intravenously, were compared with untreated controls over the 5-month experimental period. No significant change was found in the weight curves following 2.5 to 5 mg. orally, whereas intravenous administration of these doses actually induced a more rapid weight increase than in the controls. Intramuscular administration retarded weight gain, probably as a result of local tissue injury. Toward the latter part of the experiment, those animals on 10 mg. per kg. intravenously failed to gain weight in the normal fashion. These results would indicate intravenous administration of daily doses of less than 5 mg. per kg. to be relatively free from toxicity for the adult animal.

These findings do not lend support to the hypothesis of an *in vivo* thyroxine inactivation and a concurrent inhibition of the ferments concerned in metabolism, providing a constant supply of thyroxine is available by thyroid feeding. Neither do they necessarily condemn the therapeutic use of fluorides nor preclude the possibility that fluorides may prevent the elaboration of excessive thyroxine, or its precursors, by the hyperactive gland of thyrotoxicosis, either by a specific inhibitory action on the glandular cells or by a diminution of the iodine content or interference with the iodine utilization of the gland, as recently postulated by Reveno.[4]

8321 C

Normal Curve of Leucocyte Count of the Albino Rat Over a 24-Hour Period.

DOUGLAS WARNER. (Introduced by Dr. Francis Gilchrist.)

In cooperation with the Department of Zoology, Pomona College, Claremont, Calif.

Those who have reported on the rhythm of leucocyte count in man are not in complete agreement. The following work pertains to the blood of man: Reinke,[1] Galambos,[2] Mauriac and Cabouat,[3] Stetson,[4] and Medlar,[5] agree that there are variations within the day of the leucocyte count which can be considered normal. Japha,[6]

1 Reinke, J., *Beitr. z. path. Anat. u. z. Allg. Path.*, 1889, **5**, 439.
2 Galambos, A., *Folia Haemotologica*, 1912, **33**, 153.
3 Mauriac, P., and Cabouat, P., *Paris Med.*, 1921, **39**, 407.
4 Stetson, R. P., *Arch. Int. Med.*, 1927, **40**, 488.
5 Medlar, E. M., *Am. J. Med. Sci.*, 1929, **177**, 72.
6 Japha, A., *Jahrb. f. Kinderhlk*, 1900, **52**, 242.

Turk,[7] Fletcher and Mitchel,[8] Shaw,[9] Smith and McDowell,[10] and Martin[11] find that the count is normally higher in the afternoon than in the morning. Doan and Zerfas[12] and Sabin, Cunningham, Doan and Kindwall[13] report short period rhythms as well as the longer cycle. The latter report an hourly rhythm for the total count and a morning to afternoon tide with a peak in the afternoon. They find that this tide is due to an increase in the neutrophiles which have also the hourly rhythm. The lymphocytes, they report, have a 15-minute cycle and are relatively constant as to tide. These workers conclude that the possible normal variation for a given individual is covered within the day. Shaw[9] reports 2 tides a day. Each tide is of 12 hours' duration. The forenoon tide reaches a peak in the afternoon and ebbs in the evening. The night tide, starting in the evening, reaches a peak after midnight and fades in the morning. Medlar[5] finds no consistent hourly rhythm but does concede that each individual may have a rhythm of his own. He states that the total normal variation of any individual is covered within an hour.

Goldberg and Lipskaia[14] state that mental and physical labor bring about an increase in the neutrophiles at the expense of the lymphocytes. Shaw[9] and Sabin[13] and her coworkers both find the neutrophiles responsible for the tides.

In the experiments herewith reported, conditions have been devised so as to standardize the internal factors and to make uniform the external factors which might affect the cell count.

Standardization of internal factors: 1. Food and water were taken from the animal at least 2 hours before the experiment. 2. The animals in the stock room were kept on an unvarying diet. 3. Males only were used. 4. All animals were Wistar rats from the strain known as the Experimental Colony strain. 5. Each rat was of a different litter.

Uniformity of external factors: 1. The animals had been isolated from females since 10 days of age. 2. The experimental ani-

[7] Turk, W., *Dtsch. med. Wchnschr.*, 1912, **38**, 2186.

[8] Fletcher, E. G., and Mitchel, A. G., *Am. J. Dis. Child.*, 1927, **34**, 807.

[9] Shaw, A. F. B., *J. Path. and Bact.*, 1927, **29**, 389.

[10] Smith, C., and McDowell, A. M., *Arch. Int. Med.*, 1929, **43**, 68.

[11] Martin, H. E., *J. Physiol.*, 1932, **75**, 113.

[12] Doan, C. A., and Zerfas, L. C., *J. Exp. Med.*, 1926, **46**, 511.

[13] Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, **37**, 14.

[14] Goldberg and Lipskaia, from Piney, A., 1928, Recent Advances in Haematology. P. Blakiston's Son & Co., Inc., 1928, 2nd ed.

mals were placed in small individual cages 12 hours before the experiment was to begin. 3. Fairly constant temperature was maintained for 12 hours before and for the duration of the experiment. 4. Light was kept at a constant value. 5. Handling and the resultant excitation were kept at a minimum.

In all, 53 animals, with an age spread of from 60 to 550 days, were used.

All cell types common in the circulating blood of man were found. The white blood cells have been considered as divisible into 3 main groups which may in turn be subdivided. These groups and their subdivisions follow:

I. Lymphocytes. This cell varies in size from 6½μ to 15μ with a definite mode at 9.4μ. 200 cells were measured and plotted. A typical bell curve resulted, rather than a 2-mode curve as the literature would lead one to expect. The nucleus is well defined, being lumpy in appearance as contrasted with the monocytes. The nuclear material stains a dark, slightly reddish purple, and makes up all but about 1/6th of the total diameter of the cell as seen in 2 dimensions. The cytoplasm stains a faint, dull blue. Granules are occasionally present in small numbers in the cytoplasm. The lymphocyte is the predominant cell in the blood of the rat, averaging 53.24% of the total count. Occasionally there was observed a sharply defined nucleolus stained very densely and shaped either as a washer or round.

II. A. Monocytes. This cell varies in size from 11μ to 17μ with the average at 13μ. The cytoplasm is practically colorless and usually irregular in shape. The nucleus accounts for only about ½ of the total diameter of the cell. The nucleus stains more lightly than that of the lymphocyte and the color is bluer. The structure appears to be stringy, rather than lumpy. Frequently, the cell seems to have been fixed during a period of streaming. This appearance is apparent in both the nucleus and the cytoplasm. Their number accounts for 2.74% of the total count.

B. Transitional. This cell varies in size from 13μ to 17μ. It resembles the monocyte with the exception that the nuclear material is clearly split at least one-half the way through. A few transitionals were found with lymphocyte-type nuclei. Frequency of appearance is 0.39%.

III. A. Polymorphonuclear neutrophilic leucocyte. This cell varies in size from 9⅓μ to 17μ with a mode at 11¼μ and with an average of 12 1/5μ. The nucleus is always "ropy" in texture and tends to be less lobulated and of more uniform diameter than in the

human cell. The cytoplasm is usually colorless with a few granules. They are purple in color, taking both the blue and the red stain. Although there is considerable variation in the relative number of lymphocytes and polymorphonuclear cells during different parts of the day, the average frequency of the latter is 39.18%.

B. Polymorphonuclear basophilic leucocyte. The few cells of this type measured varied from 10μ to 13μ with an average size of 11½μ. The nuclear material is more homogeneous and less ropy than that of the neutrophile. The cytoplasm contains many very large granules which stain definitely blue. The average frequency of this cell is 0.13%.

C. Eosinophiles. The average size of this cell is 12.6μ with a spread of from 9⅓μ to 17μ. The nuclear material resembles that of the basophile as compared with the neutrophile. The nucleus as seen 2-dimensionally occurs in one of 3 shapes:

1. As a perfect circle in 58% of the cases.
2. As a figure eight in 29% of the cases observed.
3. With a second twisting, making 3 loops in 11% of the cases.

The cytoplasm is of a definite light red color. Although magnification of 1425× was used, only occasionally could the individual granules be made out because of their smallness. Some animals were found with a large number of eosinophiles. This was taken to indicate the presence of intestinal parasites. Eliminating these animals, the average frequency was 2.20%.

D. Metacytes. The average size of this cell was found to be 11¾μ with a variation from 7μ to 17μ. The characteristically notched nucleus is made up of material exactly resembling the lymphocyte nucleus. The cytoplasm is usually slightly more abundant and tends to stain less densely. Its frequency of appearance was found to be 1.93%.

E. Stabkernige of "Staff" cell. The average size of the few cells measured was 11¾μ with a spread from 10⅓μ to 14μ. The description of this cell would be the same as that for man. Its frequency of appearance is 0.19%.

There were also observed a few eosinophilic metacytes and eosinophilic "Staff" cells. Only 2 myelocytes were positively identified.

At 2-hour intervals, blood was taken from the animal from a single slash in the tail. A total white count was made. Two chambers were counted and an average struck. At the same time, smears were prepared. These were stained with Wright's within 4 hours and mounted under balsam as soon as dry. Counts were taken for at least 14 hours. In most cases they were continued for 20 or more

hours. One-fourth of the animals were started at 6 A. M.; one-fourth at noon; one-fourth at 18 o'clock (6 P. M.); and one-fourth at 24 o'clock (12 midnight). It was found in most cases that the first 2 or 3 readings showed a drop, regardless of what time of day the animals were started. It was also noted that after 18 hours unexpected variations occurred. For these reasons, counts taken in the intervening period were made the basis of the study. To restate, by staggering the starting time of different individuals, it has been possible to eliminate consistently, the first 3 and the last few readings from each animal and then, by recording all readings remaining for each animal, show a composite curve over a 24-hour period.

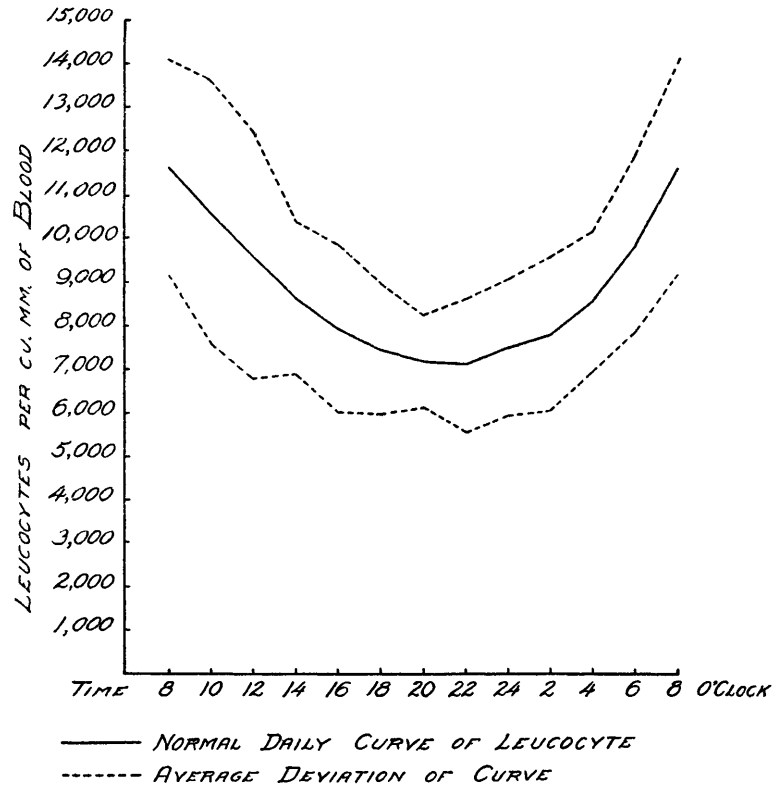
The consistent level of the red counts taken experimentally indicates that change of blood volume or compression of blood has not played any part in the white cell variation.

By this method there has been found, for total count of white cells, a definite daily tide. The low point is at approximately 22 o'clock. A gradually increasing rise (logarithmic type curve) reaches its peak at 8 o'clock and rather suddenly falls away in a straight line until about 14 o'clock when it starts to level out, returning to the low point again at 22 o'clock. The average deviation shows an overlap as plotted against the curve.

When the 3 major cell types are plotted in a cumulative graph, it is seen that all 3 follow roughly the same curve, although the majority of the change is due to the lymphocytes. The polymorphonuclear group reaches its low point at 18 o'clock, 6 hours before the lymphocytes. When the actual percentage values of polymorphonuclear neutrophilic cells are divided into the difference in percentage between that count and the subsequent count, and all these values for a given 2-hour period taken together and averaged, there results a figure which represents the rate of change. Plotting these figures reveals that this type showed a remarkably strong and persistent gain from 16 until 24 o'clock. When the lymphocytes are considered in the same manner, there is found little of significance other than the expected reciprocal drop from 16 until 24 and 8 until 16. The metacytes taken alone show their greatest increase in absolute number for a period ending 6 hours prior to the sudden rate change increase of the polymorphonuclear neutrophilic leucocytes.

Thirty-one consecutive counts were taken at 15-minute intervals in an attempt to confirm the work of Sabin, *et al.*, reporting short rhythms of the different cell types. The results were negative.

The following conclusions are indicated: 1. A diurnal tide, with



its low point at 22 o'clock (10 P. M.) ascending at an increasing rate till shortly after 8 o'clock (A. M.) and then falling at a decreasing rate until the low point is again reached, has been experimentally demonstrated for the white rat. 2. Lymphocytes are more responsible for this tide than the polymorphonuclear group, although all cell types share in the loss and gain. 3. The total normal variation of the white cell count of a rat occurs each day. 4. The lowest level is approximately 62% of the highest level. 5. Eight types of white blood cells found in the circulating blood of the normal white rat have been briefly described. 6. The frequency of appearance of these cells has been given as based on the study of 164 differential counts.

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