No. of rabbits	First blood examination	Last blood examination	Tumor inoculation	No. of blood examinations before inoculation*	No. of weeks in laboratory before inoculation
1	10†	Oct. 24, 1927	Nov. 16, 1927	Nov. 17, 1927	4	4½
2	5	Dec. 5, 1927	Jan. 5, 1928	Jan. 5, 1928	12	4½
3	9	Feb. 8, 1928	Feb. 23, 1928	Feb. 24, 1928	4	2
4	10†	Apr. 6, 1928	Apr. 18, 1928	Apr. 20, 1928	4	2
5	10	Sept. 18, 1928	Nov. 22, 1928	Nov. 22, 1928	8	8
6	5†	Dec. 29, 1928	Jan. 3, 1929	Jan. 4, 1929	3	1
7	12	Oct. 26, 1929	Nov. 19, 1929	Nov. 19, 1929	7	4
8	12	Dec. 31, 1929	Jan. 14, 1930	Jan. 14, 1930	5	2
9	8	Dec. 31, 1929	Jan. 14, 1930	Jan. 14, 1930	5	26
Total...	78					

* In an earlier paper upon the blood cytology during the course of malignant disease (8), only those blood counts made within 4 weeks of inoculation were included. In the present paper all counts before inoculation are included without regard to the time factor.

† 1 rabbit each of Groups 1 and 4 was omitted because of a complicating nephritis; and an animal in Group 6 also because it was killed for transfer material before the end of the experiment.

A second series of 13 rabbits was used as a means of testing the accuracy of the methods developed with the first group; their distribution was as follows:

TABLE II

Group	No. of rabbits	Tumor inoculation	First blood examination	Last blood examination	No. of blood examinations before inoculation	No. of weeks in laboratory before inoculation
10	8	Sept. 17, 1930	Sept. 19, 1930	Sept. 19, 1930	2	2
11	5	Dec. 13, 1930	Dec. 13, 1930	Dec. 15, 1930	10	26
Total...	13				12	28

The hematological technique employed (2) included determinations of the total red and white cells with standardized pipettes, hemoglobin estimations by the Newcomer method, and neutral red supravital differential counts. 100 white cells were counted in each differential white cell determination. The number of blood counts made upon each group of animals before inoculation varied from 3 to

12 and they were distributed irregularly over the 1 to 10 weeks immediately preceding inoculation. The values used in this analysis are the *means* of *all counts* made upon each animal *before inoculation*.[1]

The transplantable tumor is considered to be an epithelioma. It arose spontaneously in the scrotal skin of a rabbit 10 years ago (12) and has been carried from animal to animal by intratesticular inoculation for upwards of 100 generations.

Estimation of Malignancy.—Each experiment was terminated 2 months after inoculation and all surviving animals were killed by air embolism. A complete autopsy was made on every animal with especial reference to the distribution and the character of any tumor.

For the present statistical analysis, expressions of malignancy were reduced to a numerical basis by one of two methods. The first was the outcome of the experience that 2 months after inoculation, animals can be divided into four categories with respect to death or survival, namely: "deaths," "probable deaths," "probable recoveries," and "recoveries." Animals which at autopsy revealed no evidence of tumor, either at the site of inoculation or elsewhere are designated as "recoveries." Those which at autopsy showed only a slight tumor growth, usually in a necrotic condition, such as a residual primary lesion of the testicle, a circumscribed lymph node metastasis, or small peritoneal implantations, would presumably have recovered from the disease and, therefore, these individuals have been classed as "probable recoveries." The remaining animals which showed more or less widespread metastases of living tumor at autopsy have been classed as "probable deaths." In order to give a numerical value to the malignancy in each animal, the relative susceptibilities of the four classes of animals have been simply expressed as 4, 3, 2, and 1, respectively, or in percentage terms of malignancy as follows: "deaths" 100 per cent; "probable deaths" 67 per cent; "probable recoveries" 33 per cent; and "recoveries" 0 per cent.

This method of classification was used as the basis for studying the relation between the pre-inoculation blood findings and susceptibility or resistance to the tumor. The results obtained, however, were controlled by a second numerical method of appraisal. This method consists in determining the number of organs or tissues having tumor growth as determined by autopsy observation. Neither the number nor the extent of the growth in any organ nor the character of the growth is taken into consideration, but simply the presence or absence of a tumor focus. The number of possible foci has been arbitrarily set at 20, and the distribution of animal material upon this basis is as follows: "recoveries" 0 foci, "probable recoveries" from 1 to 4 foci, "probable deaths" from 4 to 12, and "deaths" from 4 to 20 foci. This second method of classification which gives a numerical value to the actual distribution of tumor in each animal has been included as

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[1] The mean values used in the present analyses differ somewhat from those mentioned in the report on the blood reaction after inoculation, as has been stated in that paper (8).

supplementary evidence of the accuracy of the first method. In general, it has been found that the analytical results obtained with both methods are in substantial agreement.

Methods of Analysis and of Recording Results.—The method of determining the relation of the blood cells before inoculation to the course of the malignant disease has been to construct a simple correlation table for each type of cell studied. The four values for tumor malignancy have been placed along the ordinate and the values of the blood cell factor under consideration along the abscissa in an appropriate number of boxes. Simple averages of the malignancy of the tumor in those cases corresponding to each box along the abscissa were then made. Owing to the inadequate number of cases in some boxes, the box means were smoothed by the formula $\frac{S_A + 2S_B + S_C}{N_A + 2N_B + N_C}$ where S_B was the summation of the ordinate values in the box to be smoothed, S_A that of the preceding, and S_C that of the succeeding box; N_A, N_B, N_C represented the actual number of cases falling in the respective boxes. The smoothed box means were then connected by lines; the result of this procedure is shown in the curves of Text-figs. 1, 2, and 3. The small numbers beside each point in the curves represent the actual number of cases in each box before smoothing was done. The vertical line down the center of each chart represents the approximate modal value obtained upon normal rabbits in this laboratory.[2]

The values in the text-figures represent an analysis of the entire series of 78 animals without regard to individual experimental groups, time of inoculation, or other factors. An analysis of this series of animals group by group by a thoroughly satisfactory statistical method is virtually impossible, owing to the small number of animals in each group. But though the specific degree of relationship by groups cannot be obtained, it is relatively simple to show that such a relationship does or does not exist. Simple correlation coefficients were calculated for each of the 9 groups between the blood cell values before inoculation and the numerical estimate of malignancy, using both methods of estimating the tumor malignancy; the results are shown in Table III. Correlations were also made upon the 2 additional groups of 13 rabbits previously referred to; the results are also shown in Table III under the headings of Groups 10 and 11.

For our present purpose, the pre-inoculation blood cell values for each animal in the analysis given in Table III have been combined and are expressed by a single numerical value which is considered to represent "mean susceptibility." The reasons for expressing the entire 15 pre-inoculation blood cell values as a single value are as follows: first, the technical inaccuracy of the mean of any particular blood cell value may be great; second, a single such inaccuracy might

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[2] Since the paper upon distributions (2) was published, a definite trend in certain classes of cells has occurred, necessitating slight changes in the modal values for total white cells, eosinophiles, and lymphocytes.

materially affect the correlation coefficient in groups of less than 10 counts; and finally, the mathematical processes involved in handling multiple curvilinear relationships are confusing to the non-statistical reader.

The method by which the entire 15 pre-inoculation blood cell means were combined into a single numerical value may be summarized as follows: first, the mean malignancy of the tumor for the 78 rabbits as expressed in Text-figs. 1, 2, and 3 was found to be about 58 per cent; second, an imaginary horizontal line was drawn through each chart in the text-figures at the level of 58 per cent; third, those points in the smoothed curves which fell above this imaginary line of 58 per cent were said to belong to boxes representing susceptible animals; fourth, any animal which for any given blood cell factor had a pre-inoculation mean falling into a "susceptible box," was said to be susceptible to tumor in so far as this particular blood factor was concerned; fifth, the total number of such susceptible factors was then determined for each animal. This total number was considered to be the numerical estimate of the susceptibility of the particular animal to the tumor. In Table III, the figures in the column under the heading "mean susceptibility" are the simple averages of the so called susceptible factors for each group of animals.

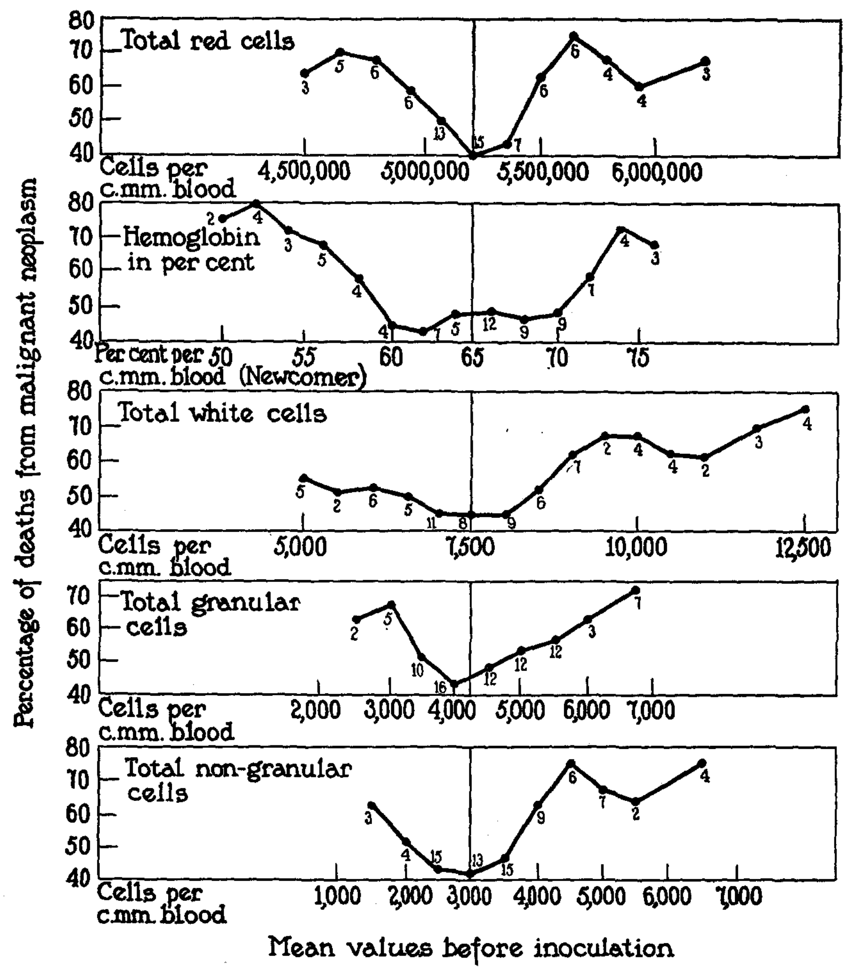
The coefficients of correlation listed at the bottom of Table III are based upon the group means given in the table and represent the relations, first, between mean susceptibility and mean malignancy, second, between mean susceptibility and mean number of foci, and third, between mean malignancy and mean number of foci respectively. A similar series of correlations upon the individual animals of each group are shown in the 3 right hand columns of Table III. Perfect correlation would be 1.000 and the absence of correlation 0.000. Neither perfect correlation nor the absence of correlation is often obtained in practice, and the coefficients obtained lie between these extreme values. The degree of correlation depends for its significance upon the number of values in the series. Since the significance of a coefficient depends upon the number of values in the series to be correlated, a correlation coefficient of +.300 obtained for the entire 78 animals would be significant while one of +.600 for 5 animals or less would not be significant. A coefficient of correlation is arbitrarily taken to be significant when the chances of its occurring by a random association of unrelated variables is less than 1 per 100. It is considered probably significant if the chances of its occurring by accident are from 1 to 5 per 100.

It should be noted that in the analysis contained in Table III, "deaths," "probable deaths," "probable recoveries," and "recoveries" are designated by the numbers 4, 3, 2, and 1 respectively while in the text-figures, these values are represented by 100, 67, 33, and 0 per cent. The use of numbers rather than percentage values in the analysis of Table III was solely for the sake of facility in making the calculations and in no way affects the results obtained.

RESULTS

The Relation of Blood Cell Values of Individual Animals to Malignancy

In Text-fig. 1 it will be noted that those rabbits with average pre-inoculation red blood cell counts varying between 5,000,000 to 5,500,-000 per c.mm. were the most resistant animals to the tumor. The



TEXT-FIG. 1. The relation of the blood cytology before inoculation to the course of malignant disease.

mode, median, and mean of more than 1,000 counts on normal animals lie between 5,050,000 and 5,250,000 red cells per c.mm. of blood. The low point of tumor malignancy occurred among animals with 5,200,000 red cells per c.mm., and it would seem, then, that rabbits with normal red cell counts were the most resistant to this malignant disease. However, 40 per cent of the animals with normal counts before inoculation died from the tumor while not more than 70 per cent of animals with abnormal counts succumbed.

The values for the hemoglobin content show that the rabbits which were most resistant had mean pre-inoculation hemoglobin[3] values of between 60 and 70 per cent (Text-fig. 1). The mean, median, and mode of the normal counts already referred to lie within these limits. Again, it would seem that rabbits with abnormal values were the most susceptible to the malignant disease. Among the animals with a normal hemoglobin content, the death rate was 45 per cent and among those with abnormal values, the actual and probable death rate was 75 per cent.

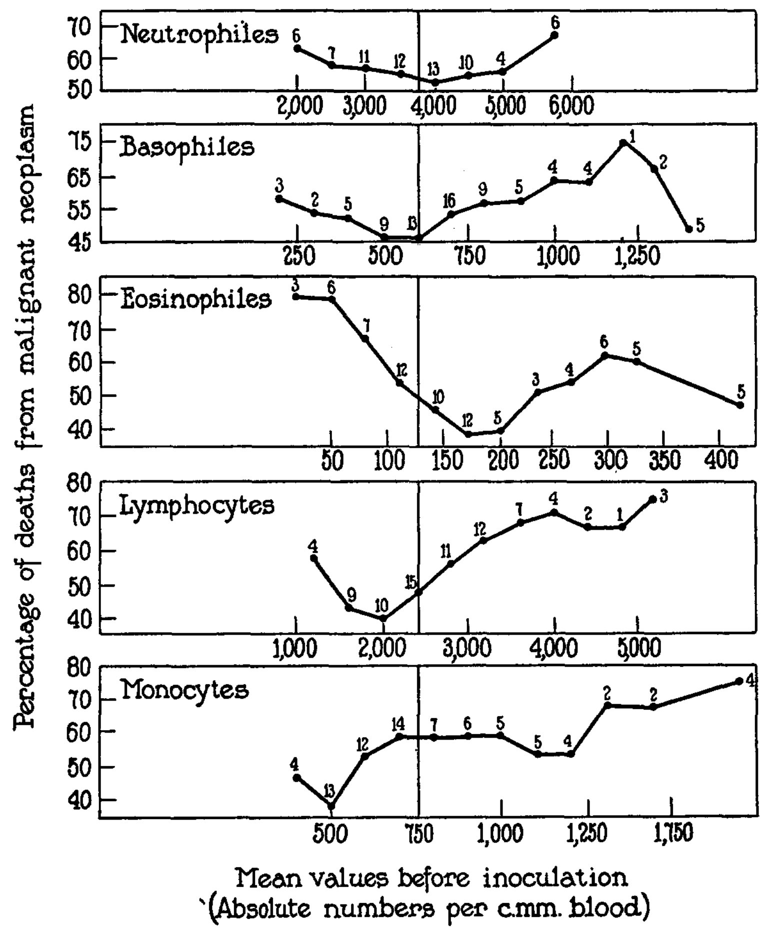
The rabbits which were most resistant had mean white blood cell counts before inoculation of between 6,500 and 8,000 per c.mm. (Text-fig. 1). The mode of the normal counts was approximately 7,500 per c.mm. Animals with mean counts below 6,500 tended to be slightly more susceptible to tumor than those within modal limits. Animals with white blood cells of more than 9,000 were definitely more susceptible than those with low and normal counts. The highest susceptibility of 70 to 75 per cent occurred in the 7 animals with total white cell counts of approximately 12,000 per c.mm.

Normal mean total granulocyte counts before inoculation were in general associated with resistance to the tumor while both high and low granulocyte counts were associated with greater susceptibility (Text-fig. 1). The rabbits which were most resistant had mean pre-inoculation granulocyte counts of between 3,750 and 4,250 cells per c.mm. These values coincide with the modal point for normal animals.

The total non-granular cells of the blood before inoculation also

[3] The values for hemoglobin are only relative, due to the wide variation between methods of estimation, readings by different individuals, and with different instruments of the same type.

seemed to be related to the outcome of the malignant disease. The rabbits which were most resistant had pre-inoculation values of between 2,350 and 3,750 cells per c.mm. (Text-fig. 1). The most sus-



TEXT-FIG. 2. The relation of the blood cytology before inoculation to the course of malignant disease.

ceptible animals were those which had pre-inoculation values above 3,750 per c.mm. Increased susceptibility was also noted in animals with total non-granular cell counts of less than 2,250 per c.mm.

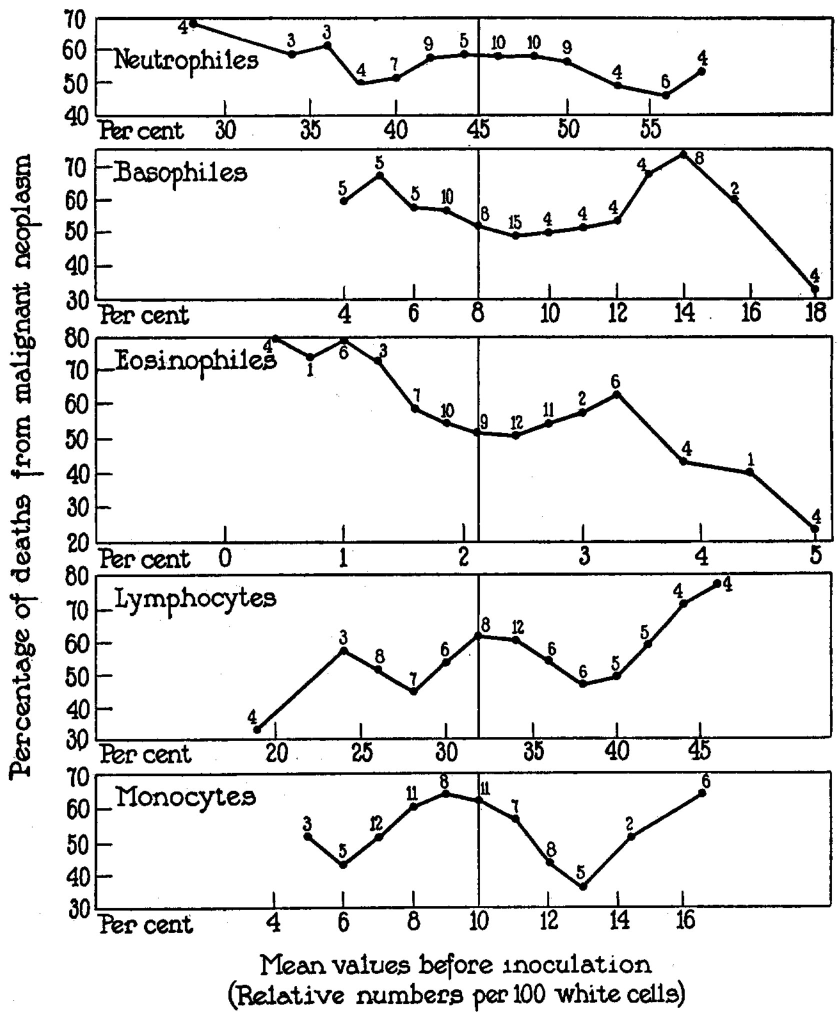
Again, deviation from the modal value seemed to be associated with lessened resistance.

Text-fig. 2 illustrates the relation of the various classes of white cells to the outcome of the malignant disease. The absolute numbers of cells per c.mm. of blood are given. It can be seen that, in general, rabbits with normal or approximately normal neutrophile, basophile, and eosinophile counts were more resistant than those with abnormal values; but it should be noted that in the case of extremely high basophile and eosinophile values, the animals tended to be resistant. In the case of the eosinophiles, however, low values were associated with an unusual susceptibility. The reaction of the various neutrophile values was of a minor order since the difference between the most susceptible and the most resistant animals was but 10 per cent.

The most resistant rabbits had pre-inoculation lymphocyte values of approximately 2,000 per c.mm.; of the 34 animals in this class, 21, or 62 per cent, were resistant to the disease. On the other hand, only 3 of the 10 animals with mean lymphocyte values of 4,000 or more per c.mm. were resistant. There was a slight tendency for animals with lymphocytes below 2,000 per c.mm. to be in the susceptible category.

In the case of the monocytes, those animals which were most resistant to the tumor had the lowest pre-inoculation values while those which were most susceptible had the highest. An exception is to be noted in the case of 4 animals with monocyte counts of below 500 per c.mm., which succumbed to the disease. The tendency for animals with low monocyte counts to be resistant and those with high counts to be susceptible is exactly the opposite to the eosinophile findings in which high values were associated with resistance and low values with susceptibility.

In Text-fig. 3, use has been made of the mean number of each white cell type per 100 cells, that is, relative values in contrast to the absolute mean numbers per c.mm. of blood previously employed. Two contrasting types of trend in relation to resistance to the tumor are to be noted. The trend of the neutrophiles, basophiles, and eosinophiles is down, that is, the more susceptible rabbits had low relative values before inoculation while the most resistant ones had high values. The trend is very similar for the three types of granular cells although it is most striking in the case of the eosinophiles. With respect to



TEXT-FIG. 3. The relation of the blood cytology before inoculation to the course of malignant disease.

the non-granular cells, however, the tendency here shown is for low values to be associated with greater resistance and high values with susceptibility. The trend in the case of the lymphocytes is in striking contrast to that of the eosinophiles. The relative monocyte values were very irregular in their relation to the outcome of the malignant disease.

It should be noted in regard to the relative values of the various white cells, as shown in Text-fig. 3, that the most resistant animals did not have counts which fell within modal limits. This is in marked contrast to the results found for the total white cell counts and those for the total granular and non-granular cells, the hemoglobin percentages, and the red cell counts, and to some extent, it is in contrast to the values for the absolute numbers of the various types of white cells (Text-figs. 1 and 2).

TABLE III

Mean Susceptibility as Determined from the Blood Cytology before Inoculation, Correlated with the Actual Course of a Malignant Neoplasm

Group	No. of animals in each group	Mean susceptibility	Mean malignancy	Mean no. of foci	r_{SM}	r_{SF}	r_{FM}
1	9	3.7	3.1	7.3	+.8974	+.6825	+.8300
2	5	2.6	2.6	5.4	+.0417	−.1379	+.8539
3	9	3.7	3.0	7.0	+.6397	+.5621	+.8669
4	9	2.3	2.4	3.6	+.5086	+.5013	+.8000
5	10	1.9	2.0	3.6	+.5770	+.7484	+.9425
6	4	4.0	2.8	7.0	+.1136	−.1199	+.8992
7	12	2.8	3.1	8.0	+.8358	+.6237	+.8548
8	12	2.4	2.6	5.8	−.1267	−.0658	+.7608
9	8	2.1	2.3	4.4	+.8017	+.8610	+.8966
10	8	3.1	2.8	5.0	+.7234	+.5757	+.9397
11	5	5.0	3.4	10.0	+.8965	+.8933	+.9863
Coefficients of correlation upon the entire 91 animals..					+.6390	+.5607	+.8858*
Coefficients of correlation upon the 11 group means.					+.8577	+.8582	+.9325

r = coefficient of correlation.
S = mean susceptibility.
M = mean malignancy.
F = mean number of foci.
* Transformed method correlations (14).

The Relation of the Blood Findings to Malignancy in Groups of Animals

In Table III which contains the analysis of the material from the standpoint of animal groups, it will be seen that the correlation between mean susceptibility and mean malignancy is greater when calculated from the 11 group means (+.858) than when calculated from the individual values for the 91 animals (+.639). In addition, the relation between mean susceptibility and mean number of foci was greater for the group means (+.858) than for the individual values (+.561). And again, the correlation of mean malignancy and mean number of foci was greater for the group (+.933) than for the individuals (+.886).

The correlation coefficients as regards pre-inoculation blood cytology (mean susceptibility) and the outcome of malignancy are comparable in magnitude with those coefficients based upon the relations between the two methods used for estimating malignancy. Both methods of estimation gave comparable results since their coefficients of correlation with the pre-inoculation blood cytology are nearly the same, namely, +.8577 and +.8582 for the 11 groups of animals and also +.6390 and +.5607 for the entire series.

DISCUSSION

No attempt has been made in this paper to give more than a brief outline of the relationships of the blood cells before inoculation to the course of the disease induced by a transplantable malignant neoplasm. The chief aim has been to show whether such relationships exist. This is the case, and it ought to be possible to predict, in the generality of cases, the eventual outcome of the disease from a cytological study of the blood before inoculation. Certain aspects of this matter and of the experimental material in relation to it will first be considered.

The Animal Material.—The use of stock rabbits of various breeds, types, and sources in these experiments was probably a benefit since a maximum number of blood variations and types of response to experimental conditions might be expected. However, the relationships encountered could conceivably be due to unknown differences in breed and type rather than to intrinsic differences in the blood formulae. In order to determine whether these relationships of pre-inoculation blood counts to resistance are dependent or independent of breed or type, other experiments with pure breeds of rabbits will have to be carried out. These are now in progress. They will serve the additional purpose of determining possible differences in cell formulae of different breeds as well as of different individuals of the same family.

The Hematological Procedure.—It is generally accepted that the numbers of blood cells in an individual animal are subject to spontaneous variations over periods of weeks or months. In attempting to relate blood findings to resistance, this feature undoubtedly plays an important rôle since an accurate blood cell formula determined upon the day of inoculation might differ significantly from one made 2, 3, or

20 weeks before inoculation. In addition, blood cell values consisting of averages of weekly determinations made during the 10 weeks preceding inoculation might differ significantly from those obtained at other intervals. The duration of the most desirable period for making the pre-inoculation counts is not known, and the appropriate number of such counts is undetermined. It seems possible, however, that with this particular tumor at least 10 counts should be made over a period of 3 weeks prior to inoculation.

The Tumor.—Any application of the results to other transplantable tumors or to the spontaneous tumors of man and the lower animals, must be made with a realization of the special material used in our experiments. From the analyses of our observations, it seems possible that a method has been evolved by means of which the cytological elements of the blood and perhaps other biometrical factors can be studied in the relation to susceptibility to transplantable tumors and other diseases. A preliminary study of such nature has already been made in experimental syphilis (16); and a more detailed report is in preparation.

In the experiments, the method of unilateral testicular inoculation was always employed. Whether the use of other sites would result in similar findings is not known. It must be borne in mind that different tumors are necessarily used as the inoculum for different experiments, and it is by no means probable that the state or condition of the tissue employed was identical in any two experiments.

The Method of Classifying Malignancy.—A satisfactory appraisal of malignancy in any given animal is admittedly difficult. The two numerical methods here employed are arbitrary and often at variance. For instance, death from inanition may result from tumor involvement of the jaw and no other tumor be found at autopsy. This might be classified as a "death" by one method or an instance of considerable resistance by another. Furthermore, an animal surviving for 2 months with widespread metastases would be described as a "probable death" while another animal dying soon after inoculation because of a single metastasis which occurred in the hypophysis would be classified as a "death." The method of counting the actual number of foci is also subject to correction since one animal with 12 foci may die in 4 weeks while another in the same group with 15 foci may live for 10 weeks. From the results of the present analyses, it seems questionable whether an animal with 10 foci is less susceptible than one with, for instance, 18 foci. It is all the more striking, therefore, that such high correlations between pre-inoculation blood findings and animal resistance have been found.

The question of the special character of the disease in relation to pre-inoculation blood cytology has also been considered, although the present material is too limited to warrant more than general suggestions. A preliminary survey, however, indicates that particular types of metastatic involvement may perhaps be associated with certain pre-inoculation blood cell values.

The Statistical Methods of Analysis.—It is quite obvious that the present problem involves multiple curvilinear correlations of a very complex sort (15). Before such an analysis can be undertaken by any of the usual methods, however, more data are necessary in order to give approximate mathematical formulae to the curves of all the various cell values in relation to resistance. Further work in the direction of normal blood cell relationships is also indicated (7). The present paper, therefore, should not be considered as an attempt to measure the *degree* of the relationships between blood cell values and resistance, nor as an attempt to express these relationships with mathematical exactness. The present data suffice merely to show the presence and the general direction of these relationships.

The Relation of the Blood Findings to Malignancy in Individual Animals

From the results, as presented, it is impossible to determine how often an animal having one blood factor indication of susceptibility will have others giving a similar indication. The following tabulation gives an idea of the frequency with which these points or values of susceptibility occurred. The number of points of susceptibility for each animal as described in the section on material and methods, are taken from the 15 blood factors representing susceptibility as given in the curves of Text-figs. 1, 2, and 3. The entire 78 animals used in Table I plus the 2 additional groups of 13 animals mentioned in Table II are classified on the basis of the number of points of susceptibility as denoted by the pre-inoculation blood cell values. The malignancy of the tumor in each class is expressed in percentage values as previously described.

TABLE IV

Points of susceptibility	No. of animals	Proportion of resistant animals
		per cent
0	6	100
1	13	93
2	22	59
3	19	26
4	12	25
5	11	27
6	5	0
7	3	0
	91	

The first fact to be noted in the above tabulation is the distribution of the numbers of animals. This distribution has a decided skew to the right, indicating that the exceptional animals of the series were more often highly suceptible than highly resistant. From this finding, it might be construed that more factors contribute toward the production of susceptibility than toward resistance.

The second point is that the blood of these animals indicated a relatively small number of points of susceptibility. Out of a possible 15 points which any animal might show, the majority had only 2 or 3 and the maximum was 7 points. A possible explanation for this finding suggests itself. The selection of normal animals for the experiments was made on the basis of weight, age, body build, and freedom from clinical signs of spontaneous disease. This implied a generally good physical condition. It might be assumed that such a healthy condition would be associated with a blood formula containing few points of susceptibility to disease, both spontaneous disease and that resulting from outside interference, such as the tumor. If this assumption is correct, it follows that rabbits with more than 7 points of susceptibility, which presumably represent a general breakdown of body resistance, would not attain the age of the animals of these experiments, or if living, would not be chosen as normal animals. This explanation is substantiated by the trends of the curves in Text-figs. 1 and 2 in which animals with normal blood values tended to be most resistant while variation either above or below normal was associated with lowered resistance to the tumor.

It is possible that susceptibility to different diseases may not be expressed by the same pre-inoculation blood formula, but by variations of a quantitative or a qualitative nature. This phase of the subject should be referred to because of the suggestion contained in the findings on the relative percentage values for the white blood cells given in Text-fig. 3. These results apparently show a certain amount of specific association in that they are independent of normal values.

The third point in the tabulation to which attention should be called is the close correlation of the number of points of susceptibility with the proportion of resistant or susceptible animals. The 6 animals which had no blood findings indicative of tumor susceptibility were entirely resistant to the disease, and the 8 animals with 6 and 7 blood factors indicative of tumor susceptibility showed little or no resistance to the tumor. In the case of the 42 animals with 3, 4, and 5 points of susceptibility, 75 per cent succumbed to the tumor. Of the 13 animals with only a single point of susceptibility, 93 per cent were resistant. The only group of animals in which it would be difficult to foretell the reaction to tumor inoculation with considerable accuracy is that with 2 points of susceptibility; 3 out of every 5 animals were resistant. This group is the largest in the series, but the succeeding one of 3 points of susceptibility is almost as large.

An index of resistance might, therefore, be devised from the above analysis in which all rabbits with 3 or more points of susceptibility are classified as susceptible and all with 2 or less, resistant. By means of such an index, 73 of the 91 animals, or 80 per cent, would be accurately classified. This accuracy can be further increased to 90 per cent for this series by the use of such quantitative biometrical methods as partial correlation. In this further analysis, account has been taken not only of the presence, but also of the degree of resistance or susceptibility as indicated by each blood factor and the relative importance of the several factors. It has been found from these calculations that certain combinations of factors seem to bear more relation to malignancy than others. These points will be discussed in subsequent papers.

The Relation of the Blood Findings to Malignancy in Groups of Animals

Probably one of the best tests of the applicability of an index of resistance derived from the values obtained on individual animals is its accuracy in representing group values. Table III represents such a test on the present material. That the coefficients of correlation upon the group values were higher than the combined coefficients upon the individual animals was to be expected; group values are less affected by errors of estimation from the standpoint of mean susceptibility, mean malignancy, and mean number of foci. The errors in calculating a coefficient based upon small numbers of animals are sometimes large as in Groups 2 and 6 with only 5 and 4 animals respectively. The very high coefficients obtained in the case of Group 11 which also had only 5 animals may be explained likewise by chance due to the small number of animals or by the fact that an especially large number of blood counts before inoculation were made.

Satisfactory coefficients of correlation depend upon accurately determined variations between individuals. In Group 8 comprising

12 rabbits, only 1 animal was a "recovery" and only 2 were "deaths." When the series came to autopsy, the distribution of living tumor in the majority of the other 9 animals was such that a classification of "probable deaths" or "probable recoveries" was unsatisfactory. It is interesting that in these particular animals, the pre-inoculation blood cell values showed few significant variations. The occurrence of such uniformity in a group with an absence of any considerable numbers of fatal or recovered cases probably explains the lack of a significant correlation. However, when these animals are considered with the rest of the 78 rabbits, either individually or as a group, their blood values are closely correlated with the course of the malignant disease. This fact is shown by the values of their group means in Table III.

SUMMARY AND CONCLUSIONS

1. The blood cytology of 91 rabbits was studied prior to inoculation with a transplantable malignant neoplasm. The following statements refer in each instance to the mean values of the pre-inoculation counts.

2. The animals which were most resistant to the malignant disease had, before inoculation, normal red and white cell counts, normal hemoglobin percentages, high eosinophile counts, and low counts of monocytes and lymphocytes. The relations of the neutrophile and basophile counts were irregular, but normal values also appeared to be associated with greater resistance.

3. The most susceptible animals were those which had, before inoculation, red cell counts above 5,500,000 or below 5,000,000 per c.mm.; hemoglobin above 70 per cent or below 60 per cent (Newcomer); white cell counts below 6,000 or above 8,500 per c.mm.; low eosinophile, high monocyte, or high lymphocyte counts.

4. No animal with any of the following findings prior to inoculation recovered completely from the tumor as determined by autopsy examination: red cells above 5,500,000 per c.mm. of blood; hemoglobin above 70 per cent; total white cells above 10,000 per c.mm.; eosinophiles below 120 per c.mm., or below the relative value of 1.5 per cent; basophiles below 400 per c.mm., or below the relative value of 6 per cent; lymphocytes above 3,600 per c.mm.; monocytes above 1,500 per c.mm.; neutrophiles above 5,000 per c.mm.; and total granular cells above 5,700 per c.mm.

In the case of each of the following pre-inoculation values, only 1 animal was completely free from tumor at autopsy: hemoglobin below 60 per cent; red cells below 4,800,000 per c.mm.; total granular cells below 3,300 per c.mm.; total non-granular cells below 2,300 per c.mm.; total non-granular cells above 3,700 per c.mm. No animal with pre-inoculation eosinophiles above 3.9 per cent, or basophiles above 16 per cent died from the tumor.

5. The blood findings before inoculation could be related to the character and outcome of the malignant disease, from the standpoint of animal groups as well as in the case of individual rabbits.

6. From the results of the experiments here reported, it seems possible to predict with an accuracy of between 80 and 90 per cent the individual resistance or susceptibility of rabbits to the tumor by a study of their blood cells before inoculation.

BIBLIOGRAPHY

1. Brown, W. H., *Harvey Lectures*, 1928–29, **24**, 106; *Arch. Int. Med.*, 1929, **44**, 625.
2. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.
3. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 23.
4. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 39.
5. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 145.
6. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 167.
7. Casey, A. E., *J. Exp. Med.*, 1931, **53**, 695.
8. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1931, **53**, 895.
9. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 799.
10. Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1923, **38**, 347.
11. Brown, W. H., Pearce, L., and Van Allen, C. M., *J. Exp. Med.*, 1924, **40**, 583.
12. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 601.
13. Wallace, H. A., and Snedecor, G. W., Correlation and machine calculation, Iowa State College, Ames, Iowa, 1925.
14. Fisher, R. A., Statistical methods for research workers, 3rd edition, London, Oliver and Boyd, 1930.
15. Ezekiel, M., Methods of correlation analysis, New York, John Wiley and Sons, Inc., 1930.
16. Casey, A. E., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 670.