

STUDIES IN THE BLOOD CYTOLOGY OF THE RABBIT

VII. OBSERVATIONS ON RABBITS INOCULATED WITH A TRANSPLANTABLE MALIGNANT NEOPLASM

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Previous papers of this series contain successive observations on the blood count of groups of normal rabbits examined over prolonged periods of time (1, 2, 3, 4, 5). The striking feature brought out by the analysis of these results was the character of the numerical variations shown by the several classes of cells and by the hemoglobin content; it appeared that certain of these variations were of an orderly nature rather than chance occurrences and that in many instances, they appeared to be related to environmental (seasonal) conditions. A further statistical analysis showed, moreover, that a significant association existed between the variations of certain cells while in other cases, no relation whatever was demonstrated (6). The magnitude of the variations, it should be pointed out, was within the limits of what is usually considered normal.

The object of the observations on normal rabbits was not only to obtain information on the character of the blood picture with respect to environmental conditions, but in addition, to provide a background for similar observations in experiments dealing with the reaction of rabbits to various disease agents. The agents employed in these experiments were *Treponema pallidum* (7), virus III disease of rabbits (8), and a transplantable malignant neoplasm (9). In connection with these studies on the blood picture of inoculated rabbits, parallel observations have also been made on tissues involved in the disease process, the idea being that the findings in the one might be related to those of the other and thus open up a method of experimental approach to the question of the nature of the host's reaction. Still other studies have been carried out with respect to the reaction of the

host from the standpoint of the character of the blood picture before inoculation. These two aspects of our work will be reported later.

The present paper contains the results of successive observations on the peripheral blood picture of rabbits inoculated with a transplantable malignant neoplasm. The tumor has been carried in this laboratory for 10 years and has been used in many experiments under diverse circumstances (10); it is considered to be of epithelial origin.

Material and Methods

The material used in this study was derived from observations on 9 groups of rabbits, a total of 78 animals. The dates of inoculation and other data are given in Table I.

TABLE I

Group	No. of rabbits	Tumor inoculation	First blood examination	Last blood examination	Number of blood examinations*	
					Before inoculation	After inoculation
I	10†	Nov. 17, 1927	Oct. 24, 1927	Jan. 18, 1928	4	9
II	5	Jan. 5, 1928	Dec. 12, 1927	Feb. 14, 1928	9	19
III	9	Feb. 24, 1928	Feb. 8, 1928	Apr. 25, 1928	4	9
IV	10†	Apr. 20, 1928	Apr. 6, 1928	June 20, 1928	4	9
V	10	Nov. 22, 1928	Nov. 2, 1928	Jan. 21, 1929	4	8
VI	5†	Jan. 4, 1929	Dec. 29, 1928	Mar. 8, 1929	3	8
VII	12	Nov. 19, 1929	Oct. 29, 1929	Jan. 13, 1930	6	15
VIII	12	Jan. 14, 1930	Dec. 31, 1929	Mar. 18, 1930	5	9
IX	8	Jan. 14, 1930	Dec. 31, 1929	Mar. 18, 1930	5	9
Total. . . .	81				44	95

* In certain series, additional counts prior to the selected preinoculation period as well as the extra counts on a few animals kept 10 weeks after inoculation are not included.

† One rabbit each of Groups I and IV omitted because of a complicating nephritis; one animal of Group VI omitted because it was killed for transfer material before the end of the experiment.

The rabbits were adult male animals approximately 6 to 8 months of age at the time of inoculation. They were representative of the usual stock received from dealers and may be described as brown, black, and Flemish crosses. In the case of Group IX, the rabbits were born and raised in this laboratory; their ages varied from 6 to 12 months and for the most part, they also were of hybrid stock. Dur-

ing the entire observation period, each animal was caged separately; the diet consisted of hay, oats, and cabbage.

The tumor inoculations were carried out with a heavy suspension of an actively growing tumor in sterile normal saline, 0.3 cc. being injected in one testicle. All animals of a group were inoculated at the same time.

The course of the disease was followed in each animal by frequent clinical examinations, special attention being paid first to the time of initiation of the growth of the primary tumor, its general rate of growth, and its ultimate condition, that is, continued growth, regression, or healing; second, the time of appearance, the location, and the course of metastases in superficial parts of the body; and third, the general physical condition including body weight determinations. The experiments were discontinued 8 to 9 weeks after inoculation, at which time all surviving animals were killed by an injection of air into the marginal ear vein. The same procedure was carried out in the case of any animal whose condition became critical before the end of the observation period. A few rabbits developed "snuffles" while under observation, but in the present analysis of results, they have not been separately considered.

The character of the disease was further appraised by the postmortem examination findings of each rabbit. The site of any macroscopic tumor growth was noted and in addition, such features as size, destructiveness, and approximate amount of living and necrotic tumor tissue were described. In the case of those rabbits in which tumor was found at the end of the experimental period, a classification of probable death or probable recovery was made, based upon the location and character of the tumor growths. The probable deaths, for example, include those instances in which both suprarenal glands or the hypophysis were involved by apparently living tumor while the probable recoveries include cases in which only necrotic and evidently healing tumors were found in such locations as the retroperitoneal lymph nodes. This classification is based upon the results of a 10 years' experience with this tumor.

The general conduct of the experiments and the technique employed in the blood examinations were similar to those carried out on normal rabbits (1). Suffice it to say here that a variable number of examinations were made during the 3 weeks preceding inoculation and once a week thereafter during the postinoculation period of 2 months. In the case of Groups II and VII, the examinations were made more frequently; in the present analysis of results, these observations have been averaged for each week. The red and white blood cell counts were made with standardized pipettes; the hemoglobin determinations were carried out with a Newcomer hemoglobinometer. Differential white blood cell counts were made with the supravital neutral red technique, 100 cells being counted in each preparation.

In the present consideration of results, each animal has been allocated to one of the four following groups depending upon the postmortem findings: deaths, probable deaths, probable recoveries, and recoveries. The findings of each weekly blood examination of each individual animal in these groups were calculated in terms of

the percentage deviation from so called standard values (1, 2). These percentage values were then combined in the form of an algebraic sum for each of the four animal groups and mean group values for each week were then calculated. By this method, individual animal and series peculiarities are minimized and in addition, the final figures may be considered as resembling smoothed values.

RESULTS

The results of nine experiments dealing with the peripheral blood cytology in rabbits inoculated with a transplantable malignant neoplasm are presented in a series of curves contained in Text-figs. 1 to 8. Because of space limitations, it has not been possible to include either the figures of individual observations or the combined weekly values. The curves represent in the form of mean percentage deviations from so called standard values, successive week to week levels of the eight blood elements studied, that is, the red blood cells, the hemoglobin content, the total white cells, the neutrophiles (pseudo-eosinophiles), the basophiles, the eosinophiles, the lymphocytes, and the monocytes respectively.¹ Each chart comprises four curves corresponding to the four groups in which the rabbits have been classified, that is, those animals which died as a result of the malignant disease, those which probably would have died had the experiments been continued, those which probably would have recovered, and finally, the recoveries or those rabbits in which no tumor was found at conclusion of the experiments.

As determined by clinical examination, a definite primary tumor developed in each rabbit with six exceptions, that is, one animal each

¹ The standard values used in this analysis are derived from 1110 blood counts on 174 normal rabbits (1, 2) and are as follows:

	<i>per c. mm.</i>
Red blood cells.....	5,200,000
Hemoglobin.....	63%
White blood cells.....	9560
Neutrophiles.....	4340
Basophiles.....	950
Eosinophiles.....	215
Lymphocytes.....	3050
Monocytes.....	1000

The curves of the present paper are drawn on the same scale as those which represent the findings in groups of normal rabbits observed for long periods of time and which were analyzed on the basis of the above values (2, 3, 4, 5).

in Groups III, VI, VIII, and IX and two animals in Group VII.² Metastases in such locations as the skin, the eyes, and the superficial lymph nodes were observed clinically in several rabbits and there were a number of cases in which paralysis of the hind quarters developed, due to metastatic growths in the spine. In the case of the recoveries and the probable recoveries, an excellent physical condition was maintained. This was also true in certain of the animals classified as probable deaths but not in others, particularly in those cases in which metastatic involvement of the jaws occurred. With respect to those rabbits which died from the disease, the physical condition was affected usually during the week preceding the fatal outcome.

Table II shows the distribution of animal material of the nine experiments; the sole criterion of this distribution, it may be pointed out again, was the character of the disease.

TABLE II

Group	No. of rabbits	Deaths	Probable deaths	Probable recoveries	Recoveries
I	9	5	2	0	2
II	5	2	0	2	1
III	9	3	4	1	1
IV	9	3	1	2	3
V	10	2	1	2	5
VI	4	2	0	1	1
VII	12	5	3	4	0
VIII	12	2	4	5	1
IX	8	1	1	5	1
Total.....	78	25	16	22	15

DISCUSSION

There are certain general features of these experiments which should be referred to before discussing the results.

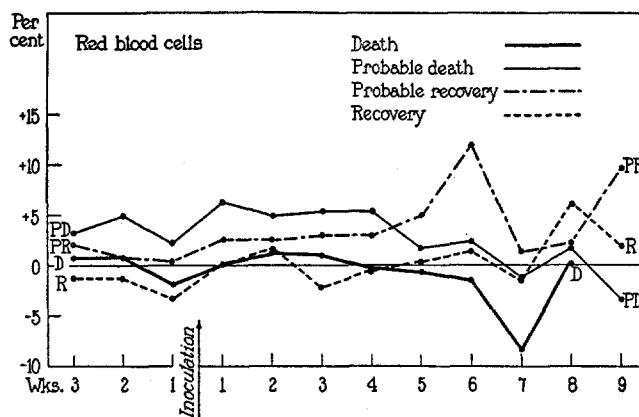
² It should be mentioned in this connection that tumor growths in other parts of the body may be found in cases in which a primary tumor fails to develop after intratesticular inoculation. Among the present six rabbits in which the primary tumor did not develop, four showed tumors elsewhere at postmortem examination 2 months after inoculation. The two rabbits in which no tumor was found have been classified as recoveries; there may have been metastatic growths which had regressed and healed.

In the first place, it must be remembered that the experiments were carried out in different years and in different seasons of the same year. It is to be expected, therefore, that some spontaneous variations in the blood picture might occur as in the case of normal rabbits observed over similar periods of time (2, 3, 4, 5). Such variations, which may be attributable to general environmental conditions as well as the occasional irregular values of individual animals, are minimized by the group method employed for the analysis of the results. Secondly, it should be pointed out that the course of this neoplastic disease is subject to considerable variation, not only as regards individual animals of the same series, but in different series as a whole. These variations are shown by such features as the rate of growth of the primary and metastatic tumors and the death rate, the time at which a fatal outcome occurs, the incidence, distribution, and extent of metastases, and the number of recovered animals. In some series, the degree of malignancy is high while in others it is low and in still others, it lies between these extremes. In the present experiments, as shown in Table II, the varying numbers of deaths, of probable deaths, of probable recoveries, and of recoveries indicate the different degrees of malignancy presented by the animals of these experiments. It is evident that the combination of individual animal observations derived from successive weekly examinations is subject to correction on the basis of the course of the disease in each particular animal. It would be difficult to follow this procedure, however, because the only available features of the disease which lend themselves to a practical method of appraisal are, first, the primary tumor, the development and growth of which is not necessarily an index of tumor growth elsewhere, and second, the occurrence of metastases in superficial parts of the body. The relatively low incidence of such superficial tumors except in cases of evident high malignancy, precludes the use of this feature. It has been found by experience, however, that gross changes in tumor growth are usually evident from week to week, and hence this time interval was used for the present observations. The combination of the results of individual animals from different series has been made on the same time basis as affording the least opportunity for error.

In the third place, the results have been considered from the standpoint of the mean values of a group rather than from that of the individual animal since exaggerated or unusual findings in particular rabbits as well as the results of technical errors of blood examination are thereby minimized. While the numbers of rabbits in each group are not large, they are sufficiently comparable to permit group comparisons, that is, 25, 16, 22, and 15 animals in the deaths, the probable deaths, the probable recoveries, and the recoveries, respectively. Finally, it must be remembered in comparing the results of the four groups that in the case of the fatal group, its numbers became progressively decreased and the reliability of the mean values is thereby lessened. 5 weeks after inoculation there were 24 animals; at the 6th week, 17; at the 7th week, 10, while there were but 3 at the 8th week. It should also be remembered with respect to the rabbits in the group of probable recoveries that all showed some evidence of tumor at postmortem

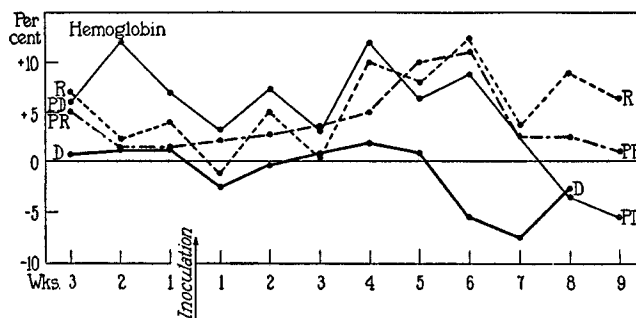
examination. In many instances, the growths appeared to be largely or entirely necrotic and in all cases what tumor there was, either living or necrotic, was in a location which presumably would not have led to the death of the animal; had the experiments continued, it was felt that ultimate regression and healing would have occurred.³ In six instances, the residual tumor comprised only necrotic nodules in the inoculated testicle.

The results obtained will now be discussed, beginning with the erythrocytes and the hemoglobin content and then taking up the total white count and the various classes of white cells. The most striking changes were observed in the fatal group as might be expected, and therefore, these results will first be considered; the recovered group, on the other hand, showed comparatively slight alterations.



TEXT-FIG. 1. Red blood cells. In this and subsequent charts, the values for the four animal groups are expressed in the form of mean percentage deviations (algebraic sum) from standard values.

³ In this connection, it should be mentioned that it has not been possible to reinoculate recovered tumor rabbits by any of the usual routes, such as the intravenous, the intracutaneous, or the intracerebral. The only site which so far has proven successful has been the suprarenal gland (unpublished experiments of Pearce and Van Allen); other tissues appear to be immune (11). It appears extremely unlikely that both suprarenals will eventually become involved when the only tumor present 2 months after inoculation is confined to the residual primary tumor or to growths in such comparatively remote sites as the eye, the connective tissue, or the retroperitoneal lymph nodes. It is also unlikely, even if one suprarenal is the seat of a metastasis, that the second will become involved after 2 months of the disease. All such cases are classified as probable recoveries.

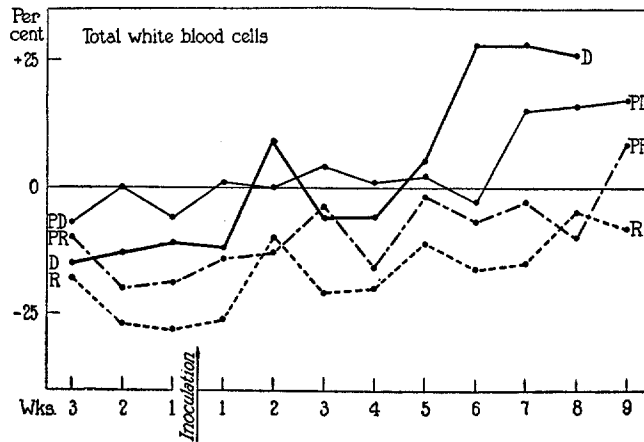


TEXT-FIG. 2. Hemoglobin content

Red Blood Cells and Hemoglobin:—The curves representing the mean levels of the red blood cells during the 3 weeks prior to inoculation (Text-fig. 1) show a range from 4 per cent above the standard value of 5,200,000 cells per c.mm. in the case of the probable deaths, to 3 per cent below this value in the recovered group; the curves representing the deaths and the probable recoveries lie between these limits. For the two first observations after inoculation, the red cell values became increased in all four groups as is shown by the upward trend of the curves. In the case of the fatal group, the curve then describes a gradual fall for 4 weeks, with an abrupt and marked drop to minus 7 per cent at the 7th week and an equally abrupt terminal rise to the base line. It will be remembered that the last two examinations present but 10 and 3 animals; the rise of the last observation was caused by the results on one rabbit, the last values of which, however, were much lower than the preceding ones. The curve of the probable deaths is maintained at approximately a plus 5 per cent level through the 4th week; it then descends in a fairly orderly fashion to a final level of 3 per cent below the base line. The findings in the probable recoveries and the recoveries were more irregular than those of the other groups, but there was a definite tendency toward progressively higher values, particularly in the last four observations; the curves for both groups end well above the base line.

The mean values of the hemoglobin content were, with the exception of the fatal group, more irregular than those of the red blood cells. This feature is brought out by comparing the curves of Text-figs. 1 and 2; it will be seen at once that in the curves representing the hemo-

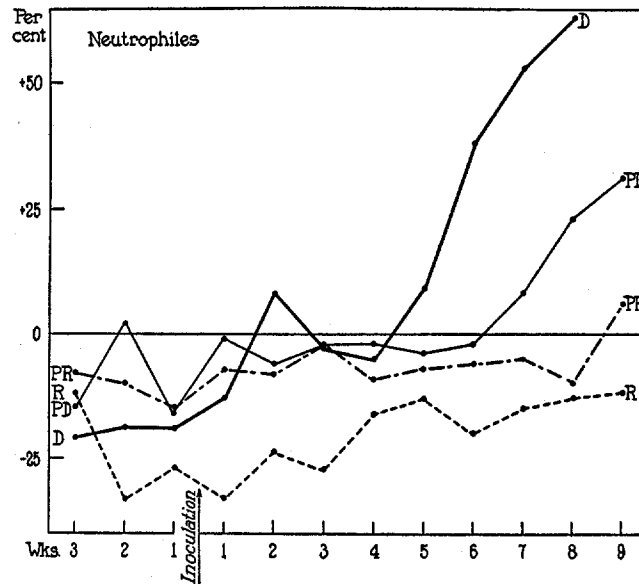
globin content, the excursions are more abrupt and the changes in level more pronounced. Before inoculation all four curves are above the base line which represents a value of 63 per cent; that of the probable death group contains one marked irregularity. After inoculation, three curves show an immediate drop followed by a rise. In the case of the deaths, a fairly constant level in the neighborhood of the base line is maintained for 5 weeks; during the next 3 weeks, the curve falls to 5, 7.5, and 2.5 per cent below the normal value. In the first half of the postinoculation period, the curve of the probable death group ranges between 3.5 and 7 per cent above the base line but during the last half, it falls steadily from this high level to a minus 5.5 per cent, a total change of 12.5 per cent. Despite irregularities, the curve of the recoveries shows a tendency toward the maintenance of higher values after inoculation. A similar tendency is seen in the probable recoveries until the last two observations when the direction of the curve appears to be definitely downward. It should be noted, however, that the values of both the probable recoveries and the recoveries are above the base line throughout the observation period while those of the deaths and probable deaths fall below the normal value during the latter part of this period.



TEXT-FIG. 3. Total white blood cells

White Blood Cells:—Before inoculation, the mean total white counts of all four groups of animals varied from 5 to 25 per cent below the

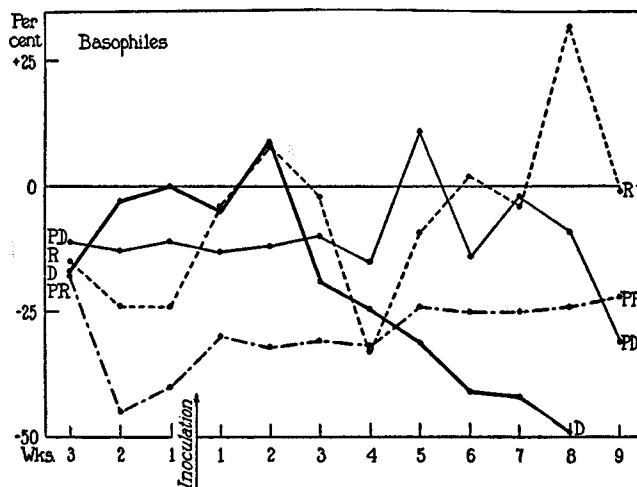
standard value of 9560 cells per c.mm.; the weekly variations of any one group, however, did not exceed 10 per cent of this value (Text-fig. 3). After inoculation, the trend in all groups was toward increased values throughout the period of observations as is shown by the upward direction of the curves. This trend was most marked in the case of the fatal group in which the highest levels were found during the last 3 weeks; the curve shows that a value of 25 per cent above the standard value was reached as compared with the average minus 15 per cent value of the preinoculation period. The curve representing the probable deaths shows a similar although slightly less marked change and in addition, it will be noted that the time of the rise occurred later. In the case of the probable recoveries, the findings were more irregular than with the deaths or the probable deaths, but as the general direction of the curve indicates, there was a similar tendency toward increased white counts which, however, were not as high as with either of the other groups. In the recovered animals, the total white cells showed a slight numerical increase after inoculation amounting approximately to 15 per cent above the preinoculation level. It will be noted, however, that at no observation did the total white count of the recovered group exceed the standard value while this was found to be the case with the other groups, particularly with the deaths and the probable deaths.



TEXT-FIG. 4. Neutrophiles (pseudo-eosinophiles)

Neutrophiles (Pseudo-Eosinophiles):—The preinoculation levels of the neutrophilic leucocytes in all four groups were below the standard value of 4340 cells per c.mm., the general group range being minus 10 to 25 per cent of this value (Text-fig. 4). The postinoculation observations showed that in the fatal group, the neutrophiles became increased, that this change became pronounced in the 5th week, and that a figure of 65 per cent above the standard value was reached at the time of the last examination. This value represents a rise of 85 per cent above the preinoculation level of the group. A similar but less marked increase to 30 per cent above the standard value occurred in the group of probable deaths and as will be seen by referring to the curves in Text-fig. 4, it occurred 2 weeks later than the decided rise of the neutrophiles of the fatal group. The curves representing the probable recoveries and the recoveries show that while there was also a tendency toward increased neutrophile counts after inoculation, the changes were comparatively slight. It is of interest to note, however, that the curve of the probable recoveries ends above the base line as was the case with the other two groups in which tumor was present

at the conclusion of the experiment. With the complete recoveries, on the other hand, the curve throughout lies below the base line.

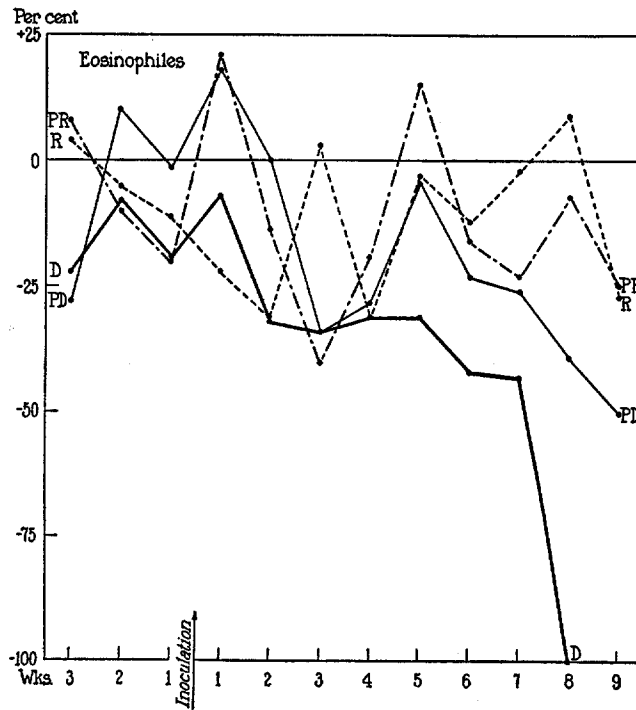


TEXT-FIG. 5. Basophiles

Basophiles:—The observations with respect to the basophiles are illustrated by the curves in Text-fig. 5. It will be seen by referring to these curves that there was considerable variation in the mean group levels of these cells during the 3 weeks prior to inoculation, the general range being from 5 to 25 per cent below the standard value of 950 cells per c.mm. In the groups of deaths and probable recoveries, moreover, the findings were comparatively irregular, the shifts in level amounting approximately to 15 and 25 per cent respectively. After inoculation it was found that in the case of the fatal group, the number of basophiles became markedly decreased, the change being pronounced at the 3rd week and continuing thereafter throughout the observation period; at the last examination, the count had fallen to 50 per cent below the standard value. With the probable deaths, a somewhat similar change was found after the 5th week of the disease, but it was less marked and less consistently followed as will be seen by comparing the respective curves. The last observation of the group of probable deaths was 30 per cent below the standard value as compared with the high point of plus 10 per cent at the 5th week and a

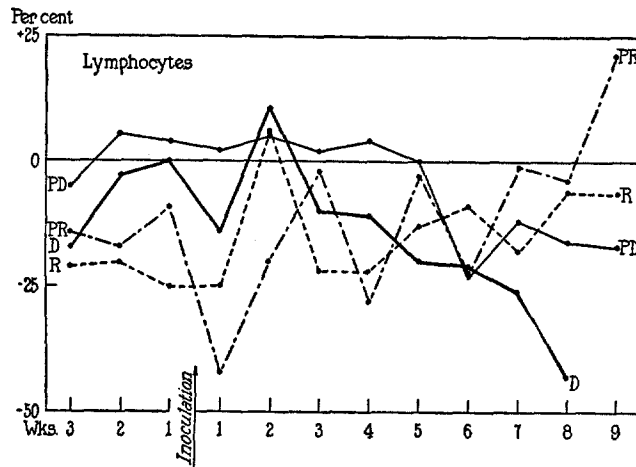
general preinoculation level of minus 10 per cent. In contrast with these findings, a sustained drop in the mean basophile count of the probable recoveries and recoveries was not observed. The curve representing the recovered group shows two major swings of considerable amplitude, but during the latter half of the observation period, that is, after the 4th week, the general trend is definitely upward and it ends on the base line. In the case of the probable recoveries, although the direction of the curve is slightly upward, it is maintained fairly constantly at levels of 30 and 25 per cent below the standard value.

It will be noted that during the latter half of the observation period, the basophile curves of the three groups of animals in which tumor was found at postmortem examination, all lie below the standard value while that of the recovered group in which no tumor was found lies at or above this value.



TEXT-FIG. 6. Eosinophiles

Eosinophiles:—The mean preinoculation eosinophile counts of all four groups were irregular. The limits of the irregularities as shown by the curves in Text-fig. 6 were 10.2 per cent above to 25.5 per cent below the standard value of 215 cells per c.mm.; the widest range of weekly variation of individual groups was 15 per cent of the standard value except in the case of the probable recoveries in which it was 35.5 per cent. After inoculation, the irregularities were generally more pronounced, a feature which is brought out by the pronounced swings of the curves. With the fatal group, there was a definite drop in the mean number of eosinophiles at the 2nd week; this new level was continued for three observations and was succeeded by even lower levels, and eventually at the last examination, no eosinophiles were found. The results in the other groups were not so clean-cut. In the probable deaths a similar but less pronounced decrease of eosinophiles occurred as shown by the general downward trend of the curve from its highest point in the 1st week after inoculation, although there is an upward swing during the 4th and 5th weeks corresponding to the stabilized period of low levels in the fatal group. With the probable recoveries and the recoveries, the results are not striking. It will be noted, however, that after the 4th week, the curves for these two groups are consistently higher than those of the deaths and the probable deaths, and that for the last four observations the mean numbers of eosinophiles in the recoveries were greater than in the probable recoveries. These features taken in conjunction with those relating to decreased values in the groups of deaths and probable deaths suggest that an increased eosinophile count is probably characteristic of the recovered state.



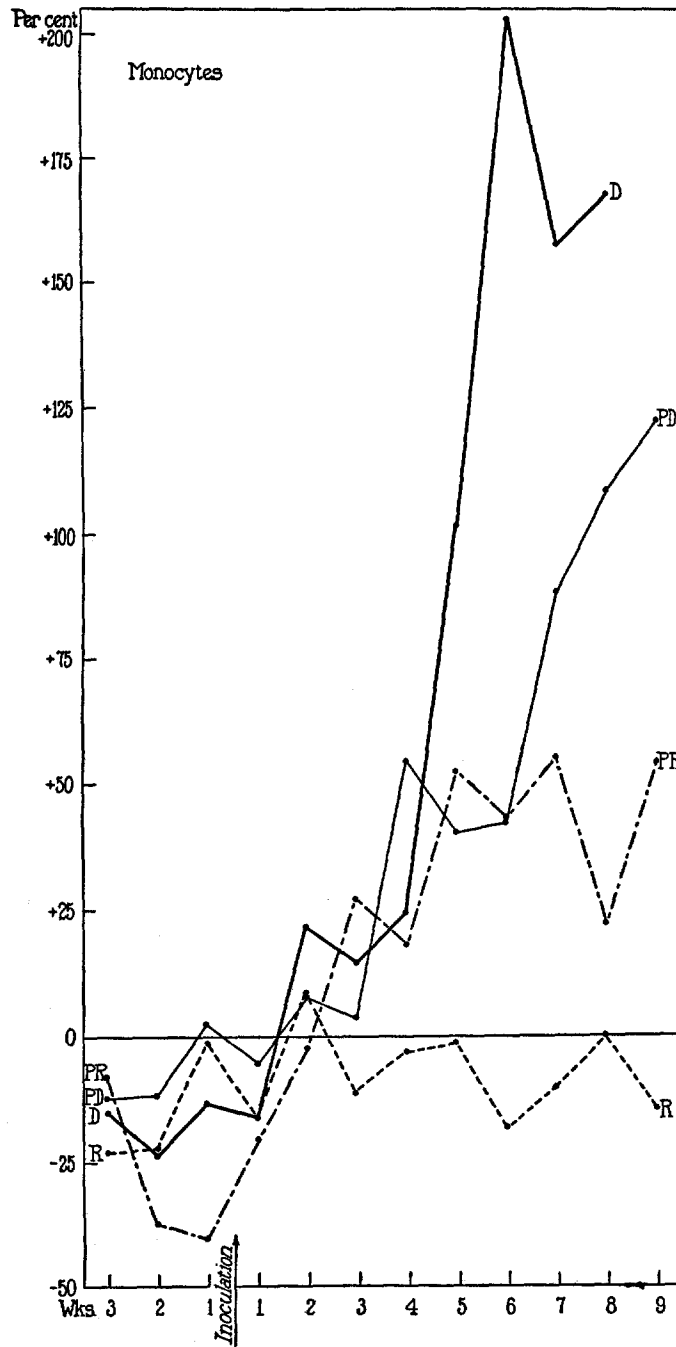
TEXT-FIG. 7. Lymphocytes

Lymphocytes.—The observations regarding the lymphocytes are illustrated by the curves in Text-fig. 7. It will be seen by referring to these curves that the average mean levels of the lymphocytes in the four groups of rabbits before inoculation were variable, their limits lying between the standard value of 3050 cells per c.mm. in the case of the probable deaths, to approximately 20 per cent below this figure in the case of the recovered group. Irregularities of individual groups ranged from 5 to 15.5 per cent on the standard value scale.

A week after inoculation, a drop in the mean numbers of lymphocytes was observed in two groups, the deaths and the probable recoveries, while in the others, little or no change was found; at the next observation, 2 weeks after inoculation, the numbers in three groups were considerably increased. From this time onward, the lymphocytes of the fatal group showed a steady numerical decline which is well brought out by the curve of Text-fig. 7; at the final observation, the level reached the low point of 40.5 per cent below the standard value as compared with an average preinoculation value of 5 per cent below and with 10 per cent above the standard value at the 2nd week after inoculation. In the case of the probable deaths, there was no significant change until the 6th week after inoculation. At this time, there

was a moderate decrease in the mean numbers of lymphocytes which persisted to the end of the observation period. The extent of the decrease amounted to approximately 20 per cent on the present scale as is shown by the curve in Text-fig. 7. The results in the probable recoveries and the recoveries are less definite. It will be seen, however, by referring to the curve representing the probable recoveries that despite frequent and comparatively wide variations, there was a distinct tendency in the latter half of the observation period for the lymphocytes to become increased over the average level of the pre-inoculation period and of the values found in the first weeks after inoculation. The curve rises from a point 25 per cent below the standard value at the 4th and 6th weeks after inoculation to a final level of plus 20 per cent. With the recovered group, the curve indicates that following the low levels of the 3rd and 4th weeks after inoculation, there was a fairly consistent trend in the direction of slightly increased lymphocyte counts which were still maintained at the last examination.

It would appear from these results that the mean number of lymphocytes in the peripheral blood tends to be somewhat decreased in rabbits succumbing to the effects of this neoplastic disease and that a similar but less marked effect is associated with a less severe process. On the other hand, the mean lymphocyte values tend to be slightly increased in rabbits in which the process is mild or in which recovery takes place.



TEXT-FIG. 8. Monocytes

Monocytes:—The observations on the monocytes are illustrated by the curves in Text-fig. 8. The preinoculation levels in all four animal groups were lower than the standard value of 1000 cells per c.mm. In the case of the deaths, the probable deaths, and the recoveries, successive weekly values showed variations of 10 to 20 per cent of the standard value; with the probable recoveries, the range extended to 30 per cent. The average levels in this period, as may be seen by the curves, were approximately 20, 7, 14, and 27 per cent below the standard value for the deaths, the probable deaths, the probable recoveries, and the recoveries respectively.

After inoculation, the monocytes in all groups except the recoveries, became markedly increased, that is, a peripheral blood monocytosis was associated with tumor growth. Beginning at the 2nd week, all four groups showed increased mean numbers of monocytes, the levels attained being higher than the standard value in three groups and equal to it in the fourth. In the case of the fatal group, this higher level was maintained for the following 2 weeks, and from this time to the end of the observation period, very large numbers of monocytes were found. 6 weeks after inoculation, the increase amounted to 200 per cent above the standard value; in the 7th and 8th weeks the figures were slightly smaller, but it will be remembered that these last two observations represent 10 and 3 animals (the most resistant of the group) as compared with 17 at the 6th week. The findings with respect to the group of probable deaths were comparable to those of the fatal group as is shown by the respective curves. It will be noted, however, that the time of the first marked monocytic increase occurred later, that is, at the 7th as compared with the 5th week in the fatal group, and furthermore, that the magnitude eventually attained at the end of the observation period was not as great, that is, 125 as compared with 170 per cent. The findings of the last three observations in the probable death group show progressively larger numbers of monocytes as is illustrated by the regularly rising curve, and this feature is undoubtedly influenced by the fact that each observation represents the same number of animals, none dying before the end of the experiment as was the case with the fatal group.

In the probable recoveries, increased numbers of monocytes were observed to the extent of 50 per cent above the standard value. This

condition was attained by the 5th week after inoculation and corresponds to a similar level on the part of the probable death group and to a value of plus 100 per cent for the fatal group. During the last four observations, the curve representing the probable recoveries is irregular but on the whole, it tends to remain at the level of 50 per cent above the standard value. In the case of the recovered animals, the highest monocyte level observed was at the 2nd week after inoculation, that is, 9 per cent above the standard value. At subsequent examinations to the end of the observation period, there was an irregular decrease in the mean numbers of monocytes, and it will be noted that after the 2nd week, the curve does not again rise above the base line and that its general contour resembles its preinoculation portion.

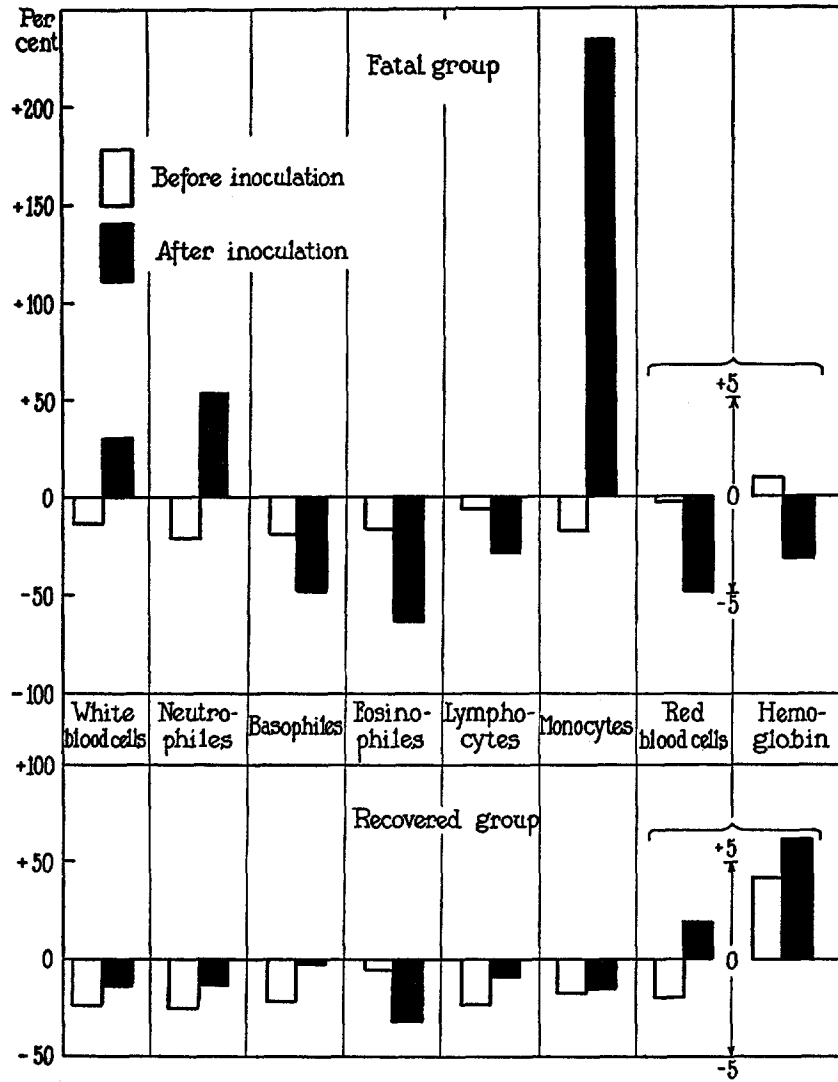
The above discussion of these results may be summarized as follows: During the course of this particular neoplastic disease, certain alterations of the peripheral blood picture were observed. The most striking changes during the course of a fatal or probably fatal condition concern the monocytes, a pronounced numerical increase of which was found, and secondly, the eosinophiles, the numbers of which were markedly reduced. In the case of the basophiles, a change similar to but less marked than that of the eosinophiles was observed (12). The relation of the numbers of lymphocytes to the course and type of disease was less definite than that of the monocytes, eosinophiles, and basophiles, but the findings showed a general correspondence to those of the eosinophiles and basophiles rather than to the monocytes. In the case of the neutrophiles, it was found that their numbers became increased in an actively progressing disease. As would be expected, the character of the total white count was greatly influenced by the neutrophiles. The very large numbers of monocytes in the fatal and the probable death groups also contributed to the increased total white counts. And finally, in conditions of pronounced or well marked malignancy, decreased numbers of red cells and a lowered hemoglobin content were observed; the change in the red cells preceded that of the hemoglobin.⁴

⁴ In connection with these results in cases of well marked malignancy, it is of interest to recall that the blood count of the original tumor rabbit taken shortly before death showed the characteristic findings here described (10).

The time of these changes should be referred to. In the fatal group, the curves representing the red blood cells, the hemoglobin content, the various classes of white cells, and the total white count show a decided trend of direction at the 4th and 5th weeks after inoculation although the trend itself may have been initiated earlier, as was the case with the monocytes. It will be noted in the group of probable deaths that, on the whole, the changes which were of the same general character as those of the fatal group, occurred somewhat later. This finding is in keeping with the differences in malignancy level of the two groups. In this connection, it should be mentioned that although the rate of growth of the primary tumor and the distribution and growth of metastatic tumors vary widely, postmortem examination of a large number of rabbits at variable periods of the disease has shown that by the 3rd or 4th week after inoculation, the tumor process is well established in cases of pronounced or average malignancy.

As far as the probable recoveries and recoveries are concerned, the results in general correspond to what might be expected in the light of the findings in the groups characterized by an actively progressing tumor process. Thus, in the case of the monocytes which is the most striking example, increased counts of a moderate degree were observed in the group of probable recoveries, but there was little change in the group of recoveries.

The differences in the blood picture of rabbits representing the two extremes of reaction to the malignant disease, that is, the fatal and the recovered cases, are brought out by the accompanying graphs in which the last observations of these groups are illustrated (Text-fig. 9). For the recovered group, the values are, of course, those given in the curves of the text-figures; for the fatal group, on the other hand, the values are different since they represent the combined last observations of each animal irrespective of the time of death.



TEXT-FIG. 9. Last observations on the fatal and recovered groups. Values are expressed in the form of mean percentage deviations (algebraic sum) from standard values.

The outstanding changes which have been noted in the present experiments far exceed the spontaneous variations found in groups of normal rabbits examined for comparable periods of time (2, 3, 4, 5). Strictly speaking, the present results cannot be compared with those of the normal animals because they are derived from an analysis in which the findings of individual rabbits from different groups are combined, while with the normal animals the entity of each group was preserved. It may be stated, however, that an analysis of each animal group of these experiments has been made and that the results are substantially the same as those presented in this paper.

It should be mentioned that all the characteristic blood picture changes associated with the malignant process as determined by the analysis of the material on a combined group basis, were not invariably found in individual animals; in certain cases, one or more of these changes were present while others were lacking. These instances were rare, however, when one takes into consideration the general character of the blood counts over the entire observation period. There was one striking example of a fatal case with a widespread distribution of tumor in which the monocytes and neutrophils were not increased, the lymphocytes were unchanged, and the basophils were only slightly diminished; there was, however, a decreased red count, a lowered hemoglobin content, and a decreased eosinophile count.

At the present time, no interpretation of the results of these experiments has been made. It is evident that in rabbits in which active and extensive tumor growth takes place, that is, in animals with a low resistance to the disease, the numbers of peripheral basophils, eosinophils, and probably the lymphocytes are low in contradistinction to the high numbers of monocytes and neutrophils. But whether these states are the result of the tumor process or are more intimately connected with the cause of its activity, cannot now be determined. The results of the tissue studies carried out in connection with observations on the peripheral blood as well as those bearing on the character of the blood picture before inoculation may throw some light on the subject.

SUMMARY AND CONCLUSIONS

Successive blood counts at weekly intervals were made on rabbits inoculated with a transplantable malignant neoplasm of epithelial origin. There were 78 animals distributed in 9 groups; the period of observation after inoculation was 2 months. The results have been considered with respect to the character of the tumor process as determined by postmortem examination, the animals being classified as deaths, probable deaths, probable recoveries, and recoveries. The blood findings have been analyzed on the basis of the values derived from normal rabbits. A comparison has also been made with the results of preinoculation counts.

In rabbits in which the tumor process was of pronounced or well marked severity, the numbers of monocytes became greatly increased; the neutrophils and the total white blood cells were also increased; the eosinophiles, and to a less extent, the basophiles and lymphocytes were decreased; the red cell count was decreased and the hemoglobin content was lowered.

In rabbits which recovered from the tumor inoculation, there were no outstanding changes in the blood picture when the findings for the entire observation period were considered. In the group of probable recoveries, the results were variable; there was, however, a definite although moderate increase of monocytes, and in general, the findings reflected the mildness of the disease when compared with those of the fatal cases and the group of probable deaths.

BIBLIOGRAPHY

1. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.
2. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 23.
3. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 39.
4. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 145.
5. Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **52**, 167.
6. Casey, A. E., *J. Exp. Med.*, 1931, **53**, 695.
7. Pearce, L., *Tr. VIIIth Internat. Cong. on Dermat. and Syph.*, Copenhagen, 1930.
8. Pearce, L., and Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1929, **26**, 666.
9. Pearce, L., and Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1929, **26**, 668.
10. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 61 ff.
11. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 799.
12. Casey, A. E., *Proc. Soc. Exp. Biol. Med.*, 1929, **27**, 135.