STUDIES IN COMPARATIVE HÆMATOLOGY.—I. CAMELIDÆ.

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APART from the tables of GULLIVER (1), which are concerned only with the diameter of dried red cells, the literature contains no systematic study of the blood of the vertebrates, particularly of the rarer species. The purpose of this series of studies is to remedy the deficiency to some extent, and to record some of the more important properties of the red cell and white cell population of the various vertebrate groups. Except where otherwise mentioned, the animals dealt with in these investigations are healthy animals under the ordinary conditions of captivity. It cannot be expected, of course, that the examination of the necessarily few specimens of each species will provide us with perfectly trustworthy information, for allowance has to be made for individual variation; we believe, however, that the data to be presented are both more representative and more to be relied upon than any at present existing.

The present paper is concerned with the blood of the Camelidæ (which includes the camels and the so-called "cameloids"). In all cases the blood was taken from a neck vein into oxalate (20 mg. to 10 c.c. of blood), without the animal being anæsthetised. The examination of the cells was commenced within about one hour from the time of withdrawing the blood.

1. RED AND WHITE CELL MORPHOLOGY.

Unless otherwise stated, all the following descriptions are based on blood-films prepared by the smear method and stained with Wright's blood-stain.

(i) *Camelus bactriens.*

(*a*) The polymorphonuclear neutrophil leucocytes (P.M.N.) are somewhat irregular in outline, tending, however, to be more round than oval in shape. The approximate mean diameter is 16 μ. The

typically polymorphous nucleus stains a light reddish purple. The cytoplasm is clear and stains a light blue colour; it is filled with evenly staining neutrophil granules of large size.

(*b*) The polymorphonuclear eosinophils (P.M.E.) are round or slightly oval in shape, and measure from 12 μ to 14 μ in diameter. The nucleus is similar to that of the neutrophil, but stains less intensely. The cytoplasm stains a very light blue, and is scarcely visible owing to the large numbers of large bright-red granules which it contains.

(*c*) The polymorphonuclear basiphils (P.M.B.) are circular in outline, and contain an irregularly and incompletely lobed nucleus which stains a reddish purple. The cytoplasm is faintly basiphil and thickly packed with coarse blue or purple granules. These cells are the smallest of the polymorphonuclear leucocytes, and measure about 8 μ in diameter.

(*d*) The lymphocytes (L.) are more spherical than any of the other blood elements, and range from 12 μ to 16 μ in diameter. The nucleus is large and slightly eccentric in position; it stains a deep blue. The thin rim of cytoplasm stains the typical sky-blue colour. Occasionally it contains a few azure granules.

(*e*) The large mononuclear leucocytes (L.M.) are irregularly circular in outline. The nucleus, which is large and deeply indented, stains a reddish purple. The basiphil cytoplasm contains many azure granules, clumped, as usual, in the indentation of the nucleus. These cells measure about 22 μ in diameter, and are very constant in size.

(*f*) The transitional leucocytes (T.) are large cells averaging about 30 μ in diameter. The nucleus is eccentrically placed, indented, and strongly basiphil, while the cytoplasm is distinctly oxyphil and filled with typical neutrophil granules.

(*g*) When seen in the fresh state in plasma, the red cells have the appearance of flattened ellipsoids. The outline of the cell is perfectly regular, and its interior is apparently devoid of structure. As might be expected from their shape, the cells do not form typical rouleaux; they remain in contact with one another, however, so as to form "chains," an end of one cell overlapping the end of the cell next to it, and so on.

The ellipsoid form is perfectly maintained when the cells are suspended in 0·85 per cent. NaCl, nothing corresponding to the spherical form of the human red cell under similar circumstances being observable (2). Crenation of a very coarse kind is seen in hypertonic saline, as also is swelling in hypotonic solutions.

In stained films the red cells appear smaller than in the fresh state, but retain their shape remarkably well. When the cells are fixed in methyl alcohol, the hæmoglobin is especially deposited in the central parts of the cell, which, as a result, take on a somewhat deeper stain than the peripheral areas. This appearance may possibly be responsible for

the erroneous statement which has sometimes been made that the cells are nucleated (3).

(ii) *Camelus dromedarius.*

With the exception of differences of size, the cells of this animal are very similar to those of *C. bactriens.*

(*a*) The neutrophils measure about 13 μ in diameter, and are fairly constant in size.

(*b*) The eosinophils are comparatively numerous. The coarse granules which fill the cytoplasm stain a deep pink rather than the characteristic bright red, and the cells show an irregular outline. In size they are about 11 μ.

(*c*) The basiphils measure about 10 μ in diameter. The coarse granules which fill the cytoplasm stain more intensely at the periphery than close to the nucleus; in the former situation they are blue-black, while in the latter they are deep purple.

(*d*) The lymphocytes, which measure about 8 μ in diameter, have a very thin cytoplasmic rim which stains the usual sky-blue. In some cases the deep-blue nucleus appears to fill the cell completely, and no cytoplasmic rim can be differentiated.

(*e*) The mononuclears measure about 13 μ in diameter. In appearance they are similar to the same type of cell of *C. bactriens*, except that light-blue staining granules are present in the cytoplasm instead of azure granules.

(*f*) The transitional leucocytes are easily recognised because of their large size, 18 μ. The eccentrically placed nucleus, however, is small in proportion, so that the cytoplasm appears abundant. The fine neutrophil granules are sparsely scattered throughout the cell.

(*g*) Except for a difference in size in the dried film, the red cells of *C. dromedarius* are identical with those of *C. bactriens.*

(iii) *Llama glama.*

(*a*) The polymorphonuclear neutrophils are about 10 μ to 12 μ in diameter. The cytoplasm is slightly oxyphil and is studded with many fine granules which are neutrophil in some cells and somewhat oxyphil in others. The nucleus shows the typical lobation.

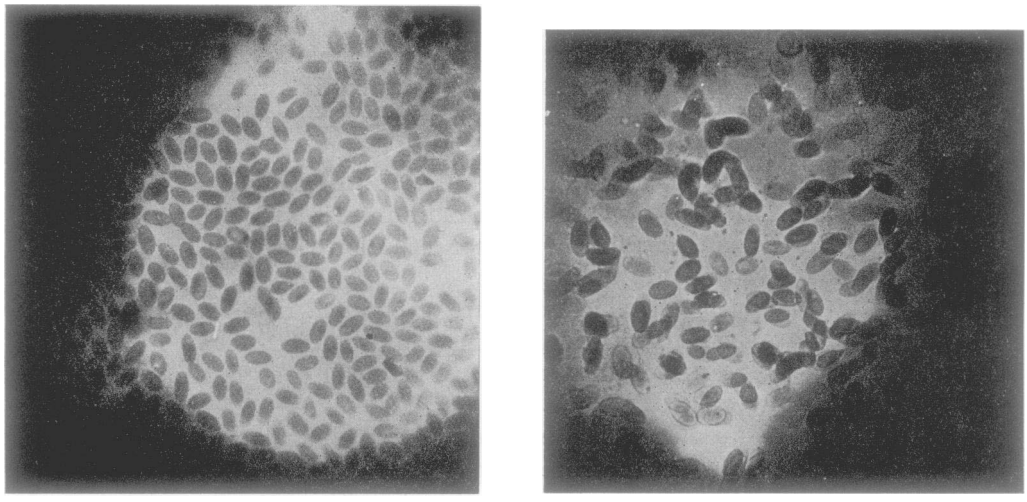
(*b*) The eosinophils are fairly constant in size and measure about 10 μ in diameter. The cell otherwise resembles that of *C. bactriens.*

(*c*) The basiphils are circular in outline and measure only about 8 μ in diameter. The nucleus, which occupies the greater part of the cell, is difficult to differentiate since it takes on a purple-blue colour only slightly less intense than that of the coarse granules which fill the cytoplasm.

(*d*) The lymphocytes are similar to those of *C. bactriens*.

(*e*) The mononuclears are variable in outline, some being perfect circles, while others are irregular ovals. The average size is 12 μ. The eccentrically placed nucleus stains a deep blue, and is slightly indented. The cytoplasm is basiphil, and always contains many azurophil granules.

(*f*) The transitional leucocytes vary from 10 μ to 13 μ in diameter. Except that the neutrophil granules are more concentrated in the notch



(*a*) (*b*)

FIG. 1.—Red cells of *L. glama* photographed (*a*) in the dried state, and (*b*) in plasma. Magnification 488. The illuminated area is the image of the ball of the pointolite. It will be observed that the cells are smaller in the dried state than they are in plasma. Some six or seven of the cells in (*b*) are suitable for measurement.

of the nucleus, this cell is identical in its staining properties with the corresponding cell of *C. bactriens*.

(*g*) Except for differences in size, the red cells of *L. glama* are very similar to those of *C. bactriens* and *C. dromedarius*. In a few cells there may be seen what appears to be a vesicle or vacuole, and in some cases this appearance may suggest a nucleus when the cells are examined in plasma.

(iv) *Llama pocas*.

The general morphology of the blood elements in *L. pocas* is very similar to that in *L. glama*. The following description will accordingly be limited to such differences as are thought worthy of note :—

(*a*) The neutrophils vary in size from 10 μ to 12 μ. The granules are regularly placed and somewhat coarse. Both the granules and the cytoplasm are poorly staining, and a large number of cells are decidedly chromaphobe.

(*b*) The eosinophils measure 8 μ to 10 μ in diameter. The granules are large and stain a brilliant red.

(*c*) The basiphils are very frequent in occurrence. They are small, measuring only 4 μ to 5 μ in diameter. It is impossible to differentiate the nucleus, which is concealed by the heavily staining basiphil granules of the cytoplasm.

(*d*) The lymphocytes measure from 6 μ to 8 μ in diameter. No azure granules are observable.

(*e*) The mononuclears measure about 10 μ.

(*f*) The transitional leucocytes range from 10 μ to 12 μ in size. The neutrophil granules of the cytoplasm, as well as the cytoplasm itself, stain excellently, this property distinguishing the cell from the Class I. polymorph.

(*g*) The red cells are similar to those of the other members of the camel family, and can be distinguished only by their size.

2. Red Cell Counts.

The red cell counts were made in the usual way, and in triplicate. The following are average results per cubic millimetre:—

Camelus bactriens . . .	10,450,000
,, *dromedarius* . .	10,800,000
Llama glama	11,300,000
,, *pocas*	19,400,000

In the young animal the red cell count does not differ significantly from that in the adult. It may be mentioned that the above figures approximate closely to those usually quoted.

3. Red Cell Sizes.

The methods used for studying the dimensions of the red cells have already been described in full (4). The optical system used in making the present measurements consists of a condenser working at N.A. 1·0 and an objective at N.A. 1·32; this combination, together with blue light from a pointolite, gives a limit of resolution of nearly 0·2 μ. The magnification employed, as found by photographing a micrometer and measuring the image with a scale, was 488. The scales and micrometer are calibrated in absolute units. The plates used were Wratten & Wainwright's process panchromatic plates, developed in elon as recommended by the makers. With the optical system described, an exposure of two seconds is sufficient.

The following table expresses the results of the measurements of cells (*a*) in plasma, and (*b*) in air-dried films. All figures are in μ ($\frac{1}{1000}$ mm.).

	Length.	σ.	Breadth.	σ.
C. bactriens, in plasma	8·1	0·62	4·5	0·41
,, dry	7·5	0·53	3·6	0·51
C. dromedarius, in plasma	8·0	0·53	4·6	0·33
,, dry	7·1	0·27	4·1	0·38
L. glama, in plasma	7·8	0·64	4·3	0·30
,, dry	7·2	0·7	3·9	0·29
L. pocas, in plasma	8·0	0·53	4·3	0·31
,, dry	7·6	0·67	4·1	0·33

It will be observed that the cells are smaller when measured in dried films than they are when measured in plasma, thus showing the typical shrinkage on drying. GULLIVER gives the following figures for the red cells of these animals: *C. bactriens*, length 8·1 μ, breadth 4·3 μ; *C. dromedarius*, length 7·8 μ, breadth 4·3 μ; *L. glama*, length 7·5 μ, breadth 4·0 μ; and *L. pocas*, length 7·5 μ, breadth 4·0 μ. These figures refer to cells in dried films, and agree quite closely with ours in the case of the two llamas; in the case of the two camels, however, we find the dried cells to be smaller than GULLIVER indicates. The difference may possibly be due to different conditions of drying. It should be noted, however, that the shrinkage on drying is not the same for the four species mentioned, although the films were prepared under the same conditions; in *C. dromedarius* the shrinkage amounts to 0·9 μ, while in *L. pocas* it is only 0·4 μ. We have already commented on the inconstancy in the shrinkage on drying of other mammalian red cells—a fact which makes it impossible to predict the true diameter of the cell from its diameter in the dried state (5).

One of the most interesting points in connexion with the cells of the camel family is the relation of their breadth to their length, for the correlation between these two dimensions is extremely imperfect. The following are the values for the coefficient of correlation:—

	Wet.	Dry.
C. bactriens	0·33	..
C. dromedarius	0·26	0·22
L. glama	0·38	0·27
L. pocas	0·23	0·29

These values are surprisingly low, and indicate that the length/breadth ratio is very far from constant; in fact, if we have information regarding the length of a cell, we can form no estimate of the breadth therefrom. This is of considerable interest in view of the

prevalent idea that the red cells of mammalia are subject to very little variation in shape. We have already shown that the correlation between the diameter and the thickness of the biconcave cells of mammalia is not particularly high (0·67), thus showing that the constancy of shape of these cells is less than is usually thought (5), and it is significant to find the same lack of constancy in shape in the cells of animals for which measurements can be much more accurately made.

The scatter of the red cell population, considered either with respect to length or to breadth, presents no points of interest. The values of σ are proportionately much the same as for the cells of man or for the cells of the rabbit, considered with respect to their diameters.

GULLIVER gives the figure 1·66 μ for the thickness of the cells of *C. bactriens* and *C. dromedarius*. Our measurements give 1·9 μ, but the accuracy with which the thickness can be measured is not very great. If this latter figure is accepted, the volume of the cell of *C. bactriens* works out at 36 μ^3 approximately, while its area is approximately 65 μ^2.

4. RESISTANCE TO HÆMOLYSINS.

The methods used for the measurement of the resistance of the cells of the four species to saponin, sodium taurocholate, and hypotonic saline have been fully considered in previous papers (6, 7). In the case of saponin and sodium taurocholate the method consists in plotting the time-dilution curves for the action of these lysins on the cells of man taken as an arbitrary standard, and on the cells whose resistance is required. The asymptotes of the two curves are found, and the concentrations of lysin corresponding to these asymptotes written down; the division of the one figure by the other supplies a resistance constant R, the magnitude of which gives the resistance of the type of cell examined in terms of that of the standard type. In the case of hypotonic saline, a special series of solutions of varying tonicity is prepared, the tonicity ranging from 0·24 per cent. NaCl to 0·68 per cent. by intervals of 0·02 per cent. To each one of these solutions is added a quantity of the suspension of cells whose resistance is to be determined. The resistance is measured by the greatest tonicity which will complete hæmolysis in sixty minutes at 25°.

The suspensions of red cells used are prepared by receiving 1 c.c. of the oxalated blood into 0·85 per cent. NaCl (saline), washing thrice, and suspending the washed cells in 20 c.c. of saline. The standard suspension of human cells is similarly prepared, except that the blood is citrated instead of oxalated. In determining the resistance to hypotonic saline, suspensions of half concentration are used; *i.e.* the thrice-washed cells of 0·5 c.c. of blood suspended in 20 c.c. of saline.

(i) *Saponin.*

The time-dilution curves, at 25°, for *C. bactriens, C. dromedarius, L. glama, and L. pocas*, are shown in fig. 2, together with the curve for

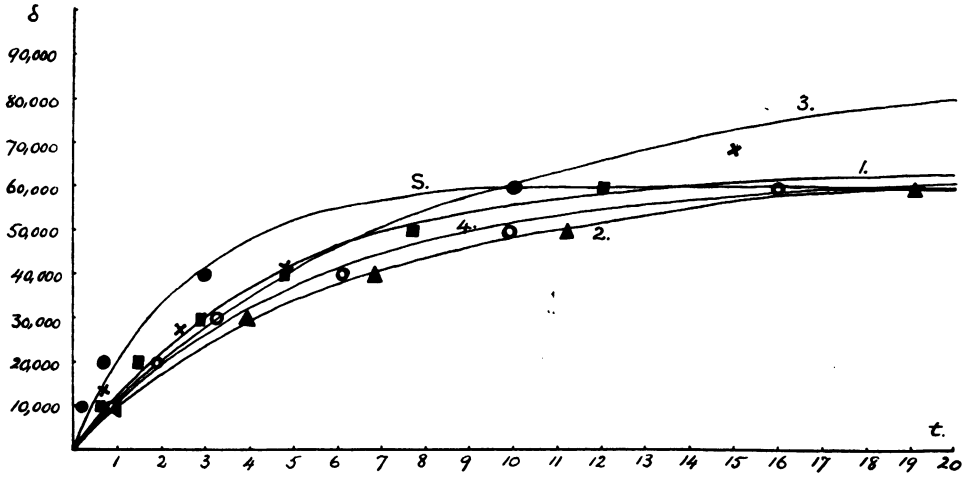


FIG. 2.—Time-dilution curves for saponin. Curve 1, *C. bactriens* (squares). Curve 2, *C. dromedarius* (triangles). Curve 3, *L. glama* (crosses). Curve 4, *L. pocas* (circles). Curve S, human cells as standard (dots).

the cells of man as a standard. In this figure the observations are shown as points, the continuous curves being calculated from the expression

$$t = \frac{1}{\kappa} \log \frac{c}{c-x},$$

where c is the initial concentration of lysin (in milligrammes), x the quantity of lysin used up in producing hæmolysis, t the time for complete lysis, and κ a constant. The curves are by no means easy to fit, but it will be seen that the experimental points lie on the calculated curves fairly well. The values of x and of κ are the following:—

	x.	κ.
C. bactriens . . .	0·031	0·213
C. dromedarius . .	0·033	0·166
L. glama . . .	0·024	0·137
L. pocas . . .	0·032	0·182
Man (standard) . .	0·032	0·370

This is the first occasion on which we have found a significant difference in the value of κ when comparing the saponin time-dilution curves for two mammals, although the occurrence is the rule when sodium taurocholate is used as a hæmolysin. As a result of the differ-

ence the usual relation $R = c_1/c_2$ does not hold, and we require to define the resistance constant as $R_\infty = c_{1\infty}/c_{2\infty}$, as in the case of time-dilution curves for systems containing sodium taurocholate as the lysin (7). Measuring the relative resistance in this way, we obtain the following values of R_∞ :—

	R_∞.
C. bactriens	0·96
C. dromedarius . . .	1·03
L. glama	0·75
L. pocas	1·00

The cells of the camels and cameloids accordingly have much the same resistance to saponin as have the cells of man, the erythrocytes of *L. glama* being the only ones whose resistance is significantly different. It may be mentioned that the surface presented by the suspension of camel cells is nearly the same as that presented by the suspension of the cells used as a standard.

The changes in shape which occur in these elliptical cells during saponin hæmolysis are in general similar to those which occur in the discoidal cells of other mammals (8). The cells remain unchanged until shortly before they hæmolyse; they then assume the form of spheres and almost immediately thereafter hæmolyse. The cell membranes remain as ghosts, but ultimately disintegrate into fragments.

(ii) *Sodium taurocholate.*

The time-dilution curves for the four species studied are shown in fig. 3, together with a curve for the cells of man as a standard. The

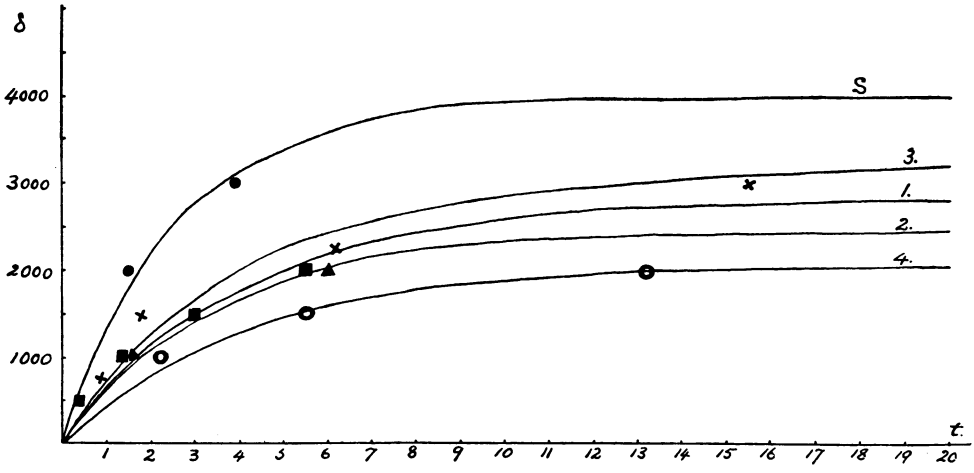


FIG. 3.—Time-dilution curves for sodium taurocholate. Numbered as in fig. 2.

continuous curves are calculated according to the same expression as in the case of saponin, the following values being used for the constants :—

	x.	κ.
C. bactriens . .	0·714	0·250
C. dromedarius . .	0·800	0·278
L. glama . . .	0·606	0·250
L. pocas . . .	0·952	0·227
Man (standard) . .	0·500	0·400

The value of κ is very constant for the camels, but differs from that for the human cells. Taking $c_{1\infty}/c_{2\infty}$ as a measure of $R\infty$, we have

	R_∞.
C. bactriens	1·42
C. dromedarius . . .	1·72
L. glama	1·20
L. pocas	1·90

The cells of the camel family are thus more resistant than the cells of man, and in most cases considerably more resistant.

In marked contrast to the changes which are seen during saponin hæmolysis, the cells of the species studied do not assume the spherical form before hæmolysing in sodium-taurocholate solutions, the elliptical form being retained both before and after the loss of the hæmoglobin. In the case of all other mammalian red cells which have been studied, the changes in form during hæmolysis by saponin and by sodium taurocholate are identical; the retention of the ellipsoidal form by the camel cell during taurocholate hæmolysis is therefore quite remarkable.

(iii) *Hypotonic saline.*

The resistance to hypotonic saline is expressed as the greatest tonicity (grammes per cent.) of NaCl which will produce complete hæmolysis of the added cells in one hour at 25°.

	NaCl per cent.
C. bactriens	0·26
C. dromedarius . . .	0·28
L. glama	0·28
L. pocas	0·28

The cells of these animals are thus much more resistant to hypotonic saline hæmolysis than are the cells of man (0·32 per cent.), and are accordingly more resistant than the cells of any other mammal which has been studied. Immersion in the hypotonic NaCl causes the cells to swell, the swelling being accompanied by quite a marked diminution in the long axis, much as in the case of the cells of man (9). This indicates that the camel red cell, like the erythrocyte of other mammalia, is a balloon-like body.

5. Hæmoglobin.

The hæmoglobin was estimated as carboxyhæmoglobin by Palmer's colorimetric method, with the blood of one of us (H. A. C.) as a standard (100 per cent.). We have found this method very satisfactory. The readings were made in triplicate, with the following average results:—

C. bactriens . . .	87 per cent.
C. dromedarius . . .	96 ,,
L. glama	89 ,,
L. pocas	106 ,,

We have found no significant difference between the young animal and the adult.

6. White Cell Counts.

(i) *The Total White Cell Count.*

The following figures represent the average total white cell count per cubic millimetre for the animals examined:—

C. bactriens	10,800
C. dromedarius	12,000
L. glama	10,300
L. pocas	12,100

(ii) *Differential Counts.*

In making the differential counts, the cells were classified according to the types described in the earlier part of this paper. The counts were based on the examination of 100 cells, stained with Wright's stain.

	P.M.N.	P.M.E.	P.M.B.	L.	L.M.	T.
C. bactriens . .	67	15	2	11	3	2
C. dromedarius . .	55	27	3	8	6	1
L. glama . . .	63	10	10	11	4	2
L. pocas . . .	51	5	37	4	2	1

The only interesting feature in connection with these differential counts is the high percentage of basiphils and eosinophils. In *C. dromedarius* the eosinophils are of very frequent occurrence, while in *L. pocas* basiphils are more frequent, and, so far as we have been able to find, these figures for eosinophils and basiphils respectively are the highest recorded for normal mammals.

(iii) *Polynuclear Counts.*

These counts were made in the manner described by COOKE (10), and on 100 cells. The stain employed was iron hæmatoxylin, but counts can be made on films stained with Wright's stain or Giemsa. The nuclear lobulation is distinct, and the counts are easy to make.

	I.	II.	III.	IV.	V.
C. bactriens .	23	36	34	6	1
C. dromedarius .	24	35	32	7	2
L. glama . .	14	29	40	13	4
L. pocas . .	22	31	37	8	2

The polynuclear count in these animals is thus very much the same as in man and the rabbit.

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