A Quantitative Comparison of the Nucleated Cells in the Right and Left Humeral Bone Marrow of the Guinea Pig

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DURING the past few years a technic has gradually been developed for the quantitative study of nucleated cells in the bone marrow, at first in the rabbit[4] and subsequently in the guinea pig.[1, 3, 5] The technic which has thus far been evolved, though it has yielded results of considerable interest, is probably subject to special errors of its own in addition to those normally associated with hemocytometric methods. Accordingly, in order to test the accuracy of the method at present in use, and to obtain control data for a new strain of experimental animals, it was decided to perform bilateral counts on the cells of the humeral marrow of seventeen guinea pigs. It seemed a reasonable assumption that the marrows of the right and left humerus would exhibit the same degree of hemopoietic activity, and therefore be likely to contain equal numbers of nucleated cells.

Material and Methods

The work was performed on young, healthy male guinea pigs, between 350 to 400 Gm. in weight, of the Mill Hill strain originated by Dunklin and Hartley. Under ether anesthesia the abdomen is opened, the abdominal aorta incised, and blood collected through a funnel into a centrifuge tube and centrifuged for 5 minutes at about 4000 rpm to obtain serum. Meanwhile a small tightly stoppered (rubber) glass tube, of about $^1/_4$ inch internal diameter and $1^3/_4$ inches in length, is weighed, then half filled with serum and weighed again. A humerus is quickly removed and cleaned, the two ends sawed off, and the marrow ejected by a blower into the serum-containing tube which is then weighed a third time. The tube thus contains known weights of marrow and serum, whose volume can be ascertained from their specific gravities. The tube is then shaken for 2 to 3 minutes in a mechanical shaker with an amplitude of about 5 inches at 400 times per minute; the bone marrow cells become free and form a uniform suspension from which smears can be prepared and stained, and absolute counts made in the usual manner. As Mechanik[2] and others have pointed out, the specific gravity of red marrow is practically 1.0, and since that of serum is also close to unity, very little error would be introduced if, for the estimation of marrow dilution, volumes were converted into weights on this basis. However, in a number of instances specific gravities were determined, and for the final calculations a correction factor 1.0111 was applied.

Determination of Specific Gravity

The ordinary pyknometric method is used to determine the S.G. of a sample of serum, and then a sample of bone marrow suspension of known wt./wt. per cent composition is made from the same serum. From the results the S.G. of the bone marrow can be calculated.

The pyknometer used is of approximately the same size and shape as a red blood cell

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dilution pipet, but has an expansion bulb at the upper end, and glass caps fitted at either end by ground glass joints. There is only one graduation, at the 1 ml. level.

A sample of clear serum is obtained from the guinea pig in the usual manner, and its temperature determined. The clean dry pyknometer is weighed accurately, and filled with blood serum in the same way as an ordinary blood dilution pipet. The lower cap is placed in position before the upper one. This allows any displaced fluid to enter the expansion bulb, so that when the upper cap is applied, none is lost. The filled pyknometer is then weighed, and after cleaning and drying, the procedure is repeated with distilled water and bone marrow suspension, the mean temperature of the fluid being recorded in each case.

TABLE 1.—*Absolute Marrow Counts. Comparison of Right and Left Guinea Pig Humerus*
(All counts are in cells per cu.mm. marrow)*

No. of exp.	Side of humerus	Erythroid	Myeloid	Lymphocyte	Monocyte	Damaged	Unclassified	Total abs. count nucleated cells	M:E ratio
ON1	L	273,409	369,906	165,777	59,383	325,369	32,165	1,237,147	1.352
	R	198,096	232,299	468,874	22,802	475,999	22,802	1,425,148	1.172
ON2	L	193,198	160,164	312,320	44,045	244,250	42,043	1,001,025	0.829
	R	261,482	355,513	188,061	38,643	369,682	66,981	1,288,090	1.359
ON3	L	—	—	—	—	—	—	—	—
	R	236,610	205,313	451,938	16,275	306,717	26,290	1,251,906	0.867
ON4	L	222,194	306,068	534,148	14,715	364,928	17,658	1,471,482	1.377
	R	239,705	300,029	500,048	49,211	474,649	17,462	1,587,456	1.251
ON5	L	286,205	324,579	482,872	41,572	426,910	33,577	1,598,913	1.134
	R	182,545	192,028	461,104	21,336	309,378	15,410	1,185,358	1.052
ON6	L	403,540	361,635	296,447	21,729	330,593	128,822	1,552,078	0.896
	R	291,847	409,943	259,268	20,361	268,770	89,590	1,357,426	1.404
ON7	L	244,289	305,651	221,134	77,571	240,816	53,257	1,157,769	1.251
	R	232,166	382,533	167,208	31,276	333,212	43,306	1,202,933	1.647
ON8	L	265,340	403,048	212,720	7,837	162,339	59,338	1,119,579	1.518
	R	368,637	560,044	432,439	37,218	269,388	93,931	1,772,292	1.519
ON9	L	324,239	354,518	291,437	45,419	118,593	121,116	1,261,630	1.0934
	R	345,209	345,209	280,955	10,079	162,526	108,350	1,259,888	1.000
ON10	L	—	—	—	—	—	—	1,011,476	—
	R	305,908	425,139	185,472	15,657	197,515	67,444	1,204,361	1.389
ON11	L	380,813	548,141	196,177	5,770	154,345	141,363	1,442,475	1.439
	R	522,201	590,660	187,865	4,776	122,590	144,879	1,592,076	1.131
ON12	L	260,348	332,126	289,546	27,981	227,500	69,345	1,216,579	1.276
	R	204,015	410,629	223,507	1,299	414,527	33,786	1,299,459	2.012
ON13	L	166,985	441,012	366,796	19,981	336,825	72,788	1,427,223	2.641
	R	179,594	291,841	396,604	11,225	290,593	59,805	1,247,182	1.625
ON14	L	336,004	465,585	323,950	18,081	277,241	70,817	1,506,746	1.385
	R	309,504	649,166	211,098	14,285	238,080	146,023	1,587,202	2.097
ON15	L	255,825	434,747	389,232	25,111	307,618	145,962	1,569,484	1.699
	R	280,229	362,649	260,997	15,111	324,186	114,014	1,373,672	1.294
ON16	L	149,190	248,650	421,152	24,865	576,559	119,663	1,554,068	1.666
	R	356,779	488,121	488,121	15,682	523,407	78,413	1,960,328	1.368
ON17	L	335,856	746,124	386,134	30,166	277,534	231,278	2,011,118	2.221
	R	113,084	423,754	239,837	13,669	344,222	98,171	1,242,682	3.747

* These data do not include a small group of miscellaneous cells (see text), and hence the column "Total absolute count" will be found to exceed slightly the sum of all the other columns.

The usual corrections for coefficient of cubical expansion of glass and buoyancy are applied.

If x = wt./wt. per cent composition of bone marrow suspension, D_1 = density of serum, D_2 = density of suspension, D_3 = density of bone marrow, then $D_3 = \frac{x}{\frac{100}{D_2} - \frac{100 - x}{D_1}}$. Conversion factor $F = \frac{D_3}{D_1} = \frac{\text{Density of bone marrow}}{\text{Density of blood serum}}$.

If N = total number of cells in 5 large squares (each 1 sq. mm. in area 1/10 cu. mm. in volume) of an improved Neubauer hemocytometer, d = dilution, a = wt. of marrow in suspension, b = wt. of serum, then the total absolute count = $N \times \frac{b}{a} \times d \times 2 \times F$ cells per cu. mm.

Results

The results are presented in table 1. A more detailed discussion of the problems of cell identification will be found elsewhere[5] and only a few observations need be made here.

Lymphocytes are virtually all small lymphocytes, morphologically indistinguishable from small lymphocytes in a teased preparation of lymph gland or thymus.

Myeloid cells are predominantly heterophile (or pseudoeosinophile) corresponding to the neutrophils in man. Taking the entire series, the heterophils averaged 329,000 per cu. mm. of marrow, eosinophils 47,000, and basophils 17,000.

An appreciable number of cells could not be classified and these are listed separately in table 1. There was also a small group of miscellaneous cells, such as plasma or reticulum cells; these have not been included in table 1. The most serious source of difficulty was the large number of damaged cells, which must of course be taken into account in a quantitative study. The identity of these damaged cells is not altogether clear, and has been discussed elsewhere.[5] The M:E ratio is about 1.5:1. Thus in the present series of experiments the mean blood lymphocyte count was 4650 per cu. mm., as compared with a marrow lymphocyte count around 300,000 per cu. mm.

Discussion

A comparison of tables 1 and 2 indicate that while in individual experiments there are on occasion quite appreciable differences between the two sides, the differences between the mean values of the two sides are not significant, except in the case of the monocytes. It is doubtful, however, whether much importance should be attached to this difference in a small group of cells showing rather wide fluctuations in the different specimens.

The results seem to indicate that despite obvious failings, which have been discussed elsewhere,[5] the technic employed appears to give fairly consistent results in a series of sufficient length.

The large number of damaged cells is perhaps one of the most unsatisfactory features of the technic in its present state of development. But though the high incidence of damaged cells is associated particularly with smear preparations,

TABLE 2.—*Comparison of Right and Left Humeral Marrow in Guinea Pigs*
(All counts are in absolute numbers per cu.mm.)

	Mean	Standard deviation	Standard error of the mean
Right			
Erythroid	272,218	96,073	23,240
Myeloid	389,687	130,000	31,464
Lymphocytes	301,131	170,470	28,460
Monocytes	19,941	12,570	3049
Damaged	316,792	117,730	28,554
Total abs. count	1,402,217	240,500	58,330
M:E ratio	1.526	.657	1.59
Left			
Erythroid	273,162	74,250	19,158
Myeloid	386,802	132,000	34,082
Lymphocytes	326,000	101,600	26,232
Monocytes	30,952	20,000	5,164
Damaged	291,427	116,000	30,131
Total abs. count	1,383,810	266,000	66,500
M:E ratio	1.452	.475	.123

we have preferred these to supravital preparations since the stained smear appeared to afford finer cell detail for purposes of cell identification. In a previous paper[5] a comparison was made between stained smear and supravital preparations, and the results in the two groups were not too dissimilar, though the number of lymphocytes was somewhat smaller in the latter than in the former.

But by either technic the number of small lymphocytes in the marrow, when calculated in absolute numbers, is remarkably high—far too high to be attributed to contamination with blood. Thus in the present series of experiments the mean blood lymphocyte count was 4650 per cu. mm., as compared with a marrow lymphocyte count of around 300,000 per cu. mm.

Finally, it should be borne in mind that the figures in table 1 are in fact a little below the true level. For although the animal is exsanguinated by division of the abdominal aorta, a little residual blood is probably still present in the marrow vessels, and dilutes the marrow tissue somewhat. An idea of the degree of contamination of the marrow with residual blood may be obtained by counting the number of red cells per cu. mm. of marrow, and this in an earlier series was found to be around 400,000 per cu. mm., as compared with a red cell count of 5,000,000 to 6,000,000 per cu. mm. of blood.

SUMMARY

In seventeen young male guinea pigs a quantitative study was made of the nucleated cells of the bone marrow in the right and left humerus. Analysis of the data shows a close correspondence between the two sides in regard to the main cell groups.

SUMMARIO IN INTERLINGUA

A fin de probar le accuratessa del technicas nunc in uso in studios quantitative del nucleate cellulas intramedullari, contos separate de tal cellulas in le medulla

del humeros dextre e sinistre de 17 juvene porcos de India esseva executate e comparate statisticamente. Le resultatos obtenite revela le expectate proxime correspondentia inter le quantitates del major typos de cellulas nucleate in le medullas del duo lateres.

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