NUCLEAR APPENDAGES IN NEUTROPHIL POLYMORPHS

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The description by DAVIDSON and SMITH (1954) of sex differences in the nuclear morphology of neutrophil polymorphonuclear leucocytes related to a specific appendage (the "Drumstick") of the nuclei has led to further detailed study of these cells by other workers(3), (4), and (5). Many types of appendages have consequently been observed, described and classified.

There are four main types of nuclear appendages termed A,B,C and D. Of particular interest is the "Drumstick" appendage, originally described by DAVIDSON and SMITH (2), found only in the neutrophil polymorphs of female subjects, and utilized by these workers for sex diagnosis. The Drumstick is now classified as the type A appendage. The appendage is a tear-drop of nuclear material remarkably constant in size, uniformly and deeply staining, and connected to a lobe of the nucleus by a fine chromatin thread of varying length. It is the only one of its kind in any particular cell (fig 1). This was the only nuclear appendage described by DAVIDSON and SMITH in England(2), and two years later by TENECZAR and STREITMATTER in America(5). The later workers also described a similar appendage in the eosinophil leucocyte.

The type B or "Sessile" nuclear appendage has all the characteristics of type A except that no chromatin thread intervenes between it and the main nuclear mass (Fig 1). It has been shown to be of equal significance in sex diagnosis as the type A appendage, being found only in females(3). It is probably an early stage in the formation of a Drumstick. The type B appendages are to be differentiated from nuclear tags and minor lobes with which they may be easily confused.

To type C, belong a large group of appendages of different forms. They are not sex specific, and include the "Intermediate" appendage which resembles the Drumstick except in size, being just less than half the size (Fig 1). Although found in both sexes, it is relatively more common in the male. Other sub-type C appendages include the "Hooks" and "rods" (of varying length and thickness) whose form are well depicted by their nomenclature (Fig 1).

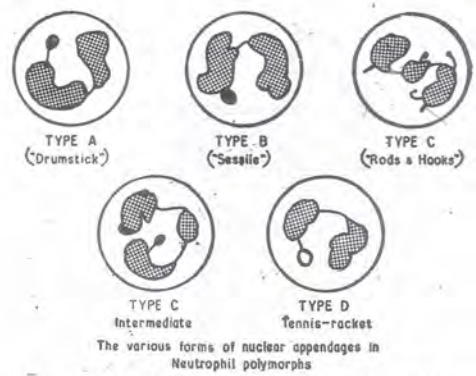
A relatively rare appendage is the "Tennis-racket" or type D (Fig I). It has been found in both sexes, and is formed by a ring of nuclear material surrounding a clear colourless zone and joined to the main nucleus by a chromatic thread.

In this paper is published the results of nuclear appendage counts in neutrophil polymorphs of the peripheral blood of 105 *female* Nigerian subjects. Their ages ranged from 3 months to 50 years. (Age was not recorded in the first twenty cases).

MATERIAL AND METHODS

Blood films were made from needle- prick specimen of female subjects attending U.C.H. General Out-Patient Department, and the U.C.I. Dispensary. Specimen were also taken from a few patients with established diagnosis in the wards. The blood films were of such quality as to allow the examination of individual blood cells and at the same time contain well over 200 neurophil polymorphs suitable for scrutiny. The dry films were immediately fixed in methyl alcohol for 3 to 5 minutes. The blood films were

Figure 1.



The various forms of nuclear appendages in Neutrophil polymorphs

stained by a modification of the Haematoxylin and Eosin (H and E) staining.

Technique – The fixed films were stained for 3 minutes in Ehrlich's haematoxylin, blued in running tap water for about 1 minute and then stained upright in a freshly made aqueous solution of Eosin and Azure II for 20 minutes. They were then flushed with tap water, allowed to dry in air at room temperature and examined under a light microscope with a ½ inch oil immersion lens.

Eosin-Azure II solution – 7 ml 0.1% aqueous Eosin
5 ml 0.1% aqueous Azure II
50 ml distilled water

200 neutrophil leucocytes were counted in each film, and the number and type of nuclear appendage encountered were recorded. In 19 blood-films (a regrettably small number), the nuclear appendage count was combined with the Arneth-Cooke count (6) the aim being to find relationship, if any, between the percentage incidence of type A and B appendages and maturity of the neutrophil polymorphs.

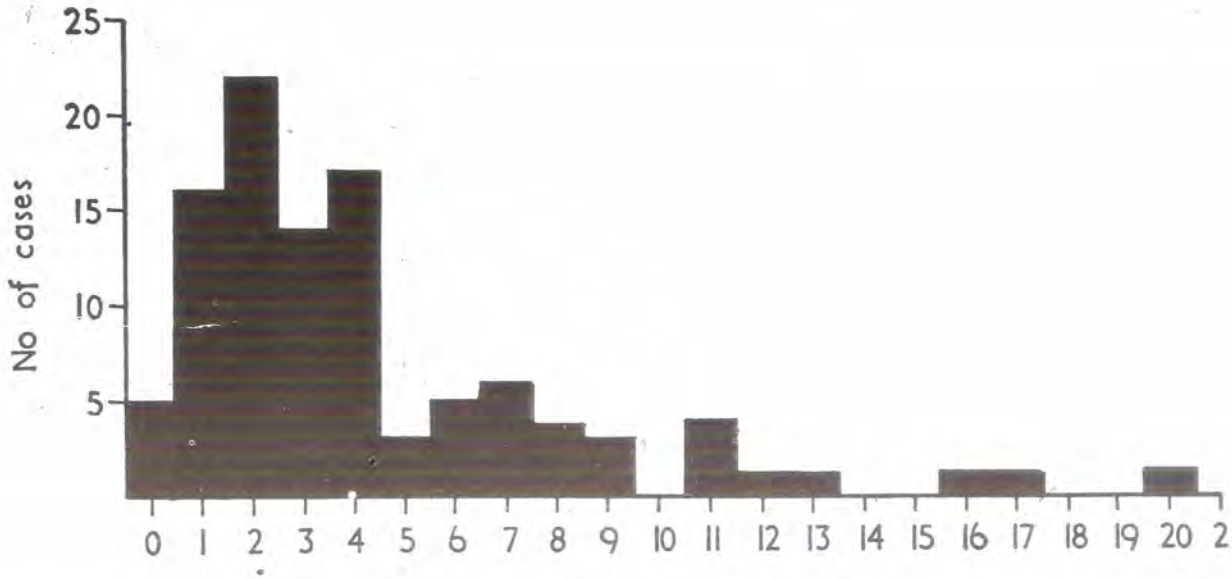
FINDINGS

The incidence of type A (Drumstick) appendage ranged from 0 to 20 per 200 cells counted (fig 2). The incidence of type B (Sessile) appendage ranged from 1 to 9 in a count of 200 neutrophils. (fig 3). The type (A & B) incidence ranged from 2 to 25 per 200 cell counted (fig 4). The incidence of cells containing only type C appendages ranged from 0 to 75 per 200 cells counted. The tennis-racket (type D) appendage was seen in only 3 films. These were from a woman of 50 with infective hepatitis, a girl of 9 with multiple tuberculosis (pulmonary, spine and knee) and a woman of about 40 with a clinical and haemotological diagnosis of multiple myelomatosis. Occassional eosinophils were encountered with the characteristic drumstick appendage; one basophil with a Drumstick nuclear appendage was an interesting finding.

Counts from 8 mothers and their respective daughters, and from one pair of siblings were compared but there was no apparent relationship between the percentage incidence of the various appendages in both individuals.

The relationship of the frequency of type A and B appendages to (a) the average maturity of the neutrophils as

Figure 2



No of type A appnedages counted per 200 neutrophils

manifested by the number of nuclear lobulations (Arneth-Cooke Count) and (b) the age of the individual, is shown in Tables 1 and 2, and fig 5. "Adults" were all over 20 years. There was only one girl in the 16-20 age group and she was therefore disregarded. A preliminary study was made on the blood film from a white mouse. The nuclei of the neutrophil leucocytes in this animal also possess appendages. These were however all of the same shape (Sessile tear-drops) though of different sizes. 5 distinguishable types were observed

DISCUSSION

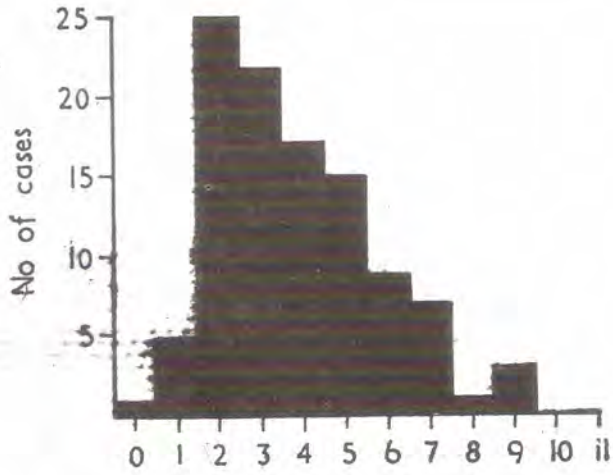
Table 1 shows clearly that the frequency of type A appendage rises with the average maturity of the neutrophils indicated by the Arneth-Cooke Count; A frequency of 1 Drumstick per 35 neutrophils in specimens with a shift to right contrasting sharply with the low average frequency of 1 Drumstick per 123 neurophils in specimens with a shift to left. Also in shifts to left, type B appendages are more frequently seen than type A but if anything, there is little difference in their incidences in shift to the right. Hence it would appear that one of the major factors influencing the type A and B incidence is the average maturity of the neutrophil polymorphs in the peripheral blood. This may be explained by postulating the extrusion of the type B and A appendages as part of the maturation (or perhaps ageing) process in a neutrophil, indicated ordinarily by lobulation of the nucleus. The higher incidence of the type B (Sessile) appendage in counts showing a left shift in the Arneth-Cooke count seem to confirm the assertion by KOSENOW (3) that this appendage is the female heterochromatin in an early stage of "Drumstick" (type A) formulation.

Of more signifcance is the relationship found in this work between appendage incidence and age. Out of the 51 "adults" counted, 28 (55%) were within the 2-5 range in the number of (A & B) appendages counted per 200 neutrophils (fig 5). In contrast, out of the 35 "under 15" counted, 21 (60%) were within the 6-15 range (fig 5). A glance at Table 2 shows that a higher frequency of Type A appendage occurs in the under 15 age-group (1 in 36) when compared with the frequency of 1 in 63 in adults. This last figure incidentally compares favourably with TENECZAR and STREITMATTER'S figure of 1 in 61 in adults. It is therefore most tempting to conclude that the number of neutrophils manifesting the Type A appendage in the peripheral blood of a female is influenced by the age of the individual. Futher investigation on this aspect is being carried out.

The nature and origin of the nuclear appendages are still a matter of conjecture. Somatic cells in the intermitotic phase show condensationss of chromatin materials in the nucleus, manifested histologically by their intense staining with nuclear dyes (e.g. haematoxylin). These are referred to as heterochromatins and according to DARLINGTON (1) each represents a relatively genetically inactive focus on specific chromosomes which in the intermitotic nucleus show an abnormal retention of nucleic acids.

In 1949, BARR and BETRAM noted and reported

Figure 3.



No of type B appendages counted per 200 neutrophils

Table I

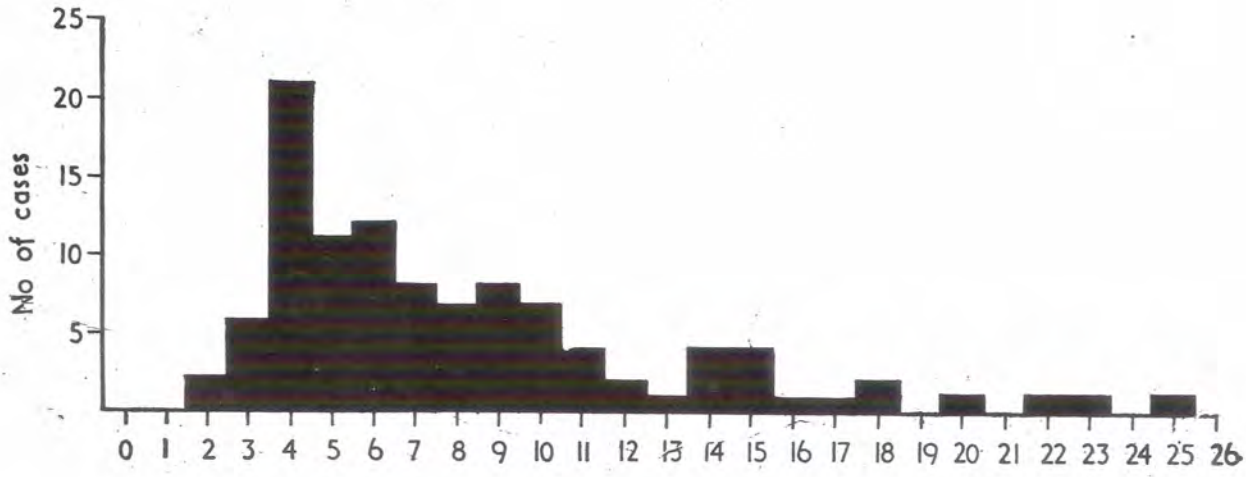
		Arneth-Cooke Count			
		Left Shift	Normal	Right Shift	Total
Number of cases combined with Arneth-Cooke count	Adults	4	7	3	14
	Under 15 yrs	4	-	1	5
	Total	8	7	4	19
Frequency of Appendage	Tyep A (Drumstick)	1 in 123	1 in 63	1 in 35	
	Type B - (Sessile)	1 in 66	1 in 116	1 in 50	

Table II

	Number of Neutrophil Counted	No of type A 9Drumstick) Appendages encountered	Frequency of Drumstolicks
All cases *	21,000	436	1 in 48
Under 15 yrs	6,800	191	1 in 36
Adults	10,200	164	1 in 63

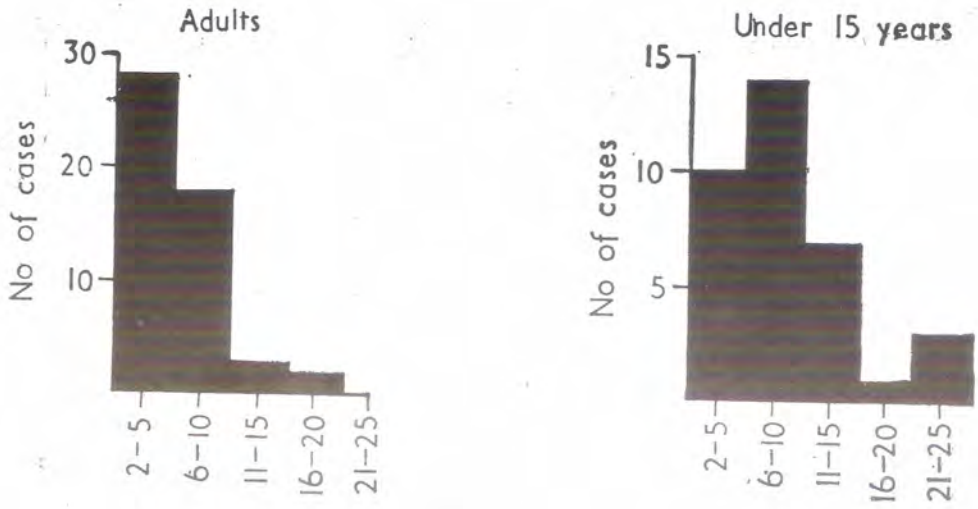
* Age not recorded in the first 20 cases

Figure 4.



No of type (A+B) appnedages counted per 200 neutrophils

Figure 5.



No of type (A+B) appnedages counted per 200 neutrophils Type (A+B) incidence and age

morphological differences in the nuclei of nerve cells in the male and female cats (7). In particular, they noticed the presence of a small spherical mass of deeply staining chromatin condensation, always in close proximity to the nuclear membrane and found only in female cats. They called it the "sex-chromatin" and further investigations have shown that there is present in the nuclei from different tissues of the cat and other mammals (including man), a chromatin peculiar to the female sex. It has been related to the heterochromatin of the female sex chromosomes. Some nuclei of the neutrophil polymorphs studied showed peripheral condensation of chromatin, the size and intense staining of which strongly suggest them to be primary foci of chromatin aggregation which, to my mind, must of necessity precede the subsequent formation. These foci may be analogous to the perinuclear sex-chromatin characteristic of squamous epithelial cells from females, and are probably a stage immeditaely preceeding the formation of the Type B appendage. Up to date, the Type C and D appendages have not been related to any phenotypic character in man.

The nuclear appendage count in neutrophil polymorphs provides an easy and fairly reliable method in the diagnosis of the chromosomal sex of an individual. It has proved of value in diagnosing the genetic sex in cases of pseudohermaphroditism, gonadal dysgenesis syndrome, hypogonadism, gross gynaecomastia and may in future be of some use in medico-legal practices.

SUMMARY

The result of Neutrophil nuclear appendage counts in 105 female Nigerian subjects are publsihed.

The incidence of Type A (Drumsticks) and B (Sessile) appendages are related to the average maturity of the peripheral blood neutrophils and to the age of the individual.

Speculations are made on the origin, nature and significance of the nuclear appendage.

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Editor's Note

B. O. Osunkoya's article was first published in the 1st Edition of DOKITA in 1960. The author went on to become a Professor of Chemical Pathology, College of Medicine, University of Ibadan.