OBSERVATIONS ON THE BLOOD CYTOLOGY IN EXPERIMENTAL SYPHILIS

II. The Period of Disease Latency

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It has been demonstrated (1, 2) that marked alterations take place in the blood cytology of syphilitic rabbits in the postinoculation period of 3 to 5 months, during which the disease is clinically characterized by the development, regression, and healing of primary and generalized lesions. Among the outstanding changes observed were an increase in the total white cell count, the platelet count, and the absolute numbers of neutrophils and monocytes, and a decrease in the number of lymphocytes. In connection with our studies on the biology of syphilitic infections, observations were made to determine the blood cell levels of infected animals after the complete spontaneous healing of lesions; that is, in the period of latency when there is no clinically demonstrable evidence of infection. It was found that the red blood cell count and the hemoglobin in per cent of rabbits with latent syphilis were both significantly lower than normal values; other significant differences were not noted. These observations, together with a brief comparison of the blood cytology in experimental and human syphilis, are presented in this report.

Material and Methods

Careful clinical examinations of a large group of syphilitic animals were made, and those rabbits selected for blood cytology studies which had been inoculated at least 5 months previously and which presented no clinical evidence of infection. Thirty-five animals from among our stock fulfilled these requirements at the time this investigation was in progress, and a study of their blood cytology forms the basis of the present report.

The 35 rabbits were all bred in this laboratory and comprised 10 hybrid crosses and 25 standard bred animals. The latter were distributed according to breed as follows: 6 Dutch, 6 Havana, 5 Himalayan, 5 English, and 3 Polish. Each

animal was housed in a separate cage and was fed a diet consisting of hay, oats, compressed food pellets, and a free supply of water.

Automatic standardized pipettes were used for the red and total white cell counts and all differential examinations were made by the neutral red supravital method, 100 cells being counted on each of two smears. The Ringer-heparin method of Casey and Helmer (3) was employed for the red cell and platelet counts, and the hemoglobin was estimated with a Newcomer hemoglobinometer.

Each animal had been inoculated with the Nichols strain of *Tr. pallidum* from 5 to 18 months (average 8 months) previous to the present study. The blood examinations of 11 of the 35 animals were conducted in April and May, 1932, and the remaining 24 animals were examined in September and October of the same year. Four complete examinations were made on each animal over a period of 2 to 4 weeks and the blood level of each animal was determined by the mean of the 4 counts. The mean and the standard error of the mean of the 35 means were then calculated for each blood element. These means were compared with normal values obtained in the following manner: In a study of the hemocytological constitution of standard bred rabbits (4), the mean blood cell levels for each of 180 normal standard bred rabbits representing 15 different pure breeds were obtained from 3 to 10 counts on each animal. The means and variance for the different cells were calculated for each breed. The means and variance of similarly obtained means for 140 miscellaneous hybrid rabbits (5) gave the normal values for hybrids. Weighted normal mean values and the standard error of these means were calculated from two formulae:

$$(1)\ \frac{\Sigma(n_{\text{sample}}\ M_{\text{breed}})}{35} = Mx_{35};$$

$$(2)\ \Sigma n_{\text{sample}}\ (\text{Var.}_{\text{breed}} + M_{\text{breed}}^2) = \Sigma x_{35}^2.$$

In these equations n is the number of animals of a particular breed used in the present investigation and M is the mean and Var. the variance for this breed obtained in the above mentioned study on hemocytological constitution. The value for each cell element obtained by the use of the first equation gave the weighted mean value with which the experimental mean was compared, while the standard error of these weighted means was derived by using both formulae. In all comparisons a difference was considered to be significant when the probability of its occurrence by chance was less than 1 in 100 ($t = 2.5$, $P = 0.01$).

RESULTS

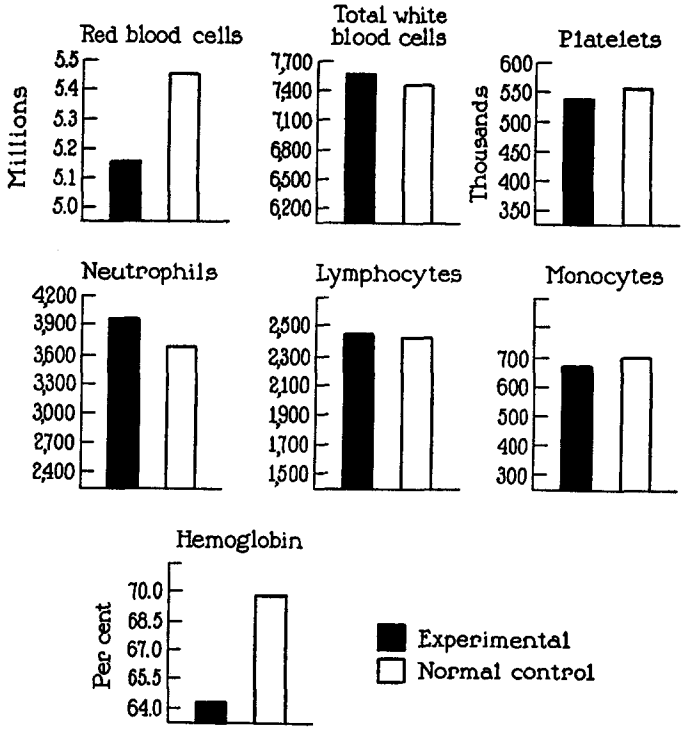
Comparisons were made between the mean blood cell formula of 35 syphilitic rabbits in which clinical evidence of the disease was absent and weighted normal values obtained as described. These comparisons are presented in Table I and Text-fig. 1. There were no signifi-

TABLE I

Mean Blood Cell Values of 35 Syphilitic Rabbits with Latent Infection Compared with Normal Values

Group	Red blood cells	Platelets	White blood cells	Neutrophils	Basophils	Eosinophils	Lymphocytes	Monocytes	Hemoglobin
	thousands	*thousands*							*per cent*
Experimental.....	5,154±86	537±12	7,570±236	3,942±162	415±30	85±7	2,450±129	678±53	64.6±1.0
Control.....	5,490±78	556±21	7,441±321	3,706±196	483±43	114±12	2,439±173	699±53	69.8±1.0

cant differences between the experimental and normal values with respect to the total number of white cells, the platelet count, and the absolute number of neutrophils, basophils, eosinophils, lymphocytes, or monocytes. The only significant differences encountered were in the red blood cell count and the hemoglobin in per cent, both of which were lower in the experimental group than the corresponding



TEXT-FIG. 1. The blood cytology in the latent phase of experimental syphilis. Mean blood cell observations on experimental group compared with normal control values.

normal values. (White blood cells: Difference = 129 ± 398. Platelets: Difference = 19,000 ± 21,600. Neutrophils: Difference = 236 ± 254. Basophils: Difference = 68 ± 52. Eosinophils: Difference = 29 ± 14. Lymphocytes: Difference = 11 ± 216. Monocytes: Difference = 21 ± 53. Red blood cells: Difference = 336,000 ± 116,000, $t = 2.9$, $P = 0.01-$. Hemoglobin: Difference = 5.2 ± 1.4 per cent, $t = 3.6$, $P = 0.01-$).

DISCUSSION

Experimental syphilis of the rabbit under the usual conditions of intratesticular inoculation with a strain of *Tr. pallidum* such as the Nichols' strain, is clinically manifested by the production of a primary orchitis, metastatic orchitis and generalized lesions. In our experience an orchitis of the inoculated testicle develops in all instances, while the incidence of metastatic orchitis in a group of inoculated animals varies from 80 to 100 per cent and the incidence of generalized lesions varies from 40 to 100 per cent. In a large majority of cases the tissue reaction with the production of lesions takes place within a 2 to 3 month period following inoculation after which there is a regression of all lesions and finally complete healing. With the appearance of this stage there is established a period of latency so far as clinical evidence of the disease is concerned, but the animal still harbors infective spirochetes as can be demonstrated by subinoculation of lymph node tissue. In a certain proportion of animals, the period of latency is interrupted by one or more periods of clinical relapse with the production of lesions. Ultimately, however, a state of permanent latency is reached in the vast majority of animals.

All the animals of the present experiment had passed through the period of lesion activity, and at the time of the blood studies the disease in all was latent. A primary orchitis had developed in all animals, and the disease in the group can be characterized as of moderate severity varying in individual animals from a very mild disease with no generalized lesions to a very severe infection with many generalized lesions.

Previous studies (1, 2) have demonstrated that the active period of experimental syphilis of the rabbit is associated with changes in the blood cytology which include an increase in the total white cell count, platelet count, and numbers of neutrophils and monocytes, and a decrease in the number of lymphocytes. These changes were shown to be of statistical significance. From the present study it is seen that when the period of latency has been established, these blood cell elements return to normal levels but the red blood cells and hemoglobin are significantly depressed. The depression of the red cells is of increased significance since the blood examinations were conducted in April and May, September and October. Observations on the sea-

sonal trends of blood cells made in this laboratory over several years has indicated that the red cell count is highest during these months.

From a study of the blood cytology in untreated and treated human syphilis (6), it was found that with treatment there is a fall in the total white cell count, the platelet count, and the numbers of neutrophils and monocytes, and a rise in the number of lymphocytes from values observed in untreated cases. These findings are noteworthy since they are paralleled so closely by the findings in the experimental disease. The blood cell values of syphilitic rabbits in the active phase of the disease differed from normal values in the following respects: higher white blood cell count, platelet count, neutrophil and monocyte counts, and lower lymphocyte count. In the present study it has been shown that these cells return to normal levels during the period of latency. With respect to these elements, therefore, the changes observed in the experimental disease after spontaneous healing of all lesions are similar in direction to the blood cell changes in human syphilis following the institution of treatment. The depression of the red cells and hemoglobin after complete healing of lesions in the rabbit has its counterpart also in the human disease. In untreated tertiary human syphilis, the red cells and hemoglobin were significantly lower than the values for treated tertiary syphilis patients, while no differences were found between any of the other blood cells of the two groups. Thus a comparison of the blood formula of syphilitic rabbits in the period of latency with normal control values revealed the same differences as were noted when comparing the blood formula of untreated with treated tertiary syphilis in man. It is quite probable that treatment in the latent period of experimental syphilis would bring about an increase in the red cell count and hemoglobin just as it does in the tertiary disease of man.

SUMMARY

The mean blood cell levels of 35 latent syphilitic rabbits in which all lesions had undergone spontaneous regression and complete healing were compared with weighted values for normal rabbits.

The only differences noted were in the red cell count and hemoglobin content, both of which were significantly lower in the experimental group than the normal values.

A parallelism was observed between the blood cell changes of the experimental disease after spontaneous regression of lesions, and the cell changes in the human disease after treatment. This parallelism lends additional weight to deductions drawn from the experimental disease as applied to human syphilis.

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