In this paper we present an instance of complete maturation of the erythrocytes during the whole of an induced rapid remission in a typical severe case of pernicious anemia (woman, age 41, Hosp. No. 43942). The remission was produced by the feeding of powdered fetal calf's liver, in amounts corresponding to 300 g. of fresh liver a day. This remission followed immediately upon an unsuccessful trial of corresponding doses of a beef liver extract, the potency of which had not been previously ascertained. As seen from Table I, there was at first during the liver extract period some definite response with a slight increase in the number of erythrocytes and an increase of the reticulocytes up to 3.9%. Both these changes were, however, transitory. As an unfavorable sign megaloblasts appeared in the circulation in increased number.

With the onset of the fetal liver feeding the erythrocytes started again to show a steady increase in number, and the megaloblasts disappeared promptly. The normoblasts which had been present in small number during the whole period of observation did not increase, and the reticulocytes remained below 2%, soon falling as low as about half of 1%. On beef liver extract the corresponding reticulocyte counts would have gone as high as 15% or more.

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Statistical Significance of Erythrocyte Counts During Responses to Liver Extract in Normal Individual.

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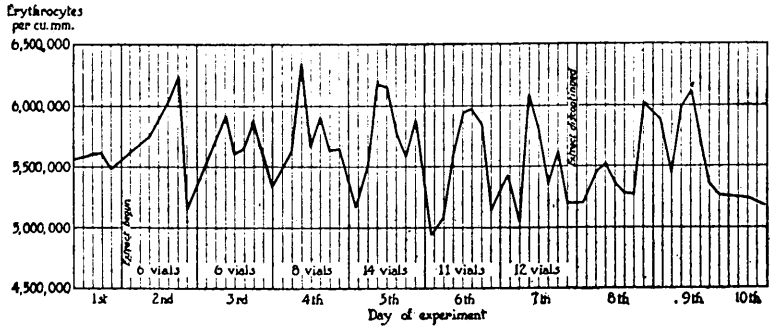
Marked increases in the number of circulating erythrocytes have recently been reported following the administration of the Cohn-Minot liver extract to normal individuals.[1] The outstanding features of the curves obtained were 2: rapid and considerable increases as well as marked irregularities in the number of erythrocytes leading to deep and abrupt depressions alternating with peaks. The curves were constructed from daily blood counts.

It seems desirable to supplement these observations by more frequent determinations of the number of erythrocytes, as well as by

[1] Watkins, C. H., Johnson, R., and Berglund, H., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 720.

an inquiry into the statistical significance of the erythrocyte counts. One of the persons (H. B-d) who had previously in 2 separate experiments responded to the liver extract both with peaks up to 7,870,000 and 7,700,000 erythrocytes respectively and with deep depressions was chosen for the experiment. The erythrocyte counts of this individual have been consistent over a number of years. As early as 1906 the counts gave values of 5,500,000 (mean of 5 or 6 determinations) and in the 2 recent experiments the curves started at a level of 5,950,000 and 5,400,000 respectively.

In order to determine the significance of the variations in the erythrocyte counts in the normal individual, observations were made at 3 hour intervals during the day before liver extract was started. Following this, the Cohn-Minot liver extract (Lilly 343; lots 29157-815244-815245) was given for 6 days in the dosages shown in Graph 1. On the 1st day counts were made at 3 hour



GRAPH 1.

A graph illustrating the variations of the means of erythrocyte counts, plotted in succession.

Abscissa: Days of experiment. Ordinate: Means of erythrocyte counts.

intervals from 9:00 a. m. to 9:00 p. m. On the 2nd and 3rd days counts were made at 3 hour intervals from 6:00 a. m. to 12:00 midnight, and on the remaining 5 days counts at 2:00 a. m. were substituted for those at midnight. Each time erythrocyte counts were made, blood was taken from 3 fingers, using a different pipette for each finger. Three counts were then made from each pipette, making only one count for each filling of the counting chamber, giving a total of 9 counts for each period of the day. In making each count the cells covering 80 small squares were counted. The mean, standard deviation and probable error of the mean were calculated for each series of 9 counts. Comparisons were made of the means for different times of the normal day with the means for all other times of that day, and the significance of the difference tested by the

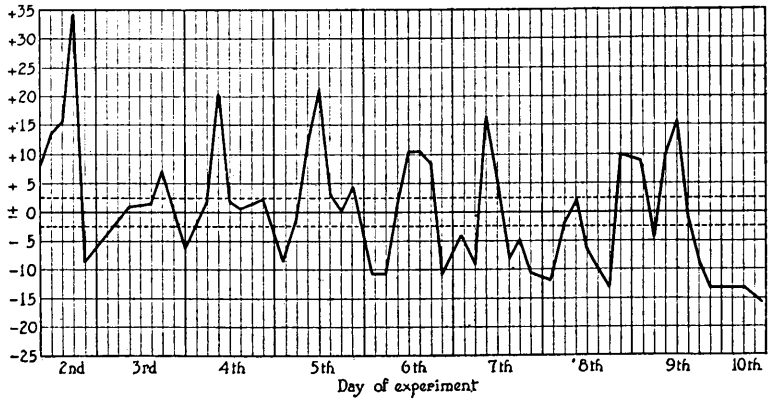
usual formula for the probable error of a difference. The differences of the means for the pre-liver day were found to be statistically insignificant. Therefore all of the data for the pre-liver day were grouped in one series to establish a mean for use as a normal base.

Similar comparisons were made for the first 2 days after liver extract was started. Only seriatim comparisons were made for the remaining 7 days. With a few exceptions, the differences show a high statistical significance. The ratios of the differences to their probable errors range from 0.06 to 21.23. Of the 64 ratios computed, but 16 are less than 2.5 and are of questionable statistical significance.

The preponderance of the large difference ratios indicates that the variation of the blood counts from time to time, under liver extract stimulation, is significantly different from the blood count prior to this stimulation, for there is no evidence of significant variation during the pre-liver day.

The series of means for the normal day and for the days of the administration of liver extract and for 2½ days following the same are plotted in succession in Graph 1.

The ratios of the differences of the means to their probable errors, for blood counts on liver days as compared with the blood counts of the pre-liver day, are shown in Graph 2. The 54 ratios range from 0.26 to 34.44. Of these 16 are less than 2.5. This con-



GRAPH 2.

A graph illustrating the variations of the ratios of the differences of the successive means from the mean to the probable errors of these differences. The conventional zone of questionable statistical significance is indicated between the dotted lines.

Abscissa: Days of experiment. Ordinate: Ratios of the differences of successive means from the initial mean, to the probable errors of these differences.

ventional zone of insignificance is indicated on the graph by the dotted line on each side of the base line. Here again the preponderance of significant differences seems to indicate that there is a marked effect on the erythrocyte count, following the administration of liver extract.

The statistical significance of the observations having been established and properly defined, Graph 1 presents itself for biological consideration. The character of the response previously established for normal individuals to liver extract is decidedly more complicated than was suspected from our previous daily observations. There is produced through the liver extract a series of reactions, which reoccur with unmistakable periodicity. This periodicity seems to be approximately of a 24 hour type. A strong factor is present tending towards increasing the concentration of circulating erythrocytes. This factor is evidently checked by another force aiming to preserve a lower erythrocyte level. With the tendency towards a high erythrocyte level remaining about constant throughout the experiment, the force to establish a lower level is gaining as the experiment progresses, leaving the subject with a final level lower than the initial one. In this connection attention should be called to the fact that the subject of the experiment during 2 previous experiments responded with blood counts one a half million higher than in this experiment. That this difference is not due to a difference in the potency of the extracts used is indicated by the clear independence of the height of the peaks in this experiment of the amount of extract taken.* An increase in strength of the lowering factor during the different experiments as well as during the course of this experiment is suggestive. The temporary persistence of the effect after the extract had been discontinued is noticeable.

It is possible that through the exaggerated conditions of the experiment an expression of the normal mechanism for the regulation of the erythrocyte level has been brought to light, a mechanism which under normal conditions is perhaps difficult or impossible clearly to observe.

* This supposition is perhaps not correct. Dr. George Minot just informs me that the lots used were checked up as being about 35% and 50% weaker than standard respectively.