NORMAL BONE MARROW AS OBTAINED BY STERNAL PUNCTURE

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THE value of sternal puncture as a diagnostic aid has been limited by a lack of definitely established normal standards. In 1944 Osgood and Seaman[1] reviewed the available data from their own studies and those in the literature and compiled a set of tentative standards. They emphasized the complete lack of uniformity of methods and the inadequacy of the records. The present paper contains the results of studies that were started originally with the hope of establishing standards that were completely unavailable at the time and that were extended with the hope that the resulting data might be useful in future computations.

MATERIAL

The experimental subjects were 50 white volunteers. All were American, but the ethnic derivation varied considerably. Irish, Italian, German, and Jewish origins were represented, but most of them were Anglo-Saxon. All subjects had been living in the city of Buffalo for at least one year prior to the collection of the samples. Ages varied from 17 to 45 years. Forty-four of them were from 19 to 24 years old and were either second year medical students or medical technicians. All were in good health. One had recovered recently from tonsillitis and one from the common cold. The nutritional status was within accepted normal limits in all of them. Six subjects might be described as stocky, 13 as slender, and the remaining 31 as very close to the accepted average for age and height. Eight of the subjects were female. Pregnancy was not present in any of them. Samples were obtained in the interval between menstrual periods.

COLLECTION OF SAMPLES

The site of the puncture was the midportion of the sternal body. Local anesthesia was produced with 2 cc. of 2 per cent novocain. A wheal was made and with the anesthetizing needle directed toward the neck at a 45 degree angle with the surface of the sternum the periosteum was infiltrated. An 18 gauge sternal puncture needle was inserted through the wheal and directed exactly as described for the anesthetizing needle. The tip of the aspirating needle entered the marrow cavity approximately 2½ cm. above the center of the sternal body.

Using a dry 5 cc. syringe, exactly 3 cc. of marrow were withdrawn in each instance. This sample was discharged into a bottle containing an appropriate amount of the dried oxalate mixture described by Heller and Paul[2] and agitated sufficiently to secure both complete mixture with the oxalate and even distribution of the cellular elements. Films were prepared on glass slides immediately. Special attention was paid to the promptness in making these films so that oxalate changes could be avoided. To secure such promptness a technician accompanied the operator to the bedside and made the films directly after the sample was secured.

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EXAMINATION OF MARROW SAMPLES

All samples were handled in a uniform manner. Total nucleated cell counts were made precisely as in blood leukocyte counts and were recorded as the number of cells per cubic millimeter of aspirated marrow. As noted previously, the amount of marrow withdrawn was always 3 cc. This amount was chosen arbitrarily several years ago as an amount convenient to handle. Reference to the review of Osgood and Seaman[1] will show that this was a fortunate choice since it falls within the range of 1 to 10 cc. where the errors are the smallest.

Reticulocyte counts were made from films spread on slides previously treated with the brilliant cresyl blue in the manner described by Castle and Minot.[3]

Differential counts were made from films stained by Wright's method, and exactly 200 cells were enumerated in each instance. Cell grouping was simplified as much as possible with the following main types represented: myeloblasts, myelocytes, juveniles, bands, filaments, eosinophils, basophils, lymphocytes, monocytes, plasma cells, reticulo-endothelial cells, normoblasts, disintegrated cells, and miscellaneous. A definite attempt was made to fit the cell into the category closest to it rather than to have additional groups for small numbers of cells. For example, promyelocytes were grouped either as myeloblasts or as myelocytes, depending upon the examiner's judgment of closest fit. In the routine counting, subgroups of different types were segregated, of course, but in the results to be described in this paper such subgroups were discarded.

Rough estimates of megakaryocyte counts were made. After trying several methods of megakaryocyte counting with poor success, the procedure of enumerating the number of these elements in 50 low power fields at the edges of the film in a uniform fashion was adopted. In each case the thinnest end of the film was found to show the highest concentration of megakaryocytes and enumeration of cells at this end was included invariably.

The data were subjected to various types of statistical analysis and those that were finally selected as most appropriate for the material are listed below.

1. Range from lowest to highest results observed.
2. 95 per cent range, which is the range of the extreme values within which 95 per cent of the observations fell.
3. Arithmetic mean.
4. Standard deviation $= \sqrt{\frac{\Sigma X^2 - M^2}{N}}$

   Σ—Sum
   X—Value or result of the observation
   M—Arithmetic mean
   N—Number of observations

5. Standard error $= \frac{\delta}{\sqrt{N}}$

   δ—Standard deviation
6. Percentage of cases falling within the range of six times the standard deviation.

7. Coefficient of variation = $100 \cdot \frac{\delta}{M}$

8. Number of cases in which cells of each type were found in the standard enumeration of 200 cells.

RESULTS AND DISCUSSION

The results are summarized in table 1 and are compared with those compiled by Osgood and Seaman[1] in table 2.

TABLE 1.—*Observations on Bone Marrow of Healthy Human Adults*

Determinations	No. of Cases	Range	Mean	Standard Error	Standard Deviation	% of Cases within the Range of 6 S.D.	Coefficient of Variation	No. of Cases in which Cells Appeared in Counts
Total Nucleated Cells..	50	9,400 to 74,000	35,300	2,075	14,670	100	41.27	50
Myeloblasts.....	50	0.0% 3.0%	1.33%	0.10	0.70	100	52.95	47
Myelocytes.....	50	2.0% 15.5%	8.92%	0.45	3.18	100	35.68	50
Young forms.....	50	3.5% 17.5%	8.75%	0.44	3.09	100	35.35	50
Band "	50	12.0% 34.0%	23.92%	0.85	5.98	100	25.01	50
Filament "	50	6.0% 35.5%	18.47%	0.86	6.08	100	32.93	50
Eosinophil cells.....	50	0.0% 6.5%	1.86%	0.15	1.03	98	55.36	48
Basophil "	50	0.0% 1.5%	0.15%	0.05	0.32		213.40	11*
Lymphocytes.....	50	7.0% 34.5%	16.22%	0.79	5.56	98	34.28	50
Monocytes.....	50	0.0% 6.0%	2.42%	0.23	1.63	100	67.43	47
Plasma cells.....	50	0.0% 1.5%	0.33%	0.06	0.45		132.54	22*
Reticulo-endothelial cells.....	50	0.0% 2.5%	0.25%	0.07	0.47		188.64	15*
Unidentified cells.....	50	0.0% 0.5%	0.02%					2*
Disintegrated "	50	0.0% 18.0%	7.85%	0.53	3.74	100	47.69	49
Normoblasts.....	50	1.5% 24.0%	9.51%	0.66	4.68	100	49.24	50
Reticulocytes %.....	50	0.1% 2.8%	0.94%	0.04	0.30	92	31.91	50
Megakaryocytes/50 l.p.f.....	50	0.0 44.0	9.62	1.23	8.73	100	90.78	49

* These cells were found too infrequently to be significant from a statistical standpoint.
All computations were carried out to 3 digits.

The total nucleated cell counts varied from 9,400 to 74,000, with a mean value of 35,300. The mean value is almost identical with that compiled by Osgood and Seaman[1] from the data of Napier and Gupta[4] and Pitts and Packham.[5] The range of values of the combined data was slightly wider than ours, although 95 per cent of the cases showed results between 10,000 and 100,000. It will be apparent to anyone familiar with statistical methods that fixing the extremes of normal variation will be difficult. Our own studies of marrow, obtained several years ago from 15 normal subjects and not included in this analysis, actually showed a range wider than the present series (9,200 to 85,000).

It should be pointed out that the similarity of the two mean values mentioned

above may be somewhat misleading. The samples are comparable only to the extent that they are all obtained from normal subjects. In other respects they can hardly be regarded as representative samples of the same universe. Our sample consisted of an uneven mixture of male and female subjects of a young adult group in the Eastern part of the United States. The sample of Napier and Gupta[4] was obtained from residents of India. That of Pitts and Packham[5] consisted of a group of young women from western Canada. A study of the three groups of data shows that the range and the mean values of Napier and Gupta[4] were somewhat greater than ours while those of Pitts and Packham[5] were somewhat smaller.

TABLE 2.—*Comparison of the Present Results with Those of Osgood and Seaman*

Determinations	MEANS		95% RANGE	
	Osgood and Seaman	Vaughan and Brockmyre	Osgood and Seaman	Vaughan and Brockmyre
Total Nucleated Cell Count	35,000	35,300	10,000–100,000	12,000–72,000
Myeloblasts	0.4	1.3	0.0– 1.0	0.0– 3.0
Myelocytes	5.6	8.9	0.0–15.0	3.0–15.0
Young forms	6.5	8.8	3.0–10.0	4.0–15.0
Band forms	24.0	23.9	17.0–33.0	12.5–33.5
Filament forms	15.0	18.5	5.0–25.0	9.0–31.5
Eosinophils	2.0	1.9	0.0– 4.0	0.0– 5.5
Basophils	0.2	0.2	0.0– 0.5	0.0– 1.0
Lymphocytes	14.0	16.2	3.0–25.0	7.5–26.5
Monocytes	2.0	2.4	0.0– 4.0	0.0– 6.0
Plasma cells		0.3		0.0– 1.5
Reticulo-endothelial cells		0.3		0.0– 1.0
Unidentified cells				0.0– 0.0
Disintegrated cells	19.0	7.9	10.0–30.0	2.5–16.5
Normoblasts	11.2	9.5	5.0–18.0	2.5–17.5
Reticulocytes %	1.9	0.9		0.4– 2.4
Megakaryocytes per 50 fields		9.6		1.0–38.0

Despite these discrepancies and the obvious need for further study of normal values, it is interesting and important that three such diverse samples show such relatively similar results. From our studies of total nucleated cell counts in various diseases it is believed that this type of observation should receive much more emphasis than it has received heretofore, not only from the standpoint of diagnostic aid, but especially as an additional means of gaining insight into abnormal bone marrow cytology and physiology. We agree with those who feel that the wide variation in normal subjects is an obstacle, but it is more than likely that in some diseases we shall find the range almost entirely abnormal, and in others we shall have reason to modify current views, particularly as to evaluation of hypoplasia and hyperplasia.

Our differential counts seem to compare favorably with those compiled by Osgood and Seaman[1] from the data of Young and Osgood[6] and Pitts and Packham.[5]

Study of the individual series shows that the results of Pitts and Packham[5] are much closer to ours than are those of Young and Osgood.[6] This points again to the fallacy of combining small samples of different workers for statistical purposes.

The percentage values of some of the different cell types are almost identical in all three series and those of other types are so similar that one is tempted to ignore the discrepancies. However, reference to table 2 will show that differences between our results and those compiled by Osgood and Seaman[1] are particularly noticeable for myeloblasts, myelocytes, juveniles, filament forms, disintegrated cells, normoblasts, and reticulocytes. In these instances, the differences exceed 9 standard errors and are regarded as significant.

Several reasons for the discrepancies may be postulated. Among them are the possible errors of sampling and differences in classifying cells by the various workers. However, it is felt that the most important reason lies in the methods used in preparing the films. It will be noted that the percentage of disintegrated cells is much greater in the preparations of the other workers than in ours. It is obvious that a true picture of the marrow cytology cannot be obtained when broken cells constitute such high proportions of those present. As one might expect, the greatest differences would be apparent in the immature cells which are more subject to trauma than the mature ones. That this is the case is substantiated by the fact that our mean percentage of myeloblasts exceeds that of the other authors threefold. The subject of preventing disintegration deserves further study.

Examination of table 1 will show that some of the cell types (basophils, plasma cells, reticulo-endothelial cells) were encountered in too few cases to make the results significant from the statistical standpoint and those given must be regarded as only rough approximations of the true values. As mentioned by Osgood and Seaman,[1] the true picture can be obtained only by enumerating many hundreds of cells in each case. While this might prove of academic interest, there is little reason to think that it would be of great practical value.

The coefficients of variation are especially revealing as regards the general accuracy of classifying cells. It will be noted that in the instances just mentioned the figures are comparatively high in all cases (myeloblasts, eosinophils, monocytes, and disintegrated cells), where the percentages are low, and where the routine counts fail to show any of the cells in a proportion of cases.

The same phenomenon is noted in the megakaryocyte counts. In this instance the chief difficulty lies in the method of counting. These authors recognize that their method could not be truly accurate, and it was only because no better one was known to them that they adopted it to gain some insight into the approximate number of these cells in aspirated marrow. It is of interest to find that the results seemed to agree approximately with the other findings as far as statistical distribution is concerned.

It is interesting that the reticulocyte counts, which were made with a well standardized technic, failed to follow the empirical rule that a range of six times the standard deviation usually includes 99 per cent or more of the observations. Otherwise they seem to fulfill the requirements of statistical distribution.

SUMMARY

A study of aspirated sternal bone marrow of 50 normal human adult volunteer subjects has been presented. Samples of exactly 3 cc. each were collected in a uniform manner. The technic of performing total nucleated cell counts, differential counts, reticulocyte counts, and megakaryocyte counts has been described and the results given. These results have been subjected to statistical analysis and compared with those of other workers.

CONCLUSIONS

1. The use of standard 3 cc. samples of marrow is recommended.

2. Our results agree substantially with those compiled by Osgood and Seaman although some significant differences are noted.

3. Total nucleated cell counts are significant from a statistical standpoint despite a wide range of results and should be included in the routine study of marrow.

4. Methods for diminishing the number of disintegrated cells in stained films would increase the accuracy of differential count results.

5. A practical method of making megakaryocyte counts is needed.

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