The Individuality of Nuclear Chromatin with Particular Reference to Polymorphonuclear Neutrophil Leukocytes

By Donald K. Briggs

When Faust makes his infernal bargain and sells his soul, Mephistopheles insists that he sign his name to the diabolical contract with a drop of his own blood. Every year more information is accumulated in the field of hematology to demonstrate the fiendish wisdom of this proviso and the inexorably binding nature of the agreement. Physical, chemical, serological and other methods demonstrate the intense individuality of human blood.

In the red cells the most widely studied aspect of individuality is the blood groups, whose multiplicity has already reached amazing proportions. Examining the staff of the Lister Institute, London, according to 9 different blood group systems, Race and Sanger demonstrated that in 132 subjects there were 129 different specific blood group combinations of which 126 occurred only once. Electrophoresis is also of great discriminatory value in differentiating individuals from the rest of the population in terms of congenitally determined abnormalities of hemoglobin. The shape of the red cells also provides material for geneticists, notably in the case of congenital ovalocytosis and congenital spherocytosis.

For some years our attention has been directed to another form of individuality of human blood, namely the specific patterns identifiable in the highly complicated morphology of the nuclei of polymorphonuclear leukocytes. The form of the nucleus, the protrusions from its surface and the appendages of chromatin are measurable and distinctive. They merit detailed study.

In the early days of descriptive hematology, much attention was paid to the form of the nucleus, but the older literature is largely speculative and lacks evidence drawn from careful objective observations. The best available studies in this domain are those which enumerate the lobes of the nucleus. The Schilling count and the Arneth count are numerical expressions of the degree of nuclear segmentation. It is known that in the bone marrow the degree of segmentation is less than in the peripheral blood, and it is widely believed that the degree of segmentation is an index of maturity. It is also known that segmentation is increased in certain diseases and decreased in acute infections as well as in certain families, notably those having the gene for the Pelger-Huet anomaly. It has been shown however by Turpin that the lobe count is a measurable aspect of individuality. Some individuals have an unusually high lobe count under physiologic conditions throughout life; others show a persistently low lobe count. Of great interest too is the entirely

From New York University–Bellevue Medical Center.
This work was aided by a grant from the United States Public Health Service.
Submitted Nov. 3, 1957; accepted for publication Apr. 25, 1958.
inexplicable finding, often reported though little known, abundantly confirmed in our laboratory, that in mongolism the lobe count is lower than normal.3,4

The lobe count is at best a crude representation of the prevailing form of leukocyte and takes no account of the remarkable intricacy of the chromatin arrangement and the rich variety of appendages and projections from the lobes. That these features are modified by physiologic and pathologic circumstances including acute infections and such metabolic disorders as megaloblastic anemias is well recognized, but it is probably true that a given subject retains certain specific nuclear appearances throughout life. Heredity is probably important.5-8 Whether these characteristics are as specific as a patient's facial appearance or his fingerprints, whether they are such as would divide subjects into categories in the same way as the ABO blood groups or whether they merely give quantitative information should be determined. The studies by Undritz7 of various animals and the different strains within each species demonstrate the wealth of individuality which may be expressed in the appearance of the leukocyte nuclei.

The Appendages of Nuclear Chromatin

Great interest in the appendages of the leukocyte nucleus was attracted by the now classical demonstration by Davidson and Smith8 of the sexual dimorphism of leukocytes. The essence of the distinction is the presence or absence of drumsticks (fig. 1). These are stalked, rounded appendages of chromatin, 1.5 microns in diameter, projecting from the nuclei of the leukocytes of female subjects only. This readily observed difference associated with so broad a distinction as male and female had escaped observation for a long time. This may have been because early studies were made with monocular microscopes without the aid of the mechanical stage or because microscopists became preoccupied with other more obviously promising matters. Not until 1949 was it known that the majority of somatic cells show morphologic sex differences. This was discovered first by Barr and Bertram9 in neurologic tissue in the cat and has been demonstrated in numerous human organs. This concept of the chromatin sex added a new dimension to investigative endocrinology and has led to a drastic reconsideration of much previously accepted endocrinologic theory.10,11

Work conducted in our laboratory in connection with the determination of chromatin sex from peripheral blood has necessitated the careful scrutiny of thousands of leukocytes in well-made smears from various normal and abnormal subjects.10 Careful note has been taken of the peculiarities of nuclear form and the curious shapes of the appendages. The observant investigator is left with the impression that no two blood smears are the same with regard to leukocyte morphology. Indeed, on occasions it has been possible to suggest the name of the patient who contributed an unknown blood smear from previous experience with leukocytes of that individual. Much study may be necessary before it can be stated how concisely the specificity of nuclear chromatin may be determined. This communication is concerned chiefly with the enumeration of drumsticks or sex chromatin masses. By counting the number of cells bearing the drumstick it has been possible not only to divide
patients into male and female but also to divide females into groups in terms of their drumstick counts. The purpose of this work then is not so much to define the statistical limits of normality as to demonstrate categorically that these differences exist between groups of women.

The Concept of Chromatin Sex

The "chromatin sex" must be established in certain patients in order to reach the correct endocrinologic diagnosis. Thus it has become an integral part of the laboratory investigation in numerous endocrinopathies and dysgenetic states. In clinical studies, biopsied skin, oral mucosa scrapings or blood smears may be examined. Since the nucleus in the normal female contains an extra piece of chromatin, the subject is said to be "chromatin positive." The normal male has cells which show very few of these extra pieces of chromatin and is designated as "chromatin negative." When biopsied skin or oral mucosa are used in chromatin sex diagnosis, differentiation depends on a quantitative method, for the chromatin-positive individual has more than 80 per cent of the cells showing the sex chromatin and the chromatin negative individual has less than 2 per cent. In the blood the difference is a qualitative one; the normal male or chromatin-negative individual has no drumsticks, whereas their presence in any number is enough to indicate a chromatin-positive individual.

Among the most interesting medical findings of recent years are the facts that certain individuals living as women have male or chromatin negative
cells and that others living as men have chromatin positive or female cells. It appears that sex may be established at the time of conception but that the physical sexual development and its corresponding secondary characteristics and psychologic orientation may develop according to the opposite sex during gestation as the consequence of some abnormal developmental influence. Knowledge of the chromatin sex is very valuable therefore in diagnosis in ovarian agenesis or Turner’s syndrome, in primary testicular agenesis or Klinefelter’s syndrome, in adrenogenital syndrome and in the syndrome of testicular feminization. Much recent literature has appeared on these and similar topics and we have shown how the chromatin sex may be used in the evaluation and management of cases of intersex and in determining the reproductive prognosis in infants of indeterminate sex. Figure 2 shows a woman whose somatic cells indicate that she is male; this is an example of Turner’s syndrome or ovarian agenesis where the phenotype is female though there are no functioning ovaries. Figure 3 shows the reverse of this, a patient with Klinefelter’s syndrome who has the male phenotype but has “chromatin positive” or female cells. (These two patients were supplied for us from Dr. Herbert S. Kupperman’s Endocrine Clinic.)
Fic. 3.—Klinefelter’s syndrome (primary testicular agenesis). Chromatin sex is female (chromatin positive). A. Before treatment. B. After treatment. (Patient supplied from Dr. Herbert S. Kupperman’s Endocrine Clinic.)

Technic of Chromatin Sex Determinations from Leukocytes

From our experience with the blood smear method of chromatin sex determination it may now be emphatically said that the drumstick is never seen in the chromatin male. (An exception to this rule has recently been described in the rare phenomenon of human chimerism where female cells become implanted in the male organism as the result of anastomoses between the placental blood vessels of binovular twins.) We had certain doubts at the beginning of our work after finding projections resembling drumsticks. We are now satisfied that the few discrepancies we encountered arose from artifacts visible in blood smears received from sources where we could not control the technic. We have learned to recognize a minimum blood smear quality and are able to recognize drumsticks with entire confidence, and, when we have done so, to state categorically that the patient is chromatin positive or female. Since it would be unwise to base so important a diagnosis on a nuclear projection of a single cell, it is our practice to identify at least six drumsticks before reaching a conclusion.

In their original report Davidson and Smith described how they had examined the neutrophils of 125 normal women and found 6 drumsticks before
they had finished the examination of 500 neutrophils in 122 cases. They have been misquoted as having said that the patient is not chromatin positive unless 6 drumsticks are found by the time 500 cells have been examined. It soon became obvious to us that this was not so, for we found a number of individuals who had far fewer than 6 drumsticks per 500 neutrophils. At first we were hesitant to diagnose female in such cases, but we would now proceed beyond the examination of 500 neutrophils in order to reach a conclusion.

The opposite diagnosis, chromatin negative or male, is more difficult. The male has no drumsticks on the neutrophils, but the important question is how far the examination must be pursued before it can be assumed that none will be found. We are now able to state that a number of females possess only 2 or 3 drumsticks per 1,000 neutrophils and here, naturally enough, an examination of 500 cells might lead fortuitously to a negative result. In our earliest work this caused one or two mistakes in diagnosis but, as a result of our preliminary studies, we have used modified criteria which have proved infallible when applied to all cases subsequently submitted to us. Using these modified criteria, no smear is said to be chromatin negative unless at least 500 cells have been examined, the lobe count found to be within normal limits and the cells themselves to be completely devoid of drumsticks and “sessile nodules” (fig. 4). (The sessile nodule is the projection described by Davidson and Smith as having the appearance of a drumstick but being devoid of any stem.) We do not make a diagnosis on the finding of sessile nodules alone, but their presence has frequently been valuable to us in prompting us to examine an additional series of 500 neutrophils. In our experience whenever sessile nodules are present drumsticks are present too. The method of diagnosis of chromatin sex just described is entirely reliable as a means of giving consistent and reproducible conclusions.

Frequency of Drumsticks

During our original investigation of chromatin sex in normal and abnormal individuals, it was found that the number of drumsticks possessed by different female subjects was widely variable. It seemed evident, however, that when a particular female was re-examined on a number of occasions the frequency of the drumsticks would vary only slightly. That is to say that a woman kept her high, medium or low drumstick count which remained fairly constant between serial examinations. This aroused some curiosity. Whenever a measurable quantity is discovered, it is reasonable to seek the factors controlling its fluctuations and to investigate its variations in physiologic and pathologic circumstances. From our first studies of patients attending the endocrine clinic we derived a strong suggestion that the high drumstick count and the low drumstick count were associated with congenitally determined disorders of the endocrine system. We were not able to reach any conclusions with confidence since the blood smears lacked uniformity of technic and, although they were adequate for the diagnosis of chromatin sex, did not lend themselves to the accurate enumeration of drumsticks. Furthermore, since the majority of the cases examined were patients with endocrinopathies, it was unjustifiable to place reliance on comparisons between the drumstick count...
and the state of the endocrine system. We lacked adequate knowledge of the normal drumstick count in the normal woman. It therefore became necessary to examine a larger number of normal women to see how the counts varied between individuals and in addition to examine a number of women at various times over a period of months to see whether the individual's count underwent wide fluctuations.

METHOD OF STUDY

It is necessary to employ a completely standardized method of making blood smears. We use slides rather than coverslips since we feel that with coverslips the distribution of the cells is less uniform and the degree to which they are spread out varies in different parts of the smear. Slides give a more even distribution, permit better reproduction of technic from day to day and are easily labeled in ink in the blood.

When the smear has been taken from the patient, it is assigned an arbitrary serial number and stored for subsequent examination. At the time of examination this serial number is covered with a working number on adhesive tape to reduce still further the possibility of the examiner's having any idea of the source of the specimen. After his examination he makes a report in writing which is later correlated with the clinical information. In this study a total of 500 neutrophil leukocytes from each case was examined and the number of drumsticks counted. The observer also commented on other features of the nuclear chromatin pattern.

CLINICAL MATERIAL

In order to derive some idea of the normal range of the drumstick count, careful objective studies were made on 100 student nurses. They were chosen since they were readily available and had passed an exacting physical examination before admission to
training. Their ages fell within a fairly close range, the majority being between 18 and 30. They comprised a group consecutively attending for routine physical examination. The slides were made at the same time as blood was taken for hemoglobin measurement so that they were in no sense volunteers at the first examination; none of them knew she was being examined and none was excluded for any reason. All the smears were taken in the early afternoon within a period of one month and examined during the ensuing months.

Reproducibility of Results

There are a number of difficulties inherent in any method which attempts to enumerate the number of drumsticks per 500 leukocytes. It has been said that the drumstick is found on the nucleus of the mature leukocyte, but since it is difficult to decide at what point a leukocyte reaches maturity it has been our policy to count all neutrophils including stab forms and juveniles. This produces an unusually low drumstick count in comparison with what would have been obtained if the less mature forms had not been included. Another difficulty arises from uncertainties in recognizing precisely what is a drumstick. The classical drumstick with its rounded head and narrow stem is never the source of error, but an appendage may be disguised when it lies close to the main body of the nucleus, has a short neck or is otherwise deformed. Because of this, two observers may disagree as to whether or not a particular structure is a drumstick, or the same observer might call it a drumstick on one occasion and not on another. Nevertheless the degree of reproducibility of counts made by different observers on the same blood smear or by the same observer on different occasions is surprising. With experience the observer develops his own standards for what is a drumstick and what is not.

Results

Figure 5 shows the distribution of the drumstick counts at the time of the first examination of a smear from each woman and shows the extent to which they differed from one another. The lowest counts amounted to 1 drumstick per 500 cells and the highest, 29 drumsticks per 500 cells. In order to determine how much this variation was due to the intrinsic error of the counting method and how much to individual conditions prevailing in each woman at the time of the examination, the counts were repeated for the five women showing the fewest drumsticks and the five showing the greatest number. Figure 6 shows the distribution of drumstick counts of these two groups when the identical slides used for compilation of figure 5 were re-examined.

To gain some idea of how far technic influenced the results, further counts were made on duplicate smears which had been taken at the same time as those used in the first examination. The results of these new counts are recorded in figure 7. Thus the only difference between figures 5 and 6 and figure 7 is that a different, though simultaneous, specimen was used in the case of each woman.

In order to determine how much the drumstick counts were dependent upon factors operative at a particular time, all 10 women were recalled 6 months after the first examination, further smears were made and these were examined by the same objective technic. Figure 8 shows the distribution of these new counts.
Figs. 5–8. (top to bottom)—Distribution of drumstick counts in 100 normal female subjects. Figure 5, when first examined. Figure 6, when the same smears were re-examined from women falling at the extremes of figure 5. Figure 7, when duplicate smears were examined from the same subjects. Figure 8, examinations from new samples taken 6 months later.

Clinical Associations

When the work had reached the stage depicted in the four figures, an attempt was made to determine what it was that distinguished the women who fell into the low drumstick count category from those in the high. The women were interviewed and their medical histories reviewed. They were assessed from various standpoints such as age, racial background, physical development, etc., but our ingenuity could find no single feature which placed the
women of the two groups in the categories into which they were relegated by their drumstick counts.

**Relationship of Drumstick Count to Lobe Count**

The first studies by Davidson and Smith and our own evaluation of pathologic material gave some indication that reduction in the number of drumsticks was associated with a shift to the left in the lobe count, that is to say a reduction in the degree of segmentation of the leukocytes. In our laboratory, lobe counts, though sufficiently reproducible to be satisfactory to us, are low when compared with those quoted by other workers. This is because we do not accept a cell as having two lobes unless they are distinctly separate except for a small strand joining them; if a cell gives the appearance of having two lobes overlapping one another these are nevertheless counted as one. Figure 9 gives an over-all picture of the relationship of the lobe count to the drumstick count in the entire group of 100 subjects. This figure fails to show any clear relationship between the lobe count and the drumstick count.

**Validity of Grouping**

Once these two groups had been separated from the rest of the subjects, an attempt was made to see how far the drumstick count was of value in distinguishing between them. This was merely an attempt to try out the accuracy of the numerical differentiation shown in the various tables. The observers were given slides under the usual objective conditions and asked to say whether the donor of the particular specimen could or could not belong to the high drumstick count or the low drumstick count group. The correct answer

Fig. 9.—Relationship of lobe counts to drumstick counts. (There is no evidence of direct relationship between the two.)
was given in each case; thus we were able to provide negative information of the kind which is used in elimination tests for paternity by means of blood group determinations.

**DISCUSSION**

The studies here described represent a preliminary investigation of one of the easily measured aspects of the individuality of the nuclear chromatin arrangement of the leukocyte. The method of study is fallible in many respects including such obvious omissions as collateral studies of total leukocyte count in the subjects examined. More penetrant statistical analysis is necessary at every stage of the work and studies of this type are presently proceeding in our laboratory.

Nevertheless it is clear that within certain limits the enumeration of drumsticks provides a method of differentiating women from one another. All the counts included in the results here reported have been made by two observers. We find that there is not enough difference between their respective results to make an appreciable difference to the tentative conclusions which we have drawn.

Comparison of figures 5 and 6 show the extent to which the method of measurement is reproducible. Though the women change their position in the figure by reason of the intrinsic error of the counting method, there is no overlap between the members of the two groups at the extremes of figure 5. It appears to us theoretically possible that, by enumerating a sufficiently large number of cells, any one member of the group of 100 eventually may be distinguished from any other. On the basis of a count done on only 500 neutrophils it is possible to delineate these particular two groups.

As shown in figure 7 the counts repeated on duplicate smears made at the same time as the first do not alter the relationships of the two groups to one another. The fact that there is no more obvious difference between figure 7 and figure 5 than between figure 6 and figure 5 tends to discount any suggestion that differences in the two groups of women are strongly influenced by smearing technic.

The results shown in figure 8 depict a reappraisal of the drumstick counts made from the two selected groups six months later. This shows that the women still fall into the categories of high drumstick counts and low drumstick counts in much the same way as they did at the original examination.

Serial examinations have been made on a number of other subjects at intervals for over one year to survey the range of counts and any possible changes from month to month. These subjects were chosen from members of the staff who were most readily available. They were volunteers in the sense that they agreed to undergo repeated blood examinations and were not selected with the impartiality exercised in the choosing of the 100 student nurses. Some of the findings have been reported and a statistically controlled analysis of the results of prolonged studies will be presented subsequently. Our extended experience has supported the conclusions which we draw from the present investigation. The women whom we have studied from time to time have mostly given drumstick counts falling well within the range of figure 5. One
INDIVIDUALITY OF NUCLEAR CHROMATIN

exception is a normal woman whose blood has been examined repeatedly and
who persistently has a count ranging between 40 and 60 drumsticks per 500 neutrophils.

The Meaning of the Drumstick Count

So far our studies of the drumstick count indicate no definite correlation
between it and any other clinically measurable aspect of individuality. We
were able to find no other single factor which was consistently different be-
tween the two groups extracted from the extremes of figure 5. The relationship
of the drumstick count to the lobe count is certainly not obvious and is not
substantiated by the present study. If any relationship between these two fea-
tures exists then it is complicated by other co-existing factors.

It is reasonable to suppose that the drumstick count, like a person's height
or weight, is the product of many interrelated factors. If so, then the distribu-
tion of drumstick counts would be represented by a bell-shaped curve of the
type to which figure 5 approximates. If the two groups of women which we
chose from the extremes of figure 5 differ qualitatively from one another, then
this distinction is as obscure to us as any clinical distinction which may exist
between women of blood group A and those of group B. None has yet been
found. In the case of blood groups, hereditary factors are of the utmost impor-
tance so that we consider it worthwhile to evaluate the hereditary aspects of
the drumstick count. We are now studying the drumstick counts of the mothers
and sisters of a number of women who have persistently high and low drum-
stick counts. If these studies give inconclusive results it may be necessary to
resort to animal experiments.

Current Applicability of the Drumstick Count

The data derived from this investigation together with material subsequently
accumulated will provide the foundation for the application of leukocyte studies
to the determination of chromatin sex in endocrinologic problems and will
increase the accuracy and reliability of such studies. The examination and re-
examination of the blood of the 100 cases here presented were performed under
objective conditions together with material from the endocrine clinic and other
sources including a number of normal males. There were no discrepancies with
regard to chromatin sex diagnosis. A slide from the woman with the lowest
drumstick count was examined and 500 cells counted without a single drum-
stick being found, but a sessile nodule, resembling a submerged drumstick,
was reported, thus prompting a further examination and subsequent finding
of drumsticks.

Another application of the drumstick count is the one already quoted
wherein the detection of drumsticks in a normal male was taken to indicate
the survival of cells which had been grafted into the bone marrow during
intra-uterine life by migrant precursors received from a female binovular
twin. In view of the wide variability of drumstick counts in normal women
and since we do not know why some cells of the female bear drumsticks and
some do not, it is at present unjustified to attempt to draw any conclusion
from the drumstick count with reference to the extent to which the leukocytes
of the male twin were originally derived from the female twin.
Much experimental work is now being directed toward the possibility of grafting bone marrow from one subject to another.\textsuperscript{16,17} It is envisaged that human transplants of this type may be effective in the treatment of leukemia in the way that has been successfully undertaken in experimental animals. If this becomes possible, the drumsticks along with other distinguishing features of leukocyte morphology will serve as valuable indicators of the extent to which the leukocytes of the surviving recipient are derived from the donor rather than originating in his own marrow.

\textit{Speculation}

The results of this study give rise to certain speculations with regard to the physiologic development of human leukocytes. There is a profound lack of reliable information regarding the pathways by which the cellular elements develop. According to the monophyletic school, the various formed elements are derived from totipotent precursors which can develop along a number of divergent pathways. The polyphyletic theory holds that the lymphocytes, eosinophils, monocytes, basophils and neutrophils are completely separate series with no developmental interrelationships and that the cells of each group are derived from separate, distinguishable precursors.

The results of the studies here presented suggest that even the polyphyletic theory understates the facts. They indicate that the neutrophils themselves do not constitute a homogenous group but rather that there are several parallel series of development to be found within the neutrophil population. Each line of neutrophil is morphologically distinguishable. The instances of human chimerism cited above show how the type of leukocyte which bears the drumstick may be transplanted to an individual who does not normally possess it. Other lines of leukocyte are distinguished by "small clubs" or the various appendages which have not yet been classified. The enumeration of drumsticks is only a part of the subject, for we are devising a method for performing a differential white count within the neutrophil series which classifies every morphologic type of neutrophil, including as one type those bearing the drumstick.

Since the relationship between chromatin sex and the true genetic sex endowment of the individual is by no means clear, the significance of the individual differences in the drumstick count are likewise obscure. It is possible that many of the leukocytes contain sex chromatin which forms part of the main body of the nucleus and is not borne on a narrow stem. The condition in which the chromatin mass becomes rounded and projects from the nucleus as a drumstick could be a trivial morphologic peculiarity, but may, on the other hand, be a consideration of major clinical importance. We considered it possible that the question of whether the drumstick projects or not might depend upon changes in the physiologic climate, but since we have been able to demonstrate none, we are more inclined to believe that the frequency of drumsticks is the consequence of some underlying biologic arrangement which persists throughout the life of the individual.

The findings of this investigation lay the basis for a critical inquiry into the whole subject of human individuality as expressed by nuclear morphology.
INDIVIDUALITY OF NUCLEAR CHROMATIN

The appendages of the nucleus which have received only scant attention in the past provide a means of anthropologic mensuration.

The intricate form of the neutrophil leukocyte should be studied in greater detail both in normal subjects and in patients with neoplastic diseases and leukemias, especially since the latter conditions probably arise from disturbances within cell nuclei.

CONCLUSIONS

1. Blood from a group of 100 normal women has been examined with regard to the frequency of the sex chromatin in the neutrophil leukocytes.
2. The application of this analysis to studies of chromatin sex and the value of chromatin sex in diagnosis are discussed.
3. The results define one of the many aspects of the individuality of nuclear chromatin arrangement. Others remain to be explored.
4. The value of the data obtained in this study is assessed.
5. No association has been discovered between frequency of sex chromatin and any other clinical factor.
6. Certain aspects of the physiology of the human leukocyte and its development are discussed in the light of the present findings.

SUMMARIO IN INTERLINGUA

1. Sanguine ab un gruppo de 100 feminas normal esseva examinate con respecto al frequentia (id chromatina sexual in le leucocytos neutrophilic.
2. Es discutite le application de iste analyse a studios die sexo secundo chromatina e le valor de sexo secundo chromatina in le diagnostica.
3. Le resultatos defini un del numerose aspectos del individualitate in le disposition de chromatina nucleari. Alteres remane a explorar.
4. Es evalutate le importantia del datos obtenite in le presente studio.
5. Esseva discoperite nulle association inter le frequentia de chromatina sexual e non importa qual altere factor clinic.
6. Certe aspectos del physiologia del leucocyto human e de su disveloppamento es discutite in le lumine del presente constatationes.

REFERENCES

The Individuality of Nuclear Chromatin with Particular Reference to Polymorphonuclear Neutrophil Leukocytes

DONALD K. BRIGGS