

THE PERCENTAGE EVALUATION OF 'DRUMSTICKS' IN THE LEUCOCYTES OF PERIPHERAL BLOOD IN CLINICALLY NORMAL MALES AT DIFFERENT AGE GROUPS 0-60 YEARS

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INTRODUCTION

UNTIL the beginning of this century, it was necessary to assess the chromosomal complement at conception in order to determine the sex of an undifferentiated foetus. Barr and Bertram in 1949, discovered nuclear sexing in the neurones of the cat—a prominent chromatin mass called the Barr body present in the female sex. Now, the sex chromatin could be demonstrated with ease in children and adults by examining stained scrapings of buccal mucosa, a drop of peripheral blood or any other easily accessible cells of tissues like in skin biopsy. The Barr body or the sex chromatin is found only in the female but not in the male carnivora, primates and man; only man, monkey and the cat have tissues generally sexable—vertebrates.

Davidson and Robertson Smith of King's College Hospital showed in 1954 that it was possible to sex an ordinary blood film. In women, more than 1% of polymorph nuclei bear "drumstick"—a small extra lobe $2-3\mu$ in diameter, solid, well rounded and with a thin single filamentous stalk. In general, the drumsticks could be equated with the sex-chromatin of Barr bodies in the neurones. It is stated that in the great majority of cases, the results of sexing by blood film can be accepted without question and the very few anomalies which appear are well worth study for their own sake. It is believed that (a) No normal fertile female has ever shown to be drumstick negative; and (b) No normal male could be chromatin positive; (c) This is not influenced by age, endocrine status or disease or any other genetic factor; and (d) Whenever the nuclear sexing is at variance with the apparent sex, the persons present defective sexual development or sex anomalies as in Klinefelter's or Turner's syndrome.

It is assured that the sex chromatin is the actual heterochromatin of the two X chromosomes. In an individual (or tissue) when it is visible,

the nuclei of that tissue should contain at least two X chromosomes no assumption can be made about the presence or absence of the Y chromosome.

This procedure of nuclear sexing has found applications in the study of intersexes, antenatal sexing, placental anatomy, grafts, tumours, pseudo-hermaphroditism, etc., etc.

A random blood smear examination of men and women with no obvious disorder of sex showed drumstick and drumstick-like appendages at certain specified age groups. This prompted a detailed study of nuclear sexing of the human leucocytes on a pilot scale under the auspices of the Madras State Research Committee.

MATERIALS AND METHODS

Blood smears from 951 males, without any complaints of sexual abnormality of age groups 0-15. Prepubertal; 15-30 pubertal; 30-45 post pubertal; 45-60 middle age; and over 60 years of those who presented themselves at the Stanley and the R.S.R.M. Hospitals were studied with reference to drumsticks and other nuclear appendages of the polymorphs. 10 cases of females in each of the age groups totalling 50 served as a miniature control.

The blood smears were fixed in ether alcohol and were stained with Ehrlich's haematoxylin stain for 20 minutes; the advantage with Ehrlich's haematoxylin is the distinct nuclear staining which affords remarkable clarity in identifying the drumsticks and other nuclear appendages. Five hundred polymorphs were scrutinised in each instance.

It was possible to classify the drumsticks and the appendages on morphological basis shown in Fig. 1 in six types. The classical drumstick corresponds to the one indicated as Davidson Major in Fig. 1.

We have ventured to call it so after the names of its discoverer. A brief description of these is given below:

1. Davidson Major is the classical drumstick described by Davidson and Robert Smith. It is almost a rounded spherical body about 2-3 μ in size with slender longish filament.

2. Davidson Minor is a miniature of Davidson Major, the body being less than 1 μ in size.

3. Davidson Like consists of an ovalish drumstick, like the major, with slightly long thick stalk (Fig. 4).

4. Davidson sessile is 2-3 μ in size, exactly like the drumstick but with very short thin stalk or no stalk (Fig. 5).

5. Sessile is a small knob-like Davidson Minor without stalk (Fig. 4).

6. Spikes are of different sizes and are self-explanatory (Fig. 2).

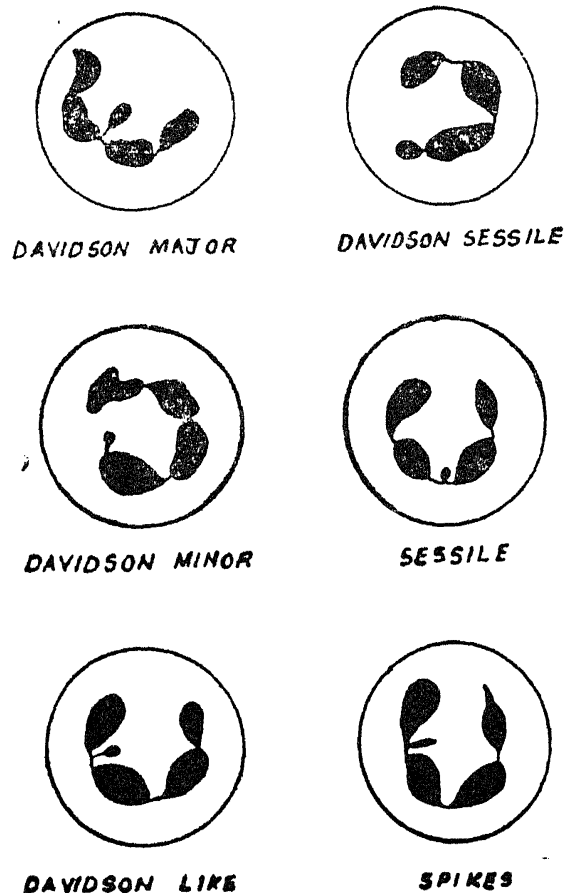


FIG. 1

OBSERVATIONS

Tables I, II and III under each of the categories present the exact findings of our observations:

Davidson Major shows an increased percentage during the pre-pubertal period, claiming an equivalent or rather more percentage than in the female. This is marked during the first three years of life.

Davidson Like presents a similar variation as the Davidson Major. The higher percentage noticed in the age group of 60 years and above is remarkable. It is present both in male and female.

TABLE I
Davidson Major

Age group in years	Males				Females			
	No. of cases studied	No. of cases positive and %	Davidson Major %	Average %	No. of cases studied	% of cases positive	Davidson Major %	Average %
0-15 ..	221	181 81.9	0- 3	1.9	10	100	0.25-0.5	0.5
15-30 ..	180	156 86.6	0- 1	0.75	10	100	1.00-3.0	2.8
30-45 ..	250	130 52	0-0.5	0- 4	10	100	2.00-5.0	3.2
45-60 ..	210	102 48.5	0- 1	0- 3	10	100	1.00-3.0	2.6
60 and above	90	27 30	0-15	0.95	10	100	1.00-2.0	1.5

TABLE II
Davidson Like

Age group in years	Males				Females			
	No. of cases studied	No. of cases positive and %	Davidson Like %	Average %	No. of cases studied	% of cases positive	Davidson Like %	Average Davidson Like positive
0-15 ..	221	179 81	1- 5	3.5	10	100	0.25-2	1.25
15-30 ..	180	166 92.2	0- 3	1.8	10	100	0.25-4	1.35
30-45 ..	250	128 51.2	0- 2	0.9	10	100	0.50-4	2.42
45-60 ..	210	196 93.3	0- 6	3.4	10	100	1.00-4	1.5
60 and above	90	90 100	1-10	6.8	10	100	1.00-4	1.8

Davidson Minor bodies could not be found in the 50 female smears except in the case of one three months old child, where it was doubtful. It is present in all the smears of the 951 cases studied. "Y" chromosome is to be soon. There is a slight increase in the percentage during the post pubertal period.

The presence of Davidson Sessile bodies shows a definite variation in the male. A marked depression is observed during the pubertal and

TABLE III
Davidson Minor

Age group in years	Males				Females		
	No. of cases studied	No. of cases positive and %	Davidson Minor positive %	Average %	No. of cases studied	No. of cases positive	Average %
0-15 ..	221	100	0.25-1	0.5	10	All negative A 20-day old female child showed one doubtfully	..
15-30 ..	180	100	0.0.5-1.5	1.5	10		..
30-45 ..	250	100	0.0.5-2	1.75	10		..
45-60 ..	210	100	0.75-1.5	1.00	10		..
60 and above	90	100	0.25-0.5	0.75	10	Positive—one cell in 500	..

TABLE IV
Davidson Sessile

Age group in years	Males				Females			
	No. of cases studied	No. of positive %	Davidson Sessile positive	Average Davidson Sessile %	No. of cases studied	% of cases positive	Davidson Sessile positive %	Average Davidson Sessile positive
0-15 ..	221	100	0.1-8	4.5	10	40	0-0.2	0.08
15-30 ..	180	100	0.5-5	1.2	10	30	0-0.5	0.15
30-45 ..	250	74	0.0-2	0.5	10	10	0-0.5	0.05
45-60 ..	210	83.3	0.0-4	1.25	10	20	0-1	0.2
60 and above	90	100	1.0-7	3.8	10	20	0-1	0.2

post-pubertal period; it starts with a good percentage in the prepubertal and rises again in the post 45 years. Very few cases in the female series showed this.

Sessile bodies } The percentage incidence of these varies and do not
Spikes } have any relation to age periods and sex.

It is obvious that these drumsticks occur in both males and females at certain specific age periods. It has been found that even in the female,

the percentage varies during the menstrual cycle. Taking the female, as such, it appears that the percentage is minimal, when the oestrogenic activity is minimal.

In one instance of male sterility, the following were the salient findings:

- (1) Sterility—10 years after marriage.
- (2) Davidson Major and Davidson Like—20%
- (3) Testicular biopsy showed active spermatogenesis.
- (4) Sperm count—Azoospermia.

On further investigation, it was found to be congenital atresia in the tract; this could not be labelled as Klinefelter's syndrome in spite of the presence of the chromatin in the blood smear and buccal smear.

DISCUSSION

The above study makes us surmise that these drumstick and other appendages seen in the leucocytes of males are of significance for they are not supposed to be present in normal males of any age group or there are of no significance at all. The exact nature of these bodies has not been assessed by the use of any selective stain. However, there appears a hormonal influences as seen by the variation in pregnancy, in the age periods, especially, with reference to the Davidson Major, Davidson Like and the Davidson Sessile.

The brilliant experiment of Jost (1947) in the rabbit indicates that there is an inherent tendency for all the embryos to become female. That is removal of the ovary does not interfere with the subsequent development of the female genital tract, while when the embryosis deprived of its tests, tends to become a female.

Jost in 1953, cultivated rat embryoses *in vitro* and found that the Wolffian ducts regress and the Mullarian ducts persist irrelevant of the sex of the embryos. Such an effect seems to be hormonal. The Mullerian persistence probably accounts for the femaleness. This indicates that the sexing is probably not entirely genetical.

The drawbacks in the above study.—The nuclear appendages seen in the leuceocytes of the male are not supposed to be present in the male of any age group as per existing literature.

The exact nature of these bodies have to be assessed both by special stains and at least in a few cases by chromosomal characterisation.

17 Ketosteroid states or any bio-chemical investigation in a randomly chosen cases was not done to denote the endocrine status.

The study has been done purely on the morphological appearance of the drumsticks and the other appendages. No stereoscopic study of the morphology was done. The sizes were measured only in a few cases. It is possible to mistake the categories from one to another purely from a two-dimension study of the bodies.

It would have been worthwhile to study the chromosome characterisation of the Davidson Minor with reference to Y chromosome.

COMMENTS

The above findings from the study, as they appear, seem to depart from the present understanding of the sex chromatin. It is wondered whether a reconsideration of nuclear sex would become a necessity, if this fundamental research is done, with all the relevant chromosome characterisation, 17 Ketosteroid and other bio-chemical estimations.

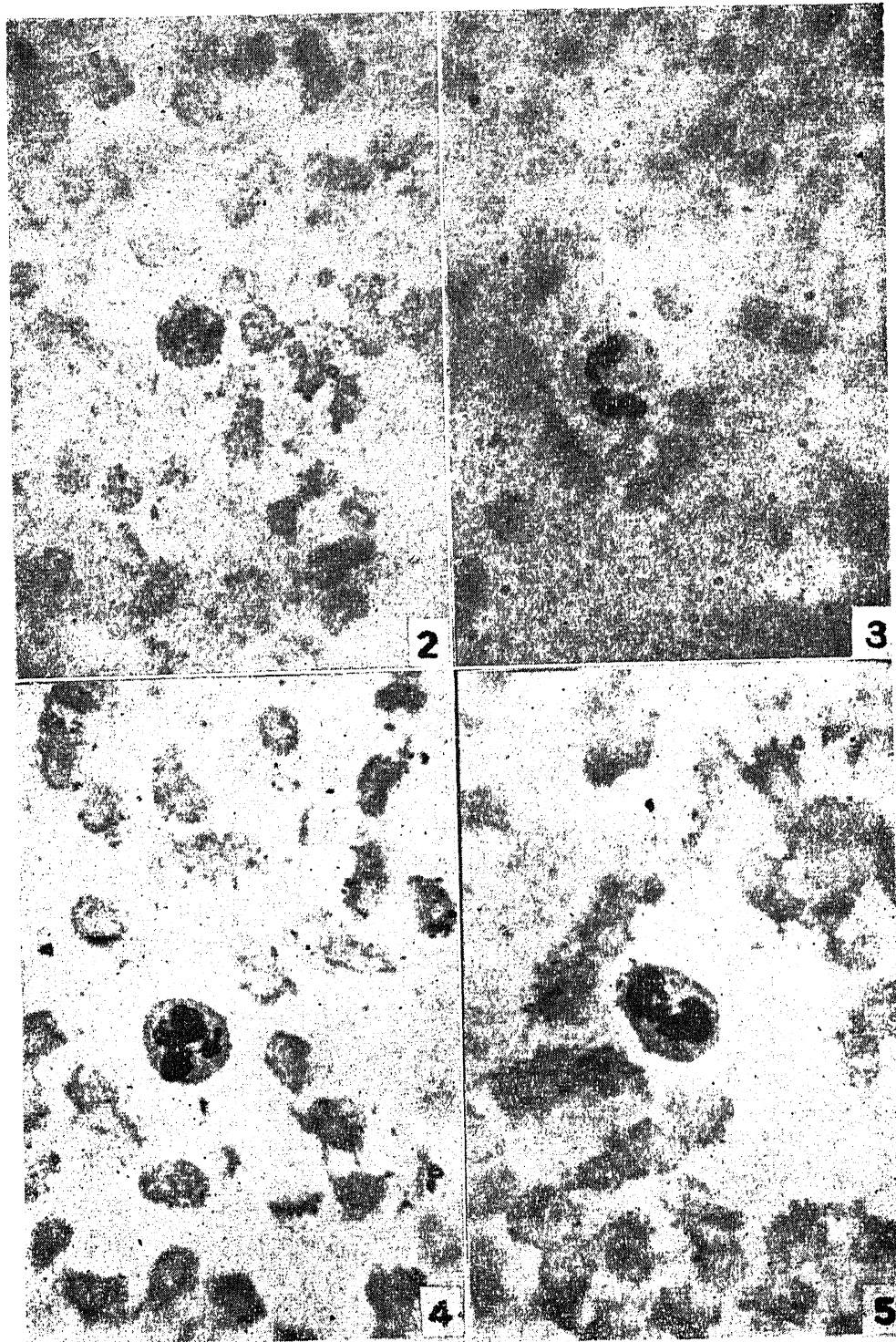
Taking the female as such, whether these count bear any relation to an imbalance in the progestin-oestrin equilibrium or not, is not clear. It is now known that at a particular threshold ratio of these two hormonal values, non-implantation of the ovum occurs. It is felt that an investigation of the cases of amenorrhoea due to various causes, simple anaemia, toxæmia of pregnancy tuberculosis the Stein-Leventhal syndrome may offer some clue to confirm such imbalance.

The headlines in the sports column of *The Hindu* daily, Madras, 20-10-1967 about the top woman sprinter Miss Ewa Keokowska being disqualified as a female on the *basis of sex chromatin test indicates another practical application* it is said that she has one chromosome too many labelling her as an intersex—an exciting popular news.

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FIGS. 2-5

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EXPLANATION OF PLATE III

- FIG. 2. Shows Davidson Major and Spike. (1) Davidson Major and (2) Spike.
FIG. 3. Davidson Minor. (1) Davidson Minor.
FIG. 4. Davidson Like and Sessile. (1) Davidson like and (2) Sessile.
FIG. 5. Shows Davidson Sessile. (1) Davidson Sessile.