

influenza in 1940 showed a sharp rise in antibodies to the Type B antigen but not to the antigen of Type A. In the case of patient Sq., no rise in complement-fixing antibodies was detected.

These observations, while limited in number, serve to emphasize the antigenic differences between the 2 viruses and to indicate that the complement-fixation reaction can be employed for the differential diagnosis of epidemic influenza due to virus of Types A and B.

### 11863

#### The Diurnal Levels of Blood Leukocytes in the Normal Rabbit.

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Extensive studies on the blood of normal rabbits,\* which were made for another purpose,<sup>1, 2</sup> seemed to offer an unique opportunity to determine whether a definite diurnal cycle in the leukocyte level actually exists in this species. The animal material used had been acclimated to the laboratory for months or years before the counts were made. The exact age of the animals, as well as their breed and physical condition, was known in most instances. Counts were not made oftener than 3 times a week, and usually only once a week. The exact time of day at which the blood was taken had been recorded for each determination. Finally, the material was sufficiently extensive to make statistical analysis possible.

*Materials and Methods.* Although blood counts on normal rabbits were available for all months of the year, the November and December counts were selected for analysis because in these months the largest samples were available for each hour of the day from 9 a.m. to 5 p.m. The variation in the formula of the blood between the 2 months was not significant.

The animal material consisted of 204 young adult male rabbits examined in the months of November and December in the years

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\* These studies were done at the Rockefeller Institute for Medical Research, in the laboratory of Dr. Wade H. Brown. They were carried out by the author, in coöperation with Drs. Louise Pearce, Paul D. Rosahn, and C. K. Hu. The technical assistance of Mr. Leslie Kish was invaluable.

<sup>1</sup> Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.

<sup>2</sup> Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L., *J. Exp. Med.*, 1936, **64**, 453.

1927-1933 inclusive. The age range was from 4 to 15 months and the average age was 8 months. All the animals were caged separately. The rabbits were usually alternated from week to week when repeated counts were made, so that counts are available for different times of the day on the same animal. In all, 963 counts (an average of about 5 per animal) were made, distributed as follows:

| 178 counts on |     | 80 rabbits between |     | 9 and       | 9:59 a.m.  |
|---------------|-----|--------------------|-----|-------------|------------|
| 226           | " " | 103                | " " | 10 "        | 10:59 "    |
| 193           | " " | 115                | " " | 11 "        | 11:59 "    |
| 30            | " " | 19                 | " " | 12 noon and | 12:59 p.m. |
| 82            | " " | 60                 | " " | 1 and       | 1:59 p.m.  |
| 124           | " " | 64                 | " " | 2 "         | 2:59 "     |
| 85            | " " | 35                 | " " | 3 "         | 3:59 "     |
| 45            | " " | 17                 | " " | 4 "         | 4:59 "     |

In all, 597 determinations on 190 of the 204 rabbits were made between 9 a.m. and 12 noon, and 366 determinations on 136 of the 204 rabbits were made between 12 noon and 5 p.m.

The blood was collected in U. S. standardized Trenner pipettes. Acetic acid was employed as a diluent. The counts were made in a Levy-Hausser counting chamber. The counting technic was standardized, so that the average coefficient of variation due to technical error in a given total white count was 9%.

The material was analyzed in 2 periods, 1927-1929 inclusive, and 1930-1933 inclusive. In the first period the counts were made before the animals had been fed or permitted access to water, and only one white cell pipette was filled from each rabbit on a given day. In this period the material consisted of generally brown-gray hybrids purchased from dealers. In the second period the animals were permitted free access to water and food at all times. From 2 to 4 pipettes were filled at a single sitting and the results were averaged. In this period the material consisted for the most part of animals bred in the laboratory and of known genetic stock. Analysis on this basis afforded an opportunity for comparing results in animals of unknown hybrid stock without free access to food and water with results in animals of known genetic origin which had usually been in the laboratory for several generations and which were permitted free access to food and water.

*Results.* The mean values of the white blood cell counts for each hour of the day between 9 a.m. and 5 p.m. are presented in tabular form, together with the standard error of the mean. The variance between the means of the hours was not significantly different from the variance within the means. The white blood cells averaged 7,891 for the morning and 7,914 for the afternoon period, a difference of  $23 \pm 194$  white blood cells (not significant).

TABLE I.  
Hourly Means of Total White Blood Cell Counts in Normal Male Rabbits.

| Hour       | 9:00<br>9:59 | 10:00<br>10:59 | 11:00<br>11:59 | 12:00*<br>1:59 | 2:00<br>2:59 | 3:00*<br>4:59 | 9-12<br>a.m. | 12-5<br>p.m. |
|------------|--------------|----------------|----------------|----------------|--------------|---------------|--------------|--------------|
| Counts     | 178          | 226            | 193            | 112            | 124          | 130           | 597          | 366          |
| Animals    | 80           | 103            | 115            | 73             | 64           | 46            | 190          | 136          |
| Mean       | 7709         | 7931           | 8033           | 8061           | 7945         | 7738          | 7891         | 7914         |
| $\sigma_m$ | $\pm 220$    | $\pm 195$      | $\pm 211$      | $\pm 276$      | $\pm 264$    | $\pm 255$     | $\pm 119$    | $\pm 152$    |

\*The data from 12 noon to 1:59 p.m. and from 3:00 to 4:59 p.m. were combined to give samples more comparable with those for the other hourly periods.

No single individual hourly mean differed from any other. Such variations as occurred could be explained on the basis of random sampling. There is therefore no evidence of a significant orderly variation in the total white blood cells of the rabbit in the hours between 9 a.m. and 5 p.m.

There is also no evidence in the rabbit that a digestive leukocytosis occurs between the hours of 9 a.m. and 5 p.m., as no statistically significant change was found in the group permitted free access to food and water.

*Discussion.* It has been observed that the lowest of repeated white blood cell counts in man are obtained early in the morning and the highest late in the afternoon.<sup>3-14</sup> The afternoon level is high even when food intake, muscular effort and emotional stress are controlled.<sup>6, 7, 10-14</sup> The controlled studies, however, are based on 12-50 counts made on the same day in the same persons, and the possible effect of the repetition cannot be discounted. It has been pointed out that successive painful punctures cause a successive rise in the leukocyte count,<sup>12</sup> and an analysis of the average hourly counts in the controlled series available on healthy individuals<sup>10-14</sup> reveals a

<sup>3</sup> Reinert, E., *Die Zählung der Blutkörperchen und ihre Bedeutung für die Diagnose und Therapie*, F. C. W. Vogel, Leipzig, 1891.

<sup>4</sup> Rieder, H., *Beiträge zur Kenntnis der Leukocytosis und verwandter Zustände des Blutes*, F. C. W. Vogel, Leipzig, 1892.

<sup>5</sup> Gregor, K., *Arch. f. Verdauungskrankh.*, 1898, **3**, 387.

<sup>6</sup> Japha, A., *Jahr. f. Kinderh.*, 1900, **52**, 242.

<sup>7</sup> Japha, A., *Deutsch. Aertz. Zeitung*, 1901, **3**, 145.

<sup>8</sup> Türk, W., *Vorlesungen über klinische Hämatologie*, W. Braumuler Vienna, 1912.

<sup>9</sup> Mauriac, P., and Cabouat, P., *Paris Méd.*, 1921, **11**, 407.

<sup>10</sup> Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwahl, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 14.

<sup>11</sup> Shaw, A. F. B., *J. Path. and Bact.*, 1927, **30**, 1.

<sup>12</sup> Garrey, W. E., *Proc. Staff Meet., Mayo Clin.*, 1929, **4**, 157.

<sup>13</sup> Smith, C., and McDowell, A. M., *Arch. Int. Med.*, 1929, **43**, 68.

<sup>14</sup> Kenyon, F., and Macy, I. G., *Human Biol.*, 1938, **10**, 511.

steady rise in the mean count until the series were variously discontinued at 4, 5, 7 and 8 p.m. The total rise for the 4 series in question averaged  $2,330 \pm 292$  white blood cells (statistically significant). Most of the studies in which variations and rhythms were reported are based on an inadequate number of instances, and no statistical consideration of possible technical variations seems to have been undertaken.

The study herewith reported seems free from these disadvantages. The animal material was accustomed to existence in the laboratory and most of it was also accustomed to the taking of blood for examination. These circumstances perhaps lessened variations which might have been caused by bringing in animals to lead an unaccustomed laboratory existence and making the blood counts while they were in a state of digestive, environmental and emotional disturbance. In addition, only one count was made on a given animal during any given 3-day period. The data therefore seemed to rule out a possible diurnal variation in the total leukocyte count of the rabbit under controlled laboratory conditions.

When the material was examined with the idea of determining a possible bias for certain breeds counted at certain hours of the day, it was found that the breeds had been so varied that no bias of this kind was evident.

*Summary and Conclusions.* Nine hundred sixty-three total white blood cell counts made on 204 normal and apparently healthy young adult male rabbits between 9 a.m. and 5 p.m. revealed no statistically significant variation in the hourly means. The blood cell level, apart from sampling variations, was the same for each hour of the day. There was also no evidence of a digestive leukocytosis in the rabbit.

The mean value for 597 leukocyte counts on 190 of the 204 rabbits between 9 a.m. and 12 noon was 7,891, as compared with 7,914 for 366 counts on 136 of the 204 rabbits between 12 noon and 5 p.m. The difference ( $23 \pm 194$ ) was not significant.

These studies are significant for several reasons: The investigation covered a wide variety of breeds and hybrids. The animals were accustomed to laboratory existence and hemocytologic technic before the counts were made. Feeding conditions were varied during the study. Only one examination was made on a given animal on a given day, and usually not more than one or two counts were made on each animal per week. Finally, the material was analyzed by acceptable biometric procedures and represents by far the most extensive study of the sort which has been made.