ABSTRACT

Early beliefs that Barr Bodies (sex chromatin; drumsticks) were due merely to the XX Chromosomes in the female were challenged as far back as 1956. That they only appear in a percentage of neutrophils, polymorphs and other somatic cells was a starting point and that they are more frequent when nuclear segmentation is increased either by ageing or folate deficiency suggested that the explanation originally held required closer investigation. A great deal of research has been undertaken to establish the factors involved. Some account of these are listed and recorded in this thesis. The comparative incidence of Barr Bodies in mammalian blood appears to have little coverage and go round an aspect worthy of investigation. Apart from humans the range of available mammals was limited largely to domestic animals; although an attempt was made (with small success) to obtain material from more exotic mammals. The range of the different groups (13 in all) is set out in the tables which includes mainly normal mammals with a small section on the megaloblastic anaemias as a readily available source of hypersegmentation associated with folate deficiency. The main difficulties encountered were the limited time available; the difficulty in obtaining material in sufficient quantity to have numerical significance; the nature of this work which is very labour intensive in that it cannot successfully be automated in any of the aspects. Within the frame work of this thesis, there is scope for a very great deal of development which the writer intends to undertake on his required return home.

I

A COMPARATIVE STUDY ON THE INCIDENCE OF BARR BODIES IN MAMMALIAN BLOOD AND SOME FACTORS INFLUENCING IT.

Being a thesis presented by BENJAMIN AGBAYEMIE AKPOGOMEH to the University of St. Andrews in application for the degree of Master of Science.

Declaration : I hereby declare that the following thesis is based on work carried out by me; that the thesis is my own composition and that no part of it has been presented previously for a higher degree.

The research was carried out in the Department of Physiology and Pharmacology in the United College of St. Salvator and St. Leonard in the University of St. Andrews, under the direction of Dr. J.H. TAYLOR.

Signature

May, 1982.

Certificate :

I hereby certify that Benjamin Agbayemie Akpogomeh has spent seven terms engaged on research work under my supervision and that he has fulfilled the Conditions of 1974, No.2 Resolution of the University Court (St. Andrews) and that he is qualified to submit the accompanying thesis for the degree of Master of Science.

Signature

ACKNOWLEDGEMENTS

The ready Co-operation of individuals; Hospitals; the Slaughter House and the Veterinary Clinic has made this project a success and my sincere thanks to Dr. J.H. Taylor for his patience, helpful advice and encouragement throughout this work. To the girls, women and mothers of babies who allowed me to collect blood samples from them. To owners of pets who also allowed me to collect blood samples from their pets. To Dr. Evans who helped in translating some of the papers and to the technicians in the department for their assistance throughout.

INTRODUCTION

The sex chromatin is a characteristic mass of chromatin within nuclei of somatic cells of normal females of mammals including man. It is usually observed in interphase cells but can be seen during early prophase of mitosis.(59)

The sex chromatin represents a single X chromosome that displays the property of positive heteropyknosis.(59, 68) It is Feulgen positive and has affinity for basic dyes.(25) It is commonly seen as a planoconvex mass against the inner surface of the nucleolus or free in the nucleoplasm in tissue cells except in some rodents.(61) In blood cells, i.e. in leucocytes; it is seen as a drumstick like nuclear appendage which has its head attached to the rest of the nucleus by a thread like neck.(26, 27) A sex chromatin-like chromocentre is rarely visible in the nuclei of normal males consequently the sex chromatin is a female cytological characteristic in man and most other mammals.(6, 60)

Originally called a nucleolar satellite[6] because of its intimate relationship to the nucleolus in cat neurons, the sex chromatin was renamed when study of neuroglial and other cells of cat and man showed the term to be inappropriate for designating the specific chromocentre in all cells.(59A) In most cells the sex chromatin was found to be located against the inner surface of the nuclear membrane and is not a satellite of the nucleolus.

Some occasionally refer to it as Barr body to commemorate the name of the Senior discoverer of the sex chromatin; this name has not been adopted widely partly because Dr. Barr has discouraged its use, but mainly because the term is neither informative nor descriptive.(59)

Reference is occasionally made to the female sex chromatin, this is inappropriate because it implies that the typical sex chromatin appears only in the cells of females; it is a well known fact that some males who appear normal have sex chromatin; similar in all respects to that seen in females.(10, 31)

The sex chromatin found in blood films has the shape of a drumstick and this is probably why it is usually called a drumstick.[27] Wilson(83) used the term sex chromatin in his work to refer to a small hypothetical part of the X Chromosome that formed a sex-differentiator concerned with sex linked heredity. He may have been the first to use this term.

After 1945, Dr. Barr(8:60) returned to his research; he tried for some time to design a method for the controlled study of the effect of activity on neurons; he then read about studies on the stimulation of axons which had shown that impulses went not only to the muscle, but antidromically to and over the cell body; these findings provided the clue to the approach he wanted. After considering various peripheral nerves that might be stimulated unilaterally, he chose the hypoglossal nerve because of its accessibility to the angle of the jaw and because the Control and Experimental evidence would be side by side in the medulla. It was at this stage of the project that Bertram, E.G.(14) was accepted for post-graduate study in anatomy. Dr. Barr discussed with him the hypoglossal-nerve stimulation approach to the investigation of possible structural changes following intense activity.(60) They worked closely during the development of the apparatus and the technique. After stimulation, the tissues were fixed in isotonic formalin solution, which reduced the distortion of cells. Sections stained with Cresyl violet which gave good nuclear details were produced. When Bertram examined the first sections, he noticed that there were chromatolytic changes on the stimulated side; whether these were due to activity or a

mild form of axon reaction or both has never been determined; he also noted that the nucleoli of chromatolytic neurons were swollen while the position of the chromatin mass changed.(14) They later found as the project progressed that there was slight increase in the cross sectional area of the cell and the nuclear volume of the chromatolytic neurons; they found that the paranucleolus was slightly larger and had moved away from the nucleolus during later and early stages of nissel depletion and restoration.(7) Intrigued by the relationship of the paranucleolus to the nucleolus and the restoration of Nissl material; they discussed in detail the movement of the chromatin mass in altered cells of several animals. Animals were soon encountered whose hypoglossal neurons lacked the nucleolar satellite as it was then called. This finding presented a real puzzle; failure of staining technique was suspected; this was checked by staining all sections from both kinds of animal at the same time in one jar. Other possibilities were considered such as differences in fixation; experimental conditions; age and sex. These facts were checked since they had records of all the experimental animals. This investigation revealed that all animals whose nuclei contained a nucleolar satellite were females and others without were males.(5:60) Sensing the significance of this finding an intensive study was made of other regions of the nerve cells from both male and female cats, and it became clear that sexual dimorphism of nuclei existed in nerve cells. To determine if a similar phenomenon existed in human neurons, sections were obtained from a neuro-pathological collection, although these sections were not made for the study of nuclear details; it was found in sympathetic ganglion cells that a sex difference comparable to that found in cat neurons existed in man. Ten years before the discovery

that the sex of a normal animal is determined not by chance but by genetic factors on certain chromosomes; the sex chromosomes had been recognized under the microscope and the behaviour in male germ cells described.[39] Henking[39] observed the first meiotic prophase in each nucleus of primary spermatocytes. One densely stained round body stood out in sharp contrast to the fine thread like chromosomes; this he regarded as the nucleolus but on examining a pair of secondary spermatocytes he noted that this densely stained body was always incorporated into one of the two daughter spermatocytes; one with 11 chromosomes and the other with this body in addition to the 11 chromosomes. He was no longer sure that it was a mere nucleolus, so he called it X for unknown. It is in his honour today that X Chromosome denotes that Sex Chromosome that determines the development of the homogametic female sex; his findings were obtained from work done on the heteropteran insect Pyrrhocoris opterus.

Heitz[38] defined heterochromatin as being those chromosomal regions which stained darkly and remain condensed to form chromocentres in interphase nucleus while the remaining regions are said to be made of euchromatin; he found the X chromosome of Drosophila melanogaster to be structurally divided; the proximal part; the centromere and a nucleolus organiser are made of heterochromatin while the distal part is made of euchromatin. Ohno et al[68:69] found that the sex chromatin of female somatic interphase nuclei represents a single X chromosome condensed along its entire length at early prophase; the sex chromatin of preceding interphase was revealed to be a single condensed chromosome folded upon itself and straightened out at prophase; this was the first suggestion made on how the sex chromatin is formed. The significance of this observation was enhanced by the demonstration of asynchronous labeling of the X chromosomes on autoradiographic studies.

These two findings are now accepted as facts and form the basis of the Lyon Hypothesis[48] which states that (a) one of the two X chromosomes in the cell of a normal female mammal is genetically inactivated; (b) the inactive X can be either the maternal or the paternal one in different cells of the same animal; (c) the inactivation occurs early in embryonic development and once it has occurred, remains fixed throughout the development of each cell line. Point (c) i.e. the fixed differentiation of the X chromatin forming sex was also put forward by Grumbach and Morishima[33a] and others.[79a;75a]

It has also been suggested that the drumstick found in leucocytes (neutrophilpolymorphonuclear) represented a rejection phenomenon, a small mass of dense inactive chromatin being squeezed out of the nucleus during segmentation.[27]

The phenomenon of nuclear sexual dimorphism is similar in many representatives of the mammalian kingdom but there is considerable variation in sex chromatin patterns from one class to another and in some cases variation in sex chromatin patterns exists in cells of the same animal.[61] Sexual dimorphism is clearest in vesicular nuclei; difficult to detect in condensed nuclei and not recognizable in pyknotic nuclei or in nuclei with many large chromocentres. This is the basis of variation in chromatin patterns in various mammals.[61] Technical factors may also influence the number of nuclei found to show a sex chromatin body and the pattern. Fixatives vary greatly in their preservation of nuclear detail; delay in fixation of cells will make the nuclei shrink; the chromatin condense and become pyknotic. Formalin may often but not always produce such homogeneity of the chromatin pattern that sex chromatin may be obscured. Other fixatives may act this way or block the staining of the sex chromatin. Water or hypotonic solutions when in contact with the tissue or blood films before fixation, will

lead to inability to demonstrate the presence of sex chromatin. Long periods in strong acid fixatives may cause nucleotides to be hydrolyzed out, so that staining characteristics will be poor. If the stain used strongly colours the nucleolus, other cell components, or bacteria, the sex chromatin will be difficult to recognize or maybe masked. Tissue sectioning and embedding procedures must be given careful consideration when being carried out.(27,45,61)

In man all cells except those with pyknotic nuclei exhibit sexual dimorphism based on the presence of sex chromatin in females and its virtual absence in males; it is most obvious in vesicular nuclei, such as in oral epithelial cells and cells of the zona fasciculata of the adrenal cortex.(23:28) In apes a sex difference similar to that in human cells is present. In Gorillas, sex chromatin in oral cells appears coarser and larger than in man.(24;35;44)

In monkeys (Rhesus) nuclei of various tissues have a distinctive pattern of chromatin according to sex as in man. Chromatin is coarser than in human cells except in nerve cells, making recognition of sex chromatin more difficult.(24;71) Marmosets are similar in this respect to that existing in the Rhesus monkey and other primates and also present in some adult and embryonic cells.(12)

In canivores, sex chromatin comparable to that in man is present in various cell nuclei of females. The position in nerve cells varies considerably from one species to another and in non-nerve cells the sex chromatin is usually seen as a plano-convex mass adjacent to the inner surface of the nuclear membrane.(37;58;41;76)

In bison only oral epithelial cells have been examined in both sexes and no sexual dimorphism is recognizable.(61) In cattle sexual dimorphism is present and cells of non nervous tissues contain much coarser chromatin which obscures sexual dimorphism.(41;61)

In deer, goat and sheep it is present in most cells examined[41;55;61] but was not observed in the epithelial cells of the sheep.[75a] In swine it is present in nerve cells but most cells contain so much chromatin that sex chromatin cannot be identified with certainty.[61]

In horses, donkeys, mules and zebronkey, sex chromatin is recognizable in cells cultured from the skins, nerve cells, epithelial and smoth muscle cells of females. Other cells contain multiple coarse chromatin masses and a sex difference cannot be observed.[61]

In rabbits no sexual dimorphism of nuclei can be recognized in various regions of the nervous system. Nuclei of both sexes is said to contain from one to four large chromatin masses while the Purkinje nerve cells of females contain two large chromatin masses, similar to sex chromatin while most males have one. From these findings, it was suggested that one of the chromocentres in cells of the female rabbit must be the sex chromatin.[23;61] In other tissues and organs, sexual dimorphism has been detected.[61] In the adrenal cortical cells, sex chromatin was identified but the presence of one to four irregular polyangular masses made identification difficult.[42] In rodents, the nuclei contains much coarse chromatin which obscures sexual dimorphism but exceptions occur which makes sex chromatin identifiable.[40;81]

In mice sexual dimorphism is not recognizable in any nerve cell.[55] The nucleus of both sexes is said to contain several large masses of chromatin usually adjacent to the nucleolus. Similar observations have been made in other tissue and organ.[37]

Sexual dimorphism in the neutrophils leucocytes in the peripheral blood in man was first demonstrated in 1954 by Davidson and Smith[26] and this observation has been confirmed from various parts of the world.[2;66;74] The sex specific structure is a drumstick-like nuclear appendage which is

found in leucocytes of females. Some have been able to identify drumsticks in lymophocytes, esonophils and monocytes.[27,73,74] The typical drumstick has a round or oval head with an average diameter of between 1.4-1.6u and is attached to the rest of the nucleus by a fine thread like neck. Its chromatin which is as dense as the rest of the nucleus is arranged in clumps. Very rarely is the size of the drumstick altered when there are translocations or deletions affecting the X chromosome.[27] Nodules of similar size but not sex specific and sessile nodules are common in female blood. Occasionally a drumstick-like nodule is found in films from males, but a critical examination shows that it is not characteristic. Even if they are of right sizes, they are usually not drop shaped but have a flattened end or even a triangular outline.[26] The possibility of a chromosomal aberration should be borne in mind if more than one such structure and maybe some sessile nodules are found in males. Other structures that may be confused with drumsticks include the tennis racket appendage which is only an outline of chromatin. Small lobes usually connected by two strands to the rest of the nucleus and a single strand when at the end or terminal and a lesser pedunculated nodule which may easily be mistaken for a drumstick. Metamyelocytes rarely have drumsticks but may show specific sessile nodules; myelocytes and myeloblast do not appear to show any sex specific nodules.[27] Extrusion of drumstick from the nuclei is said to be linked to the process of nuclear lobing. Severe shifts to the right in the Arneth Count increase the number and conversely shifts to the left decrease the number.[26,27,53] The frequency of drumsticks is said to be increased in inherited oversegmentation of neutrophils.[53] The determination of the frequency of drumsticks in human females and

other mammals has long been tedious, and a range has not been established on a proper statistical basis; but it has been suggested that there is one in every 36 neutrophils but a wide variation from 1 in 5 to 1 in over 300 neutrophils has been reported.[27] It has also been reported that the number of drumsticks seem to be influenced by female sex hormones, and that there is variation in sex chromatin frequency during the menstrual cycle.[16,20,29,34,51,70,74,78] Paintal[70] et al found three peaks in the incidence of sex chromatin on studies carried out on buccal smears from 25 women through one menstrual cycle. The peaks coincided with the first day of their menses, 14th post menstrual day and the 22nd day of the cycle. The highest incidence was on the 14th day. Hagy[34] found from studies on pre and post menopausal women that variation in post menopausal women does not occur as is pre-menopausal women. Dolan[29] found no relation between sex chromatin frequency and female sex hormone. Spasor[77,1] found that the sex chromatin frequency varied in women who had specific conditions and were being treated with sex hormones. He stated that progesterone decreased sex chromatin in women with secondary amenorrhoea; synthetic oestrogen increased sex chromatin in women who had secondary amenorrhoea due to ovarian deficiency. Oestrogen also gives rise in drumstick frequency but this falls after a prolonged treatment in rabbits.[1] An increase in drumstick frequency has been reported at birth, especially in premature infants.[50] A decrease with old age has been reported but not confirmed.[27] An increase of drumstick frequency has been reported during pregnancy.[27] It being probable that multiple factors affect the incidence of Barr bodies, there appeared justification in making a comparative study both in depth and breadth on the incidence of sex chromatin in mammalian blood.

MATERIALS & METHODS - I

The experiments described were first prepared for examination under the scanning electron microscope. This was followed by carrying out a total white cell count and making blood films which were stained and mounted. An Arneth count was done on 200 neutrophils counted on each slide. The number of drumsticks was noted using a C. Baker microscope with oil immersion lens with a magnification of x1600 and a Ferrari counter with ten channels.

COLLECTION AND PREPARATION OF SAMPLES FOR THE SCANNING ELECTRON MICROSCOPE

1. ANTICOAGULANT
   (3 gms (EDTA) Ethylene Diamine tetraacetic
   (acid.
   (100 ml of 0.7% (Nacl) Sodium Chloride
   0.5 ml anticoagulant was mixed with 9.5 ml blood.

2. BUFFERS
   A. 0.2M monopotassium phosphate
   B. 0.2M disodium phosphate
   C. Final buffer
   The buffer A was titrated into buffer B until a pH of 7.8 was obtained.

3. FIXATIVES
   i Glutaraldehyde
   1% solution made from stock of 10% ampoules
   1 ml made up to 10 ml with Buffer C.

   ii Osmium Tetroxide
   1% solution made from stock of 25%
   2ml made up to 50ml in a fume cupboard.

All solutions prepared were stored in $4^{o}C$; samples were transferred into glass centrifuge tubes; centrifuged at 2000 RPM for about 15 minutes. The buffy coat was carefully pipetted out with a pasteur pipette and transferred into a 25ml beaker containing 1% glutaraldehyde in an ice pack.

This was allowed to stand for 30 minutes. It was then transferred into a SWNNEX 25 millipore chamber containing a pre-boiled millipore filter to remove detergent and open up the pores. The glutaraldehyde solution was drained by pumping air through one end of the chamber with a 2ml syringe without the needle. The cells on the millipore filter and filter were transferred into a petri dish containing buffer C and allowed to stand for 15 minutes with occasional aggitation to wash. This was done twice. The cells and filter paper were transferred into a vial containing 1% osmium tetroxide for 30 minutes in an ice pack and in a fume cupboard. This was the second fixation; draining and washing in buffer for 15 minutes. This also was done twice. Dehydration in 70%, 80%, 90% and 100% methanol for 15 minutes was carried out twice in each grade. The cells were completely dried in CO_2 gas. The cells were glued to a stub and connected to it by a small spot of conducting paint (silver). The specimen was coated with a thin layer of gold alloy to avoid accumulation of electrons on the specimen. This could cause a -ve charging and consequent image problems. The coating with gold alloy was done in a vacuum by direct evaporation of metal prior to observation in the scanning electron microscope.

The scanning electron microscope is essentially an electron probe instrument whose characteristic is a point to point correspondence between object and image at a time. The electron beam is produced as in a transmitting electron microscope by a tungsten filament and similarly controlled by a cathode; anode and magnetic lenses. The accelerating voltage can be varied from 1.5KV to 25KV. The vacuum 10^{-5} is maintained by a rotary and diffusion pump system. The signals produced when an incident electron beam impinges on a specimen are many, but on the scanning electron microscope, one is concerned with the secondary electron emissions which are produced in greater numbers than others (Fig.I).

These are attracted to the collector which is at a 1KV potential and pass through the scintillator which converts them to photons. These are then amplified in the photo-multiplier to give an electrical signal. This signal passes to the cathode ray tube and is seen as a spot on the cathode ray tube screen. This signal is from one spot on the specimen surface. The beam is scanned over the specimen by the scan coils; each spot producing a corresponding spot on the cathode ray tube screen which is also scanned across the screen by the linked deflection coils (Fig.2). The object and image are divided into picture elements and this point to point correspondence will cover the object and image by means of sweeps of the electron beam on the surface of the object and simultaneous sweeps of the cathode ray tube and a visual image results. There are 1000 spots/line and 1000 lines from each picture.

Fig.I - Signals produced by the interactions between incident electrons and substances.

Fig.2 - FORMATION OF IMAGE ON THE SEM (Scanning Electron Microscope).

TOTAL WHITE CELL COUNT

HAEMOCYTOMETER;
WHITE CELL PIPETTE; rubber tube and mouth piece
DILUTING FLUID (1.5% acetic acid in distilled water, tinted with methyl violet).

After mixing blood by carefully inverting the container about 10 times, the white cell pipette's tip is held horizontally on the open container. Blood is sucked slightly above the 0.5 mark; excess blood is wiped with a tissue paper. Suck up white cell diluting fluid to the 11 mark.

Rotate the pipette vigorously to mix blood and solution thoroughly after removing the rubber and mouth piece. After mixing, the pipette is laid aside for complete haemolysis of red cells to take place. The acid destroys the red cells while the dye colours the nuclei of the white cells making them more prominent. The unmixed portion in the capillary tube is discarded by blowing out some of the contents. The tip of the pipette is gently placed on the surface of the counting platform where it projects beyond the cover-glass. A small amount of the solution will be drawn under the coverslip by capillary action. The platform should be completely covered, without air bubbles or over-flow into the central trough. Using the low power (x10) objective, all the white cells seen over the whole ruled area were counted; i.e. nine squares each 1 sq mm in area. The number of white cells per cubic mm is given by N x 200 ÷ 9.

Preparation of blood films: Using chemically cleaned slides, which were stored in a mixture of alcohol and ether (equal volumes), dried and wiped free of dust immediately before use. A small drop of blood is placed in the centre line of a slide about 2 cm from one end. The spreading slide is placed at an angle of about 45^{o}C to the slide and then moved back to make contact with the drop of blood. The drop spreads out quickly along the line of contact of the spreader with the slide. The moment this occurs, the film is spread by a rapid, smooth, forward movement of the spreader. The drop of blood should be of such a size that the film has a maximum length of 4-5 cm. It is important that the slide used as a spreader should have an absolutely smooth edge and should be narrower in breath than the slide on which the film is made. If the edge of the spreader is rough, films with ragged tails containing many leucocytes will result. Care must be taken while making films to avoid too thin or too thick films. The faster or slower a film is

spread, the more even and thicker or uneven and thinner it will be. These errors could result in irregularity of the distribution of blood cells; the polymorphonuclear neutrophils and monocytes would be found at the margins and tail while lymphocytes are seen in the middle of the films. This could be due to differences in stickness, size and specific gravity among the different classes of cells (C).

Due to distance, Mr. Walter MacLennan of the large animal practice teaching unit in Midlothian, agreed to make fixed films from zoo mammals. Some slides were also received from the Victoria Hospital and Ninewells Hospital. Correct concentrations of anticoagulant must be used at all times as excess would affect both red cells and leucocytes, causing shrinkage and degenerative changes which affect packed cell volume and the platelet count. The correct volume of blood must be added, followed by repeated inversions of the container to make sure the anticoagulant and blood is thoroughly mixed. Films made from blood that have stood at room temperature for more than 3 hours show changes in cell morphology; neutrophils are affected; their nuclei may stain more homogeneously; the nuclear lobes become separated and their cytoplasmic margin appear ragged or less well defined (C). Changes are retarded but not abolished in samples kept at $4^{o}C$.

FIXATION. Blood films were fixed once they were dry to prevent or inhibit autolysis and bacterial decay. Fixation should render insoluble the substance of cell and give optical differentiation and produce minimum distortion during subsequent treatment. All films for these experiments were fixed in absolute methanol after labelling in pencil on the film and in a Coplin jar for 20 minutes.

A fixative may be described as a substance which will preserve after death the shape, structure relationship and chemical constitutents of

tissue and cells. The preservation should be such that fixed tissues and cells resemble as closely as possible the form which they had during life. Fixatives are grouped into two; simple and compound fixatives and maybe grouped under headings according to their action upon the cell and tissue constitutents (D). Those which preserve the tissue in a manner which permits the general microscopical study of the tissue structure and allow the various layers of tissue and cells to retain relationship with each other are termed microanatomical fixatives. While those which are employed for their specific action upon specific part of the cell structure are termed cytological fixatives. This group maybe sub-divided into nuclear and cytoplasmic fixatives depending upon where they act. Research workers these days prefer to make use of the freeze drying technique for the preservation of tissue which is to undergo histochemical tests. Temperature has an important effect upon the action of fixatives, a low temperature will slow fixation but will reduce the autolytic action of enzymes released after death, while a high temperature will decrease the time required in the fixative but will also increase autolysis. The fixatives shown below have been grouped according to their major constituents (D).

1. Aqueous formaldehyde solution
2. Alcoholic fixatives
3. Picric acid fixatives
4. Mercuric chloride fixatives
5. Chromate solutions
6. Chromate and mercuric chloride fixatives
7. Osmium tetroxide

Methanol comes under the second group, details of the action are unknown but the basic principle is that it forms a cross linkage between

between the proteins thereby forming a gel; keeping every part in their invivo relationship with each other. Soluble proteins are fixed to the structural proteins making them insoluble and easy to handle. The following stains and staining techniques were tried :-

STAINING

1. LEISHMAN
2. GIEMSA
3. MAY GRUNWALD
4. JENNERS GIEMSA
5. MAY GRUNWALD'S GIEMSA

Composition of Stains; Method and results.

Leishman's Stain

0.2gm of the powdered dye is weighed out and transferred to a conical flask of 250 ml capacity. 100 ml of methanol is added; this is warmed in a water bath at 50°C for 60 minutes. The flask is then allowed to cool to room temperature; shaken several times during the day; filtered and allowed to stand for at least 7 days before use. The longer it stands the better its staining ability.

BUFFER. A phosphate buffer of pH 6.8 was made by dissolving a buffer(BDH) tablet in a volumetric flask and made up to 100 ml with distilled water.

METHOD:

1. Air dry blood film, 20 minutes
2. Fix film with stain, 2 minutes
3. Add to undiluted stain on slide an equal volume of buffer solution; mix by gentle rocking; leave for 10 minutes.
4. Wash and differentiate with buffer solution till slide turns salmon pink (about 3 minutes washing).
5. Slides are stood upright to dry; clean back of the films with soft tissue paper.
6. Mount using No.1 cover slide and DPX as mountant.

RESULTS:	Nuclei of all leucocytes	-	Purple
	Neutrophilic granules	-	Red to Purple
	Eosinophlic granules	-	Orange Red
	Basophilic granules	-	Dark Blue
	Lymphocytes & Monocytes	-	Dark Purple nuclei with pale blue cytoplasm
	RBC	-	Salmon Pink

2. Giemsa

1 gm of the powdered stain is weighed out and transferred into a conical flask of 250 ml capacity; 66 ml of glycerol is added and the mixture is warmed in a water bath at 56-60^{o}C for 120 minutes. 66 ml of methanol is then added; mix and allow to stand at room temperature for 7 days before being filtered. It is then ready for use.

METHOD:

1. Air dry film, 20 minutes
2. Fix in absolute methanol, 5 minutes
3. Stain in freshly prepared stain

   5 ml stain)
   45 ml buffer solution) - 20 minutes
4. Wash and differentiate with buffer solution in a Coplin jar. 3 changes and allow to stand in buffer for 5 minutes.
5. Slides are stood upright to dry; wipe back of films
6. Mount using No.1 cover slides and DPX as mountant.

RESULTS:	Nuclei of leucocytes	-	reddish purple
	Neutrophilic granules	-	red to orange
	Eosinophilic granules	-	red to orange
	Basophilic granules	-	blue
	Lymphocytes/monocytes	-	dark purple nuclei with light blue cytoplasm.

3. May-Grünwald

0.3 gm of the powdered dye is weighed out and transferred to a conical flask of 250 ml capacity. 100 ml of methanol is added and the mixture is warmed in a water bath at 50°C for 60 minutes. Allow the flask to cool to room temperature; shake the mixture several times during the day; allow to stand for 24 hrs; filter. It is then ready for use.

METHOD:

1. Air dry film, 20 minutes
2. Fix in absolute methanol, 5 minutes
3. Stain in freshly prepared stain

   25 ml stain)
   25 ml buffer) 60 minutes
4. Wash and differentiate with buffer, 2 minutes
5. Slides stood upright to dry; wipe back of films
6. Mount using No.1 cover slides and DPX as mountant.

RESULTS:

Nuclei of leucocytes	-	dark blue
Eosinophilic granules	-	orange to red
Neutrophilic granules	-	red
Basophilic granules	-	dark blue
Lymophocytes	-	dark blue nuclei with pale blue cytoplasm
RBC	-	yellowish red.

4. Jenners Giemsa

0.5 gm of the powdered dye is weighed out and transferred into a conical flask of 250 ml capacity; 100 ml methanol is added. The mixture is warmed in a water bath at 50°C for 30 minutes; allow flask to cool to room temperature; shake mixture several times during the day. Allow to stand for 24 hours; filter. It is then ready for use.

METHOD:

1. Air dry films, 20 minutes
2. Fix in methanol, 15 minutes
3. Stain in freshly prepared stain (Jenner)

   Jenner's stain - 10 ml)
   Buffer solutions - 40 ml) 6 minutes

4. Transfer with out washing into another jar containing freshly prepared Giemsa stain.

   Giemsa - 5 ml)
   Buffer solution - 45 ml) 10 minutes

5. Transfer slides to a jar of buffer solution; wash, 3 changes, allow to stand in buffer, 3 minutes
6. Slides stood upright to dry; wipe back of films
7. Mount, using No.1 cover slides and DPX as mountant.

RESULTS:

Nuclei	-	red
Neutrophilic granules	-	purple pink
Eosinophilic granules	-	red
Basophilic granules	-	deep blue
Lymphocytes	-	blue.

5. <u>May Grunwald Giemsa</u>

METHOD:

1. Air dry films, 20 minutes
2. Fix in methanol, 15 minutes
3. Stain in freshly prepared stain (May-Grunwald)

   25 ml stain)
   25 ml buffer) 5 minutes

4. Transfer without washing into another jar. Contain freshly prepared Giemsa stain

   Giemsa 5 ml)
   Buffer 45 ml) 15 minutes

5. Transfer slides to a jar of buffer solution, wash; 3 changes; allow slides to stand in buffer for 3 minutes.

6. Stand slides upright to dry; wipe back of films

7. Mount, using No.1 cover slides and DPX as mountant.

RESULTS: Same as Jenner's Giemsa but better staining quality with a high magnification.

Stains: The stains used in the experiments are often referred to as Romanowsky stains and are almost universally employed for staining blood films. The property of these stains making distinctions in shades of staining and of staining granules differentially depends upon the presence in the stains of derivatives of the interaction of methylene blue and eosin. The original combination of methylene blue azure is of particular importance in that it stains the granules of leucocytes and the nuclei of malaria parasites a reddish colour (C & D). Methylene blue azure is added to the methylene blue and eosin mixture in Giemsa stain. Jenner's stain is the simplest of Romanowsky dyes while Leishman's stain occupies an intermediate position and Giemsa is the most complex. They are all sensitive to H ion concentrations; a pH to the alkaline side of neutrality makes methylene blue component more obvious at the expense of eosin and vice versa; a uniform pH is therefore desirable for good results. The mechanism by which certain components of a cell's structure stain with particular dyes and others fail to do so although staining with other dyes depends upon complex differences in chemical structures and affinities. With Romanowsky dyes, the acidic groupings of the nuclei acid and proteins of the cell nuclei and primitive cytoplasm determine their uptake of the basic dye methylene blue. Conversly the presence of basic groupings on the haemoglobin molecule results in its affinity for acidic dyes and its staining by eosin. The granules in the cytoplasm of neutrophil leucocytes are weakly stained by the azure complexes.

Eosinophilic granules contain a spermine derivative with an alkaline grouping which stains strongly with the acidic components of the dye. Basophilic granules contain heparin which has an affinity for the basic components of the dye (C).

MOUNTING MEDIA:

Media for the mounting of microscopical preparations may be divided into two :- i) Aqueous media; ii) Resinous media. The media in group i) are designed to make either temporary or permanent mounts of water miscible preparations. Their formulae consists of a solidifying agents such as gelatin or gum arabic to which glycerol is added to prevent drying and cracking. Sugars are also added to bring about an increase in the refractive index and preservation. The second group may be divided into natural; semi-synthetic and synthetic media. The mountant used in this experiment falls into the second group. DEPEX POLYSTYRENE (DPX) contains about 20% resin in xylene. It has a refractive index of 1.522 and a density of 0.938 g/ml and a drying rate of about 2 days for cover slide setting. It has a melting point of 80-88^{o}C and when carefully handled air bubbles are not trapped on slides (D). The purpose of mounting the specimen is to enable it to be handled for examination and photography. The immersion oil used was cedarwood oil; this has been found to be suitable and its refractive index is approximately the same as that of the cover glass and does not dry out quickly. A ray of white light passing through a substage condenser, slide, specimen cover slide and oil to the objective, suffers little refraction at each glass-oil interface since all these media have approximately the same refractive index.

MATERIALS & METHODS - II

EXAMINATION OF FILMS, ARNETH COUNT, IDENTIFICATION OF LEUCOCYTES AND DRUMSTICKS

Once stained, dried and mounted, the slides were examined using C. Baker microscope under a low power magnification to get an idea of the quality of the preparation, number, distribution and staining of the leucocytes. Slides with more than five crushed leucocytes or organisms were discarded. On selecting a suitable area of distribution, bearing in mind that the edges of the slide must be avoided since distribution in these areas could be irregular, with 4 mm oil immersion objective to give a magnification of x1600. A differential leucocyte count was carried out, after this count only neutrophils were counted in lengthways strip of the slide. An Arneth count was carried out on the 200 neutrophils counted; nuclear appendages, i.e. drumsticks on cells were noted. It must be remembered while counts are made that the typical drumstick has a round oval head with a diameter of between 1.4 and 1.6u and is attached to the rest of the nucleus by a single fine thread-like neck. Care must be taken so as not to confuse nodules with thick necks, sessile nodules, tennis racket appendage and small lobes with drumsticks. To be sure that only true drumsticks were counted, an accurately calibrated circle with a diameter of 1.6u was fitted over the drumstick or over any drumstick-like object.

LEUCOCYTES: There are five kinds of leucocytes which come from two families. Those with granular cytoplasm and those without. There are three kinds of granular leucocytes which vary in size and staining properties of the granules.

1. Eosinophils - granules stain with acidic dyes
2. Basophils - granules stain with basic dyes
3. Neutrophils - granules stain with acidic dyes.

They are often called polymorphonuclear leucocytes which indicates that the nucleus has a complex shape with variable number of lobes. These nuclear lobes are arranged at random within the cytoplasm depending on how they are spread. They often assume the shape of an S, Y, Z, E or C. The number of granules is also variable; one could see granulocytes with few or no granules and others with number of granules which appear to be more than in the average cell (A).

NON GRANULAR LEUCOCYTES. Two kinds.

i) Lymphocytes

ii) Monocytes

There are two kinds of lymphocytes; the large and small ones. The large lymphocytes have a nucleus that is generally central or slightly eccentric, but always completely encircled by the cytoplasm. The small lymphocytes have a nucleus that is slightly oval and sometimes kidney shaped and the nucleus occupies about 90% of the cell diameter. One occasionally encounters a binucleated lymphocyte (A).

Monocytes also have two principal appearances, i.e. the large and small ones. The large ones have a voluminous nucleus which may be central or peripheral and is usually irregular or kidney shaped. It is made up of fine filaments and a nucleolus is not present. The small ones are sometimes difficult to distinguish from large lymphocytes; the nucleus may be round or oval. The cytoplasm has a distinct outline and sometimes stains ash blue.

RESULTS

Blood samples from cattle, pigs and sheep were prepared for examination under the Scanning Electron Microscope but it was not possible to identify drumsticks under the SEM (Fig. 2a).

In the experiments carried out to determine the total white cell count, it was found the cattle had an average of 10,282.4 cells per mm^3; sheep 6,518.7 per mm^3 and pigs 18,084 per mm^3 (tables 6, 7 and 8).

Initial experiments were carried out to determine the most suitable stain to be used for staining blood films on slides. Five smears made from one sample were stained in five Coplin jars containing the five different stains as described in the materials and methods section. It was found that Leishman's stain stained the cells faintly to give a detailed picture of the drumsticks. Fiemsa stained better than Leishman but with a high magnification, nuclear appendages became faint. May-Grünwald's stain gave almost the same picture and the time required for staining was very long, considering the number of slides to be stained. The combination of Jenner Giemsa and May-Grünwald's Giemsa stains gave an almost identical result, but with a higher magnification May-Grünwald's Giemsa stain gave a better result. It was also easier to identify drumsticks since both the nucleus and drumsticks were clearly stained and drumsticks could not be mistaken for other nuclear appendages. Thus all slides counted to give the results shown on the tables were stained with May-Grünwald's Giemsa stain; apart from slides of patients with megaloblastic (mostly pernicious) and haemolytic anaemias, and anaemias associated with Hb.s and patients with suspected tuberculosis, all of which were mechanically stained in the haematology department of Ninewells Hospital, Dundee.

The results for the investigation of the incidence of drumsticks in all the various samples are shown on tables 1-13. It can be seen

were 3 peaks; on the 1st, 14th and 22nd day of the cycle at 6.5%, 7.0% and 6.5%. In subject (c) there were 2 peaks on the 1st and 14th day at 5.0% and 4%. In subject (d) there was only a peak on the first day at 5% and in subject (e) there were two peaks on the 1st and 22nd day at 6.0% and 5.0%.

The enlarged photographs are at a magnification of X 1200 and are for this reason not as clear as the actual vision down the optical microscope. Specs of dust and scratches show up on the photographs which do not appear in the picture seen down the microscope.

The photographs show :-

Fig. 6(a) A normal female three lobed neutrophil showing drumstick.

(b) A female patient with megaloblastic anaemia showing drumstick.

Fig. 7(a) Pig drumstick.

(b) Sheep drumstick.

Fig. 8(a) Cattle drumstick.

(b) Dog drumstick.

Fig. 9(a) Cat drumstick.

(b) Monkey drumstick with some sessile nodules and rod shaped appendage.

TABLE I

(Pre-pubertal)

ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
					(3.57)	(5.21)	(8.82)	(33.33)		
B1	30 min	May-Grün wald's Giemsa	200	4	28	48	17	3	12	6
					(1.87)	(4.08)	(8.57)	(10.0)		
B2	30 "	"	"	2	26.5	49	17.5	5	9	4.5
					(1.64)	(4.30)	(6.67)	(14.29)		
B3	30 "	"	"	4.5	30.5	46.5	15	3.5	8	4.0
					(5.08)	(4.0)	(10.71)	(12.5)		
B4	30 "	"	"	2.5	29.5	50	14	4	11	5.5
					(7.02)	(5.21)	(9.68)	(22.22)		
B5	30 "	"	"	3.5	28.5	48	15.5	4.5	14	7.0
					(2.08)	(5.62)	(4.35)	(7.69)		
B6	7 Yrs	"	"	2	24	44.5	23	6.5	8	4.0
					(3.70)	(2.83)	(6.67)			
B7	$3\frac{1}{2}$ "	"	"	2.5	27	53	15	2.5	7	3.5
						(4.71)	(6.90)	(16.67)		
B8	6 "	"	"	4	33	42.5	14.5	6	7	3.5
					(1.79)	(4.85)	(3.57)	(14.29)		
B9	$8\frac{2}{3}$ "	"	"	3	28	51.5	14	3.5	9	4.5
					(4.44)	(5.32)	(4.76)	(14.28)		
B10	11 "	"	"	2.5	22.5	47	21	7	11	5.5
					(3.85)	(3.57)	(4.00)	(7.14)		
B11	2 mths	"	"	0	26	42	25	7	8	4.0
					(1.61)	(6.02)	(6.98)	(9.09)		
B12	5 days	"	"	0.5	31	41.5	21.5	5.5	10	5.0
					(1.79)	(4.35)	(5.56)	(14.29)		
B13	8 Yrs	"	"	4.5	28	46	18	3.5	8	4.0
					(3.77)	(5.95)	(6.82)	(6.67)		
B14	$5\frac{1}{2}$ "	"	"	2	26.5	42	22	7.5	11	5.5
						(3.03)	(6.90)	(14.29)		
B15	2 "	"	"	3.5	29	49.5	14.5	3.5	6	3
					(2.81)	(4.58)	(6.73)	(13.05)		
MEAN			200	4.23	27.87	46.73	16.16	4.8	9.27	4.63
S^2 VARIANCE									4.46	1.12
S STANDARD DEVIATION									2.11	1.06

TABLE 2

Pregnant Women

ID NO.	AGE(Wks)	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
P1	28(15)	May-Grün wald's Giemsa	200	3.0	(1.85) 27.0	(4.30) 46.5	(16.67) 18.0	(27.27) 5.5	14	7.0
P2	30(14)	"	"	4.5	(1.59) 31.5	(4.55) 44.0	(12.12) 16.5	(14.29) 3.5	11	5.5
P3	22(17)	"	"	4.0	(3.45) 29.0	(5.15) 48.5	(13.79) 14.5	(25.00) 4.0	13	6.5
P4	22(30)	"	"	1.5	(2.13) 23.5	(4.17) 48.0	(10.00) 20.0	(7.14) 7.0	10	5.0
P5	21(12)	"	"	3.0	(2.00) 25.0	(6.59) 45.5	(7.69) 19.5	(14.29) 7.0	12	6.0
P6	27(8)	"	"	2.5	(2.56) 19.5	(5.43) 46.0	(7.02) 28.5	(6.67) 7.5	11	5.5
P7	35(18)	"	"	3.5	(4.55) 22.0	(4.21) 47.5	(9.30) 21.5	(9.09) 5.5	11	5.5
P8	25(20)	"	"	3.0	26.0	(7.59) 39.5	(10.87) 23.0	(11.76) 8.5	13	6.5
P9	25(24)	"	"	2.0	(3.33) 30.0	(5.05) 49.5	(7.14) 14.0	(11.11) 4.5	10	5.0
P10	23(28)	"	"	2.5	(1.85) 27.0	(4.94) 40.5	(10.00) 20.0	(15.00) 10.0	12	6.0
P11	19(19)	"	"	3.0	(2.04) 24.5	(4.08) 49.0	(11.11) 18.0	(18.18) 5.5	11	5.5
P12	29(24)	"	"	2.0	(1.56) 32.0	(4.71) 42.5	(7.69) 19.5	(12.50) 4.0	9	4.5
MEAN			200	2.88	(2.24) 26.42	(5.06) 45.58	(10.28) 19.42	(14.36) 6.04	11.42	5.71
S^2									0.69	0.48
S									0.40	0.69

TABLE 3(a)

Pre-Menopausal Women/Menstrual Cycle

DAY	ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
8th	MT1	23	May-Grün wald's Giemsa	200	6	23.5 (2.04)	45.0 (5.56)	16.5 (9.09)	8.0 (6.25)	10	5.0
9th	MT2		"	"	6.5	29.5 (1.69)	48.0 (4.17)	14.0 (7.14)	7.0 (7.14)	8	4.0
10th	MT3		"	"	6	27.5 (1.82)	44.0 (3.41)	14.5 (3.45)	8.0 (6.25)	7	3.5
11th	MT4		"	"	5	23.5	46.5 (4.30)	11.0 (4.55)	14.0 (7.14)	7	3.5
12th	MT5		"	"	6.5	24.0 (2.08)	48.5 (4.12)	13.0 (7.69)	8.0 (12.5)	9	4.5
13th	MT6		"	"	5.5	28.0 (1.79)	45.5 (6.59)	15.5 (6.45)	5.0 (10.0)	10	5.0
14th	MT7		"	"	6.0	19.5 (2.56)	48.0 (6.25)	18.5 (5.41)	8.0 (6.25)	10	5.0
15th	MT8		"	"	2.5	31.5 (3.17)	49.0 (6.12)	11.5 (8.70)	3.0 (16.67)	11	5.5
16th	MT9		"	"	8.0	21.0 (2.38)	44.0 (3.41)	17.0 (5.88)	10.0 (5.00)	7	3.5
17th	MT10		"	"	5.0	19.5 (2.56)	44.5 (4.49)	18.0 (2.78)	13.0 (3.85)	7	3.5
18th	MT11		"	"	4.5	23.0 (2.17)	48.5 (5.15)	16.5 (6.06)	7.5 (6.67)	9	4.5
19th	MT12		"	"	5.5	21.0 (2.38)	53.0 (4.72)	13.5 (3.70)	7.0 (7.14)	8	4.0
20th	MT13		"	"	1.5	28.0 (3.57)	49.0 (4.08)	19.0 (5.26)	2.5	8	4.0
21st	MT14		"	"	4.5	28.5 (3.57)	49.5 (5.05)	10.5 (4.76)	7.0 (7.14)	9	4.5
22nd	MT15		"	"	5.5	29.5 (3.39)	40.0 (5.00)	19.0 (5.26)	6.0 (8.33)	9	4.5
23rd	MT16		"	"	3.5	32.0 (3.13)	43.5 (6.90)	14.5 (10.34)	6.5 (7.69)	12	6.0
24th	MT17		"	"	5.0	26.0 (1.92)	45.5 (5.49)	18.0 (8.33)	5.5 (9.09)	10	5.0
25th	MT18		"	"	4.0	27.5 (1.82)	44.5 (3.37)	16.5 (6.06)	7.5 (6.67)	7	3.5
26th	MT19		"	"	6.0	23.5	46.5 (3.23)	16.0 (6.25)	8.0 (6.25)	6	3.0
27th	MT20		"	"	6.0	18.0 (2.78)	48.5 (4.12)	19.0	8.5 (11.76)	7	3.5

							(2.38)	(5.10)	(2.78)	(7.69)	
28th	MT21	23	May-Grün wald's Giemsa	200	5.5	21.0	49.0	18.0	6.5	8	4.0
							(4.55)	(6.06)	(8.70)		
1st	MT22		"	"	9.0	19.0	44.0	16.5	11.5	8	4.0
							(3.27)	(4.49)	(7.69)	(7.14)	
2nd	MT23		"	"	5.0	30.5	44.5	13.0	7.0	9	4.5
							(4.44)	(5.13)	(5.71)		
3rd	MT24		"	"	9.0	22.5	39.0	17.5	12.0	8	4.0
							(1.67)	(2.83)	(6.67)		
4th	MT25		"	"	-	30.0	53.0	15.0	2.0	6	3.0
							(1.47)	(3.94)	(3.85)		
5th	MT26		"	"	6.0	34.0	38.0	13.0	9.0	5	2.5
							(2.27)	(6.45)	(7.69)		
6th	MT27		"	"	4.5	29.5	44.0	15.5	6.5	5	2.5
							(1.64)	(2.11)	(2.56)	(20.0)	
7th	MT28		"	"	-	30.5	47.5	19.5	2.5	5	2.5
							(3.32)	(4.50)	(5.68)	(7.25)	
MEAN		23		200	5.07	25.08	46.09	15.71	7.39	8.04	4.02
s^2											0.785
s											0.886

TABLE 3(b)

DAY	ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTA %
9th	SK1	21	May-Grün wald's Giemsa	200	3.5	(2.17) 23.0	(4.08) 49.0	(5.26) 19.0	(9.09) 5.5	8	4.0
10th	SK2		"	"	3.5	(3.17) 31.5	(3.40) 45.5	(3.23) 15.5	(12.5) 4.0	8	4.0
11th	SK3		"	"	4.5	(3.28) 30.5	(5.38) 46.5	(6.90) 14.5	4.0	9	4.5
12th	SK4		"	"	4.0	(3.33) 30.0	(5.62) 44.5	(6.06) 16.5	(10.00) 5.0	10	5.0
13th	SK5		"	"	3.0	(4.76) 31.5	(6.25) 48.0	(7.14) 14.0	(14.29) 3.5	12	6.0
14th	SK6		"	"	5.5	(3.92) 25.5	(7.45) 47.0	(8.33) 18.0	(25.00) 4.0	14	7.0
15th	SK7		"	"	4.0	(5.17) 29.0	(6.59) 45.5	(8.82) 17.0	(11.11) 4.5	13	6.5
16th	SK8		"	"	4.5	(3.33) 30.0	(5.56) 45.0	(9.38) 16.0	(11.11) 4.5	11	5.5
17th	SK9		"	"	5.0	(3.23) 31.0	(4.21) 47.5	(7.69) 13.0	(14.29) 3.5	9	4.5
18th	SK10		"	"	3.0	(3.51) 28.5	(4.21) 47.5	(2.78) 18.0	(16.67) 3.0	8	4.0
19th	SK11		"	"	3.0	(2.94) 34.0	(5.68) 44.0	(6.67) 15.0	(12.5) 4.0	10	5.0
20th	SK12		"	"	4.0	(3.64) 27.5	(6.19) 48.5	(5.71) 17.5	(20.0) 2.5	11	5.5
21st	SK13		"	"	4.0	(3.77) 26.5	(5.68) 44.0	(7.89) 19.0	(7.69) 6.5	11	5.5
22nd	SK14		"	"	5.0	(3.92) 25.5	(6.32) 47.5	(7.41) 13.5	(14.29) 3.5	13	6.5
23rd	SK15		"	"	4.5	(3.57) 28.0	(5.56) 45.0	(5.56) 18.0	4.5	9	4.5
24th	SK16		"	"	5.5	(3.39) 29.5	(4.49) 44.5	(5.56) 18.0	(16.67) 3.0	9	4.5
25th	SK17		"	"	3.0	(3.39) 29.5	(5.38) 46.5	17.0	(12.5) 4.0	8	4
26th	SK18		"	"	2.5	(3.57) 28.0	(4.12) 48.5	(3.23) 15.5	5.5	7	3.5
27th	SK19		"	"	3.5	(2.00) 25.0	(5.94) 50.5	16.5	(11.11) 4.5	8	4.0

						(2.99)	(6.25)	(6.90)	(8.33)		
28th	SK20	21	May-Grün wald's Giemsa	200	3.0	33.5	48.0	14.5	6.0	11	5.5
						(4.62)	(5.62)	(10.00)	(25.00)		
1st	SK21		"	"	4.0	32.5	44.5	15.0	4.0	13	6.5
						(3.23)	(6.10)	(6.90)	(11.11)		
2nd	SK22		"	"	4.5	31.0	41.0	14.5	9.0	11	5.5
						(3.04)	(5.21)	(2.63)	(16.67)		
3rd	SK23		"	"	2.5	27.5	48.0	19.0	3.0	9	4.5
						(3.13)	(5.06)	(4.76)			
4th	SK24		"	"	3.0	32.0	39.5	21.0	4.5	8	4.0
						(1.67)	(5.21)	(3.23)			
5th	SK25		"	"	2.0	30.0	48.0	15.5	4.5	7	3.5
						(4.00)	(4.44)	(2.56)	(5.88)		
6th	SK26		"	"	2.0	25.0	45.0	19.5	8.5	8	4.0
						(1.67)	(3.53)	(4.65)	(12.5)		
7th	SK27		"	"	2.0	30.0	42.5	21.5	4.0	7	3.5
						(1.69)	(3.45)	(5.41)	(9.09)		
8th	SK28		"	"	3.0	29.5	43.5	18.5	5.5	7	3.5
						(3.25)	(5.25)	(5.53)	(10.58)		
MEAN		21		200	3.63	29.11	45.89	16.13	4.59	9.57	4.80
s^2											1.04
s											1.02

TABLE 3(c)

DAY	ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
21st	PL1	25	May-Grün wald's Giemsa	200	4.0	30.5	44.0 (3.41)	17.0 (2.94)	4.5 (11.11)	5	2.5
22nd	PL2		"	"	3.0	28.0	48.0 (1.79)	15.5 (4.17)	5.5 (6.45)	7	3.5
23rd	PL3		"	"	4.0	27.5	45.5 (3.64)	20.0 (3.30)	3.0 (5.00)	7	3.5
24th	PL4		"	"	2.5	30.5	44.5 (3.37)	19.5 (5.13)	3.0 (16.67)	6	3.0
25th	PL5		"	"	4.5	31.5	46.0 (1.59)	14.5 (4.35)	3.5 (3.45)	6	3.0
26th	PL6		"	"	5.5	28.5	39.5	22.0	4.5	-	-
27th	PL7		"	"	4.0	24.5	43.0 (3.49)	19.5 (5.13)	9.0 (11.11)	7	3.5
28th	PL8		"	"	4.5	28.0	47.5 (1.79)	16.0 (4.21)	4.0 (9.38) (12.5)	9	4.5
1st	PL9		"	"	-	29.5	48.5 (3.39)	18.5 (5.15)	5.5 (5.41) (9.09)	10	5.0
2nd	PL10		"	"	4.5	23.5	44.5 (2.13)	24.5 (4.49)	3.0 (6.12) (16.66)	9	4.5
3rd	PL11		"	"	3.0	26.0	45.0 (1.92)	19.5 (4.44)	6.5 (5.13) (7.69)	8	4.0
4th	PL12		"	"	5.0	27.5	48.0 (1.82)	17.0 (5.21)	2.5 (5.88)	8	4.0
5th	PL13		"	"	3.5	31.5	43.5 (4.45)	17.0 (5.88)	4.5 (11.11)	6	3.0
6th	PL14		"	"	4.5	24.0	41.5 (2.08)	22.5 (3.61)	7.5 (4.44) (6.67)	7	3.5
7th	PL15		"	"	3.5	26.5	45.5 (2.20)	20.0 (5.00)	4.5 (11.11)	5	2.5
8th	PL16		"	"	3.5	27.5	49.5 (2.02)	14.0 (3.57)	5.5	3	1.5
9th	PL17		"	"	3.0	30.5	44.5 (1.64)	18.0 (3.37)	4.0 (2.78) (12.5)	6	3.0
10th	PL18		"	"	4.0	33.0	46.5 (1.52)	13.5 (4.30)	3.0 (7.41)	7	3.5
11th	PL19		"	"	5.5	29.5	39.5 (1.69)	21.0 (3.80)	4.5 (4.76) (11.11)	7	3.5
12th	PL20		"	"	4.0	23.5	48.5 (3.09)	20.0 (5.00)	4.0 (12.5)	6	3.0

							(2.04)	(4.55)	(4.55)	(9.09)	
13th	PL21	25	May-Grün wald's Giemsa	200	4.5	24.5	44.0	22.0	5.3	8	4.0
							(1.82)	(5.26)	(5.71)		
14th	PL22		"	"	4.0	27.5	47.5	17.5	3.5	8	4.0
							(1.67)	(3.33)	(3.13)		
15th	PL23		"	"	4.0	30.0	45.0	16.0	5.0	5	2.5
							(1.72)	(3.30)	(3.45)	(6.67)	
16th	PL24		"	"	3.5	29.0	45.5	14.5	7.5	6	3.0
							(2.04)	(4.55)	(4.17)		
17th	PL25		"	"	4.5	24.5	44.0	24.0	3.0	7	3.5
							(1.82)	(2.90)	(3.33)		
18th	PL26		"	"	8.0	27.5	34.5	30.0	4.0	5	2.5
							(1.64)	(3.37)	(6.25)	(11.11)	
19th	PL27		"	"	4.5	30.5	44.5	16.0	4.5	7	3.5
							(1.75)	(4.40)	(2.94)		
20th	PL28		"	"	1.5	28.5	45.5	17.0	7.5	6	3.0
							(1.41)	(4.26)	(4.33)	(6.31)	
MEAN		25		200	3.95	27.98	44.77	18.20	4.73	6.46	3.23
s^2											0.90
s											0.94

TABLE 3(d)

DAY	ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
						(3.51)	(3.25)	(6.25)	(10.00)		
21st	PC1	26	May-Grün wald's Giemsa	200	4.0	28.5	46.5	16.0	5.0	8	4.0
						(3.45)	(4.49)	(5.71)	(7.69)		
22nd	PC2		"	"	2.5	29.0	44.5	17.5	6.5	9	4.5
						(4.69)	(3.90)	(4.88)	(11.11)		
23rd	PC3		"	"	4.5	32.0	38.5	20.5	4.5	9	4.5
						(1.67)	(5.81)	(5.26)			
24th	PC4		"	"	3.5	30.0	43.0	19.0	4.5	8	4.0
						(2.13)	(4.40)	(5.00)			
25th	PC5		"	"	5.5	23.5	45.5	20.0	5.5	7	3.5
						(2.17)	(4.21)	(2.17)	(14.29)		
26th	PC6		"	"	3.0	23.0	47.5	23.0	3.5	7	3.5
						(1.92)	(4.49)	(2.33)			
27th	PC7		"	"	4.5	26.0	44.5	21.5	3.5	6	3.0
						(2.08)	(4.40)	(5.13)	(7.69)		
28th	PC8		"	"	4.5	24.0	45.5	19.5	6.5	8	4.0
						(2.26)	(5.05)	(5.56)	(9.09)		
1st	PC9		"	"	3.5	23.5	49.5	18.0	5.5	10	5.0
						(2.33)	(5.83)	(4.76)	(11.11)		
2nd	PC10		"	"	1.5	21.5	5.15	21.0	4.5	10	5.0
						(2.00)	(5.74)	(4.17)	(11.11)		
3rd	PC11		"	"	3.0	25.0	43.5	24.0	4.5	9	4.5
						(4.55)	(4.17)	(8.33)	(7.14)		
4th	PC12		"	"	5.0	22.0	48.0	18.0	7.0	10	5.0
						(1.47)	(5.60)	(4.44)	(9.09)		
5th	PC13		"	"	3.5	34.0	34.5	22.5	5.5	8	4.0
						(1.56)	(2.67)	(4.26)	(20.00)		
6th	PC14		"	"	4.5	32.0	37.5	23.5	2.5	7	3.5
						(1.72)	(3.37)	(2.56)			
7th	PC15		"	"	2.5	29.0	44.5	14.5	4.5	5	2.5
8th	PC16		"	"	3.5	28.5	46.5	16.0	5.5	-	-
						(2.22)	(5.05)	(4.76)			
9th	PC17		"	"	4.0	22.5	49.5	21.0	3.0	8	4.0
						(4.08)	(4.49)	(4.55)	(16.67)		
10th	PC18		"	"	6.0	24.5	44.5	22.0	3.0	9	4.5
						(2.17)	(3.15)	(2.56)	(11.11)		
11th	PC19		"	"	5.5	23.0	47.5	19.5	4.5	6	3.0
							(2.30)	(2.43)			
12th	PC20		"	"	4.5	28.0	43.5	20.5	3.5	3	1.5

							(4.35)	(5.56)	(11.11)		
13th	PC21	26	May-Grün wald's Giemsa	200	2.5	20.0	46.0	18.0	4.5	7	3.5
							(1.82)	(5.10)	(2.86)		
14th	PC22		"	"	2.5	27.5	49.0	17.5	3.5	7	3.5
							(1.85)	(3.96)	(3.23)		
15th	PC23		"	"	3.5	27.0	50.5	15.5	3.5	6	3.0
							(1.92)	(4.12)	(5.41)	(12.50)	
16th	PC24		"	"	3.0	26.0	48.5	18.5	4.0	8	4.0
							(2.15)	(5.26)	(9.09)		
17th	PC25		"	"	4.5	24.5	46.5	19.0	5.5	5	2.5
18th	PC26		"	"	5.0	23.5	43.5	21.5	6.5	-	0
							(2.11)	(2.63)	(14.29)		
19th	PC27		"	"	5.5	24.5	47.5	19.0	3.5	4	2.0
							(3.26)		(11.11)		
20th	PC28		"	"	1.5	27.5	46.0	20.5	4.5	4	2.0
							(1.84)	(3.84)	(3.93)	(1.29)	
MEAN		26		200	3.82	26.39	45.48	19.71	4.59	6.71	3.36
s^2											1.47
s											1.21

TABLE 3(e)

DAY	ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
						(1.72)	(3.37)	(5.41)	(10.00)		
2nd	LB1	29	May.Grün wald's Giemsa	200	3.0	29.0	44.5	18.5	5.0	7	3.5
						(1.61)	(5.06)	(2.43)	(11.11)		
3rd	LB2		"	"	4.5	31.0	39.5	20.5	4.5	7	3.5
						(1.87)	(4.90)	(6.06)			
4th	LB3		"	"	4.5	26.5	51.0	16.5	4.5	8	4.0
							(3.09)	(2.85)	(9.09)		
5th	LB4		"	"	4.0	24.5	48.5	17.5	5.5	5	2.5
						(1.64)	(3.66)	(5.00)	(14.29)		
6th	LB5		"	"	5.0	30.5	41.0	20.0	3.5	7	3.5
						(1.79)	(5.49)	(5.13)			
7th	LB6		"	"	3.0	28.0	45.5	19.5	4.0	8	4.0
						(1.82)	(3.19)	(3.13)	(9.09)		
8th	LB7		"	"	4.0	27.5	47.0	16.0	5.5	6	3.0
						(1.69)	(3.66)	(2.33)			
9th	LB8		"	"	3.5	29.5	41.0	21.5	4.5	5	2.5
							(2.04)	(4.12)	(5.26)		
10th	LB9		"	"	3.5	24.5	48.5	19.0	4.5	7	3.5
						(3.51)	(2.53)	(2.38)	(7.69)		
11th	LB10		"	"	4.5	28.5	39.5	21.0	6.5	6	3.0
						(3.17)	(3.37)	(3.45)			
12th	LB11		"	"	5.5	31.5	44.5	14.5	4.0	6	3.0
						(2.17)	(4.81)	(6.45)			
13th	LB12		"	"	4.5	23.0	52.0	15.5	3.0	8	4.0
						(3.51)	(5.95)	(2.70)			
14th	LB13		"	"	6.5	28.5	42.0	18.5	4.5	8	4.0
						(1.69)	(3.53)	(5.00)			
15th	LB14		"	"	3.5	29.5	42.5	20.0	4.5	7	3.5
						(1.67)	(5.13)	(4.88)	(10.00)		
16th	LB15		"	"	5.5	30.0	39.0	20.5	5.0	8	4.0
						(1.89)	(4.81)	(5.56)			
17th	LB16		"	"	4.5	26.5	52.0	18.0	3.5	8	4.0
						(2.08)	(1.03)	(5.13)	(11.11)		
18th	LB17		"	"	3.5	24.0	48.5	19.5	4.5	6	3.0
						(1.92)	(3.29)	(5.56)	(7.14)		
19th	LB18		"	"	3.5	26.0	45.5	18.0	7.0	7	3.5
						(1.75)	(4.30)	(6.06)	(11.11)		
20th	LB19		"	"	4.0	28.5	46.5	16.5	4.5	8	4.0
						(2.04)	(5.21)	(5.13)	(20.00)		
21st	LB20		"	"	5.5	24.5	48.0	19.5	2.5	9	4.5

						(3.17)	(4.30)	(10.34)	(14.29)		
22nd	LB21	29	May-Grün wald's Giemsa	200	4.0	31.5	46.5	14.5	3.5	10	5.0
						(1.67)	(4.30)	(9.68)			
23rd	LB22		"	"	4.5	30.0	46.5	15.5	3.5	8	4.0
						(4.65)	(4.12)	(4.88)	(11.11)		
24th	LB23		"	"	5.0	21.5	48.5	20.5	4.5	9	4.5
						(1.75)	(2.22)	(6.45)	(10.0)		
25th	LB24		"	"	6.0	28.5	45.0	15.5	5.0	6	3.0
						(3.23)	(4.08)	(7.14)			
26th	LB25		"	"	4.5	31.0	49.0	14.0	1.5	8	4.0
27th	LB26		"	"	4.5	25.5	51.5	15.5	3.0	-	0
						(1.64)	(5.49)	(3.57)	(12.50)		
28th	LB27		"	"	6.0	30.5	45.5	14.0	4.0	8	4.0
						(3.64)	(5.74)	(6.98)	(22.22)		
1st	LB28		"	"	3.0	27.5	43.5	21.5	4.5	12	6.0
MEAN		29		200	4.41	27.75	47.36	17.91	4.30	7.21	3.60
s^2											1.006
s											1.002

TABLE 4

Post-Menopausal Women

ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
01	91 Yrs	May-Grün wald's Giemsa	200	4	28 (1.79)	47.5 (2.11)	17.5 (8.57)	3.0 (16.67)	7	3.5
02	82	"	"	3.5	31.5 (3.17)	44.0 (3.41)	16.5 (3.03)	3.0	6	3.0
03	89	"	"	2.0	29	43.5 (1.15)	14.0 (3.57)	11.5	2	1.0
04	85	"	"	2.5	24.5 (2.04)	49.0 (3.06)	15.0 (6.67)	9.0 (5.56)	7	3.5
05	87	"	"	4.0	27.5	48.0 (3.13)	13.5 (3.70)	7.0 (7.14)	5	2.5
06	95	"	"	3.5	28.5	44.5 (1.12)	18.0 (2.78)	5.5 (9.09)	3	1.5
07	86	"	"	2.5	30.5 (4.91)	46.5 (3.23)	14.5 (3.45)	6.0 (8.33)	8	4.0
08	83	"	"	3.5	23.5 (2.13)	48.0 (3.13)	18.0 (2.78)	7.0 (7.14)	6	3.0
09	90	"	"	3.0	31.0 (1.61)	41.5 (3.61)	21.0 (4.76)	3.5 (14.29)	7	3.5
010	72	"	"	3.0	29.5 (1.69)	44.5 (3.37)	18.5 (2.70)	4.5	5	2.5
011	76	"	"	4.5	27.5	45.5 (3.30)	16.5 (6.06)	6.0 (8.33)	6	3.0
012	73	"	"	1.0	33 (1.52)	39.5 (3.80)	23.0 (4.35)	3.5 (14.29)	7	3.5
013	76	"	"	5.5	35.0 (1.43)	39.0 (5.13)	14.5 (6.90)	6.0 (8.33)	8	4.0
014	78	"	"	2.5	28.0	43.5 (3.45)	21.5 (4.65)	4.5	5	2.5
015	80	"	"	4.5	24.0	48.0 (5.21)	16.0 (6.25)	7.5 (6.67)	8	4.0
016	67	"	"	4.0	28.0 (1.79)	47.0 (1.06)	14.5 (10.34)	6.5 (7.69)	10	5.0
017	74	"	"	3.5	25.0 (2.00)	44.5 (4.49)	24.0 (4.17)	3.0 (16.67)	8	4.0
018	76	"	"	5.5	23.0	50.5 (4.95)	16.0 (6.25)	5.0	7	3.5
019	72	"	"	2.0	28.0	49.0 (2.04)	17.5 (2.86)	3.5	3	1.5
MEAN	80.63		200	3.39	28.16 (1.27)	45.45 (3.19)	17.37 (4.94)	5.5 (6.85)	6.21	3.11
S2									0.22	0.96
S									0.47	0.98

TABLE 5

Patients with Megaloblastic (mostly Pernicious) & Haemolytic Anaemias; Hbs .; and Suspected tuberculosis

ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
MA1	80	Mechanically Stained	200	1.5	(6.06) 16.5	(7.25) 34.5	(8.51) 23.5	(12.5) 24.0	17	8.5
MA2	85	"	"	1.0	(7.41) 13.5	(7.79) 38.5	(12.00) 25.0	(11.36) 22.0	19	9.5
MA3	79	"	"	0	(6.45) 15.5	(7.69) 32.5	(11.86) 29.5	(15.56) 22.5	21	10.5
MA4	63	"	"	1.5	(2.86) 17.5	(4.41) 34.0	(13.64) 22.0	(14.00) 25.0	17	8.5
MA5	71	"	"	2.5	(5.13) 19.5	(10.00) 35.0	(14.80) 25.0	(19.44) 18.0	23	11.5
PA6	66	"	"	1.5	(3.70) 13.5	(6.17) 40.5	(13.73) 25.5	(15.79) 19.0	19	9.5
PA7	69	"	"	0.5	(7.14) 14.0	(7.59) 39.5	(13.64) 22.0	(17.39) 23.0	22	11.0
PA8	65	"	"	1.5	(5.41) 18.5	(5.48) 36.5	(12.5) 24.0	(10.26) 19.5	16	8.0
HA9	Unknown	"	"	2.5	(6.06) 16.5	(4.94) 40.5	(16.67) 18.0	(15.56) 22.5	19	9.5
HA10	"	"	"	2.0	(8.33) 18.0	(8.70) 34.5	(18.18) 22.0	(19.15) 23.5	16	13.0
HA11	"	"	"	3.0	(7.41) 14.5	(9.33) 37.5	(15.38) 19.5	(15.69) 25.5	23	11.5
Hbs12	"	"	"	1.5	(9.68) 15.5	(4.94) 40.5	(12.5) 20.0	(13.33) 22.5	18	9.0
Hbs13	"	"	"	(25.00) 2.0	(5.13) 19.5	(5.81) 43.0	(16.67) 18.0	(20.00) 17.5	21	10.5
TB14	77	"	"	1.0	(9.09) 16.5	(7.89) 38.0	(16.28) 21.5	(19.57) 23.0	25	12.5
TB15	82	"	"	0.5	(2.38) 21.0	(6.41) 39.0	(14.29) 21.0	(29.73) 18.5	23	11.5
MEAN			200	(1.67) 1.5	(6.15) 18.67	(6.96) 37.6	(13.99) 22.43	(16.62) 21.73	20.6	10.3
s^2										2.16
s										1.47

TABLE 6

Cattle

ID NO.	AGE	STAIN	T.WBC	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTA %
C1	18 mth	May-Grün wald's Giemsa	12,422	200	4	(2.08) 24	(3.61) 41.5	(2.27) 22.0	(11.76) 8.5	7	3.5
C2	27	"	10,444	"	3.5	(2.56) 19.5	(2.25) 44.5	(2.50) 20.0	(8.0) 12.5	6	3.0
C3	24	"	10,111	"	3.5	(4.0) 25.0	(2.60) 38.5	(4.17) 24.0	9.0	6	3.0
C4	24	"	11,144	"	2.5	26.5	43.0	(4.76) 21.0	(7.14) 7.0	3	1.5
C5	15	"	13,322	"	4.5	27.5	46.0	17.0	5.0	0	0
C6	27	"	13,088	"	4.5	(3.17) 31.5	(1.69) 29.5	(6.00) 25.0	(10.53) 9.5	8	4.0
C7	18	"	13,488	"	5.5	(2.13) 23.5	(2.56) 39.0	(6.25) 24.0	(12.5) 8.0	8	4.0
C8	24	"	9,488	"	2.0	(4.17) 24.0	(3.70) 40.5	(4.08) 24.5	(5.56) 9.0	8	4.0
C9	30	"	8,911	"	2.5	(1.79) 28.0	(2.90) 34.5	(4.17) 24.0	(4.55) 11.0	6	3.0
C10	24	"	8,244	"	1.5	(3.77) 26.5	(3.90) 38.5	(4.00) 25.0	(11.76) 8.5	9	4.5
C11	20	"	9,311	"	3.5	(2.27) 22.0	(2.74) 36.5	(3.64) 27.5	(4.76) 10.5	7	3.5
C12	20	"	7,289	"	3.5	(2.63) 19.0	(2.44) 41.0	(1.72) 29.0	(6.67) 7.5	5	2.5
C13	24	"	6,933	"	3.5	(3.03) 16.5	(1.12) 44.5	(2.17) 23.0	12.5	3	1.5
C14	27	"	7,844	"	5.5	25.5	(2.20) 45.5	(2.44) 20.5	(16.67) 3.0	4	2.0
C15	24	"	13,422	"	3.0	27.0	(1.12) 44.5	(8.22) 18.0	(13.33) 7.5	6	3.0
C16	20	"	11,888	"	4.0	(2.17) 23.0	(2.15) 46.5	(5.56) 18.0	8.5	5	2.5
C17	27	"	6,311	"	4.5	(2.08) 24.0	(3.09) 48.5	(5.56) 20.0	(16.67) 3.0	7	3.5
C18	24	"	9,488	"	4.5	18.5	(2.27) 44.0	(4.35) 23.0	(5.00) 10.0	5	2.5
C19	30	"	11,289	"	3.5	(2.56) 19.5	(5.56) 45.0	(4.88) 20.5	(8.70) 11.5	10	5.0
C20	27	"	12,111	"	5.0	(3.23) 31.0	(3.49) 43.0	(3.03) 16.5	(11.11) 4.5	7	3.5
MEAN	23.7		10,282.4	200	3.73	(2.08) 24.1	(2.47) 41.73	(3.99) 21.23	(7.74) 7.63	6.0	3.0
S2											1.28
S											1.13

TABLE 7

Sheep

ID NO.	AGE	STAIN	T.WBC	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTA %
						(6.90)	(4.29)	(5.00)	(5.71)		
S1	15 mths	May-Grün wald's Giemsa	6,933	200	3.0	14.5	35.0	30.0	17.5	10	5.0
						(3.03)	(3.66)	(4.08)	(3.70)		
S2	9 "	"	7,755	"	4.5	16.5	41.0	24.5	13.5	7	3.5
						(3.70)	(4.71)	(6.00)	(3.70)		
S3	9 "	"	6,622	"	5.5	13.5	42.5	25.0	13.5	9	4.5
						(3.44)	(4.49)	(5.45)	(4.55)		
S4	12 "	"	7,288	"	2.5	14.5	44.5	27.5	11.0	9	4.5
						(2.86)	(6.59)	(6.38)	(5.00)		
S5	18 "	"	5,755	"	3.5	17.5	45.5	23.5	10.0	11	5.5
						(6.06)	(2.63)	(3.70)	(3.33)		
S6	18 "	"	8,244	"	3.5	16.5	38.0	27.0	15.0	7	3.5
						(2.38)	(3.70)	(5.66)	(6.25)		
S7	6 "	"	4,622	"	4.0	21.0	40.5	26.5	8.0	8	4.0
						(3.23)	(5.62)	(6.00)	(3.85)		
S8	18 "	"	7,488	"	2.0	15.5	44.5	25.0	13.0	9	4.5
							(1.45)	(5.00)	(8.33)		
S9	18 "	"	7,282	"	4.0	19.5	34.5	30.0	12.0	6	3.0
						(2.50)	(2.74)	(5.66)	(3.85)		
S10	20 "	"	6,044	"	4.0	20.0	36.5	26.5	13.0	7	3.5
						(3.57)	(2.30)	(4.08)	(6.67)		
S11	18 "	"	7,200	"	3.0	14.0	43.5	24.5	15.0	7	3.5
						(6.90)	(3.53)	(3.57)	(8.00)		
S12	20 "	"	6,155	"	2.5	14.5	42.5	28.0	12.5	9	4.5
							(2.20)	(6.90)	(4.00)		
S13	18 "	"	4,777	"	4.5	23.0	45.5	14.5	12.5	5	2.5
						(9.67)	(5.06)	(6.00)	(3.23)		
S14	6 "	"	7,244	"	4.5	15.5	39.5	25.0	15.5	11	5.5
						(5.56)	(3.41)	(5.00)	(3.57)		
S15	15 "	"	5,156	"	4.0	18.0	44.0	20.0	14.0	8	4.0
						(3.33)	(3.49)	(6.67)	(8.70)		
S16	12 "	"	6,022	"	3.5	15.0	43.0	22.5	11.5	9	4.5
						(3.45)	(4.60)	(5.66)	(8.70)		
S17	9 "	"	6,088	"	4.0	14.5	43.5	26.5	11.5	10	5.0
						(4.55)	(5.56)	(9.09)	(12.5)		
S18	9 "	"	4,655	"	3	22.0	45.0	22.0	8.00	13	6.5
						(2.86)	(3.23)		(7.41)		
S19	18 "	"	7,288	"	2.5	17.5	46.5	20.0	13.5	6	3.0
						(3.03)	(3.45)	(4.26)	(6.67)		
S20	6 "	"	7,755	"	1.5	16.5	43.5	23.5	15.0	8	4.0
						(3.85)	(3.84)	(5.21)	(5.87)		
MEAN	13.7 mths		6,518.65	200	3.48	16.98	43.7	24.6	12.78	8.45	4.23
S2											0.91
S											0.95

TABLE 8

Pigs

ID NO.	AGE	STAIN	T.WBC	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
G1	6 mths	May.Grün wald's Giemsa	20,333	200	3.5	(2.86) 17.5	(3.53) 42.5	(5.88) 25.5	(9.09) 11.0	9	4.5
G2	6 "	"	15,888	"	5.5	13.5	(2.74) 36.5	(5.66) 26.5	(8.33) 18.0	8	4.0
G3	9 "	"	21,822	"	4.5	(3.22) 15.5	(3.57) 44.5	(6.12) 24.5	(9.09) 11.0	9	4.5
G4	9 "	"	17,755	"	4.0	(3.03) 16.5	(4.40) 45.5	(6.25) 24.0	(10.0) 10.0	10	5.0
G5	9 "	"	17,733	"	3.0	(2.70) 18.5	(6.49) 38.5	(5.77) 26.0	(7.14) 14.0	11	5.5
G6	6 "	"	3,866	"	5.5	(7.14) 14.0	(6.80) 43.0	(8.89) 22.5	(10.0) 15.0	15	7.5
G7	6 "	"	21,755	"	2.5	(3.23) 15.5	(5.74) 43.5	(5.36) 28.0	(9.52) 10.5	11	5.5
G8	9 "	"	20,822	"	3.5	18.0	(4.35) 46.0	(5.88) 25.5	(14.29) 7.0	9	4.5
G9	9 "	"	17,755	"	3.0	14.0	(4.55) 44.0	(5.66) 26.5	(4.00) 12.5	8	4.0
G10	12 "	"	17,888	"	4.5	(3.03) 16.5	(6.58) 38.0	(5.00) 30.0	(4.55) 11.0	10	5.0
G11	9 "	"	20,755	"	5.0	(2.44) 20.5	(4.82) 41.5	(6.38) 23.5	(5.26) 9.5	9	4.5
G12	12 "	"	21,822	"	1.5	(3.57) 14.0	(6.74) 44.5	(8.16) 24.5	(3.23) 15.5	12	6.0
G13	9 "	"	17,755	"	3.5	18.5	48.5	(5.00) 20.0	(5.26) 9.5	3	1.5
G14	6 "	"	21,822	"	2.5	13.0	(4.95) 50.5	(3.92) 25.5	(5.88) 8.5	8	4.0
G15	6 "	"	20,333	"	4.5	14.5	(2.63) 38.0	(5.88) 25.5	(5.71) 17.5	7	3.5
G16	9 "	"	15,866	"	3.0	16.0	(1.20) 41.5	(2.04) 24.5	(6.67) 15.0	5	2.5
G17	12 "	"	7,822	"	3.0	(2.17) 23.0	(5.88) 34.0	(7.84) 25.5	(9.67) 15.5	12	6.0
G18	12 "	"	20,333	"	5.5	(3.70) 13.5	(4.00) 37.5	(6.52) 23.0	(6.45) 15.5	9	4.5
G19	9 "	"	21,822	"	4.5	(2.56) 19.5	(6.25) 40.0	(6.78) 21.5	(3.45) 14.5	10	5.0
G20	6 "	"	17,733	"	4.0	15.0	43.5	(2.56) 19.5	(2.78) 18.0	2	1.0
MEAN	8.55		18,084	200	3.83	(1.98) 16.35	(4.25) 42.08	(5.78) 24.6	(6.70) 12.95	8.85	4.43
S2											1.37
S											1.17

TABLE 9

Dogs

ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
						(4.29)	(9.30)	(9.43)		
D1	1 Yr	May-Grün wald's Giemsa	200	5.5	11.5	35.0	21.5	26.5	12	6
						(3.57)	(3.03)	(7.14)	(5.41)	
D2	1½	"	"	6.5	14.0	33.0	28.0	18.5	9	4.5
						(2.53)	(4.08)	(6.82)		
D3	7	"	"	4.5	9.5	39.5	24.5	22.0	9	4.5
						(3.61)	(6.38)	(6.67)		
D4	8	"	"	3.5	16.5	41.5	23.5	15.0	8	4.0
						(3.38)	(5.36)	(5.88)		
D5	3	"	"	6.0	19.5	29.5	28.0	17.0	7	3.5
						(3.57)	(2.78)	(5.08)	(6.67)	
D6	3	"	"	5.5	14.0	36.0	29.5	15.0	7	3.5
						(2.44)	(4.55)	(6.82)	(10.00)	
D7	5	"	"	3.5	20.5	44.0	22.0	10.0	10	5.0
						(2.86)	(2.46)	(2.04)	(3.70)	
D8	5	"	"	4.0	17.5	40.5	44.5	13.5	6	3.0
						(3.44)	(3.53)	(4.44)	(10.00)	
D9	7	"	"	5.5	14.5	42.5	22.5	15.0	9	4.5
						(2.78)	(4.78)	(3.39)	(6.90)	
D10	2½	"	"	4.5	18.0	33.5	29.5	14.5	8	4.0
						(3.70)	(3.90)	(3.27)	(7.69)	
D11	4	"	"	5.0	13.0	38.5	30.5	13.0	8	4.0
						(3.13)	(3.49)	(4.08)	(3.85)	
D12	6	"	"	3.5	16.0	43.0	24.5	13.0	7	3.5
						(3.23)	(4.88)	(3.57)		
D13	9 mths	"	"	4.5	14.5	46.5	20.5	14.0	6	3.0
						(2.67)	(3.64)	(6.67)		
D14	7 yrs	"	"	4.5	15.4	37.5	27.5	15.0	6	3.0
						(4.35)	(3.45)	(1.89)	(3.85)	
D15	11 "	"	"	5.5	11.5	43.5	26.5	13.0	6	3.0
						(1.07)	(4.30)	(4.79)	(6.47)	
MEAN	4.78		200	4.0	21.93	38.93	25.53	15.67	7.87	3.93
s^2										0.70
s										0.83

TABLE 10

Cats

ID NO.	AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
A1	2½ Yrs	May-Grün wald's Giemsa	200	3.0	14.5	38.0 (2.56)	28.5 (5.26)	15.0 (6.67)	8	4.0
A2	6 mths	"	"	4.5	15.5	41.0 (1.22)	26.5 (3.77)	17.5 (5.71)	5	2.5
A3	4 yrs	"	"	4.0	14.0	44.5 (3.57)	23.0 (3.37)	14.5 (4.35) (3.45)	7	3.5
A4	4½ "	"	"	5.5	18.0	38.0 (2.78)	25.0 (2.63)	13.5 (6.00) (3.70)	7	3.5
A5	3 "	"	"	4.5	16.5	41.5 (3.61)	26.5 (3.77)	11.0 (4.55)	6	3.0
A6	2½ "	"	"	4.5	18.5	43.5 (2.70)	22.5 (3.45)	11.0 (6.67) (4.55)	8	4.0
A7	9 mths	"	"	3.5	14.5	39.5 (5.06)	29.0 (5.17)	13.5 (3.70)	8	4.0
A8	5 yrs	"	"	4.0	12.5	40.5 (3.70)	26.5 (3.77)	16.5 (6.06)	7	3.5
A9	3 "	"	"	6.0	21.0	34.5 (2.38)	26.0 (2.90)	12.5 (1.92) (4.00)	6	3.0
A10	1½ "	"	"	3.0	18.0	38.5 (2.63)	29.5 (3.90)	10.0 (1.69) (5.00)	6	3.0
A11	3 "	"	"	4.0	15.5	46.0 (3.23)	25.5 (2.17)	9.0 (1.96) (5.56)	5	2.5
A12	7 "	"	"	2.5	13.5	43.0 (3.70)	26.0 (5.81)	15.0 (5.77) (6.67)	11	5.5
A13	5 "	"	"	7.0	16.0	42.5 (3.13)	29.5 (4.71)	5.0 (5.08) (10.00)	9	4.5
A14	5 "	"	"	5.5	18.0	39.5 (2.78)	20.0 (3.80)	17.0 (5.00) (2.90)	7	3.5
A15	4½ "	"	"	4.5	14.5	40.5 (3.44)	22.5 (3.70)	18.0 (4.44) (5.56)	8	4.0
A16	5 "	"	"	4.5	17.5	45.0 (3.33)	25.0 (8.00)	7.0 (14.29)	9	4.5
MEAN	3.55		200	4.41	17.09	41.0 (1.90)	25.72 (3.50)	12.88 (4.54) (5.77)	7.32	3.66
s^2										0.59
s										0.77

TABLE 11

Monkeys

ID NO.	APPROX AGE	STAIN	POLY MORPHS	ARNETH COUNT% DRUMSTICKS(%) 1	2	3	4	5+	TOTAL DRUM STICKS	TOTAL %
					(2.56)	(1.59)	(8.70)	(12.24)		
M1	6 yrs	May-Grün wald's Giemsa	200	1.5	19.5	31.5	23.0	24.5	12	6.0
						(2.82)	(9.52)	(17.02)		
M2	6 "	"	"	2.5	17.5	35.5	21.0	23.5	14	7.0
					(5.41)	(2.99)	(7.69)	(12.77)		
M3	6 "	"	"	2.5	21.0	33.5	19.5	23.5	11	5.5
					(2.17)	(4.11)	(11.36)	(17.50)		
M4	4 "	"	"	3.0	18.5	36.5	22.0	20.0	17	8.5
					(2.03)	(2.90)	(9.52)	(15.38)		
M5	4 "	"	"	2.0	23.0	34.5	21.00	19.5	13	6.5
					(2.43)	(2.88)	(9.36)	(14.98)		
MEAN			200	2.3	19.9	34.3	21.3	22.5	13.4	6.7
S^2										7.11
S										2.67

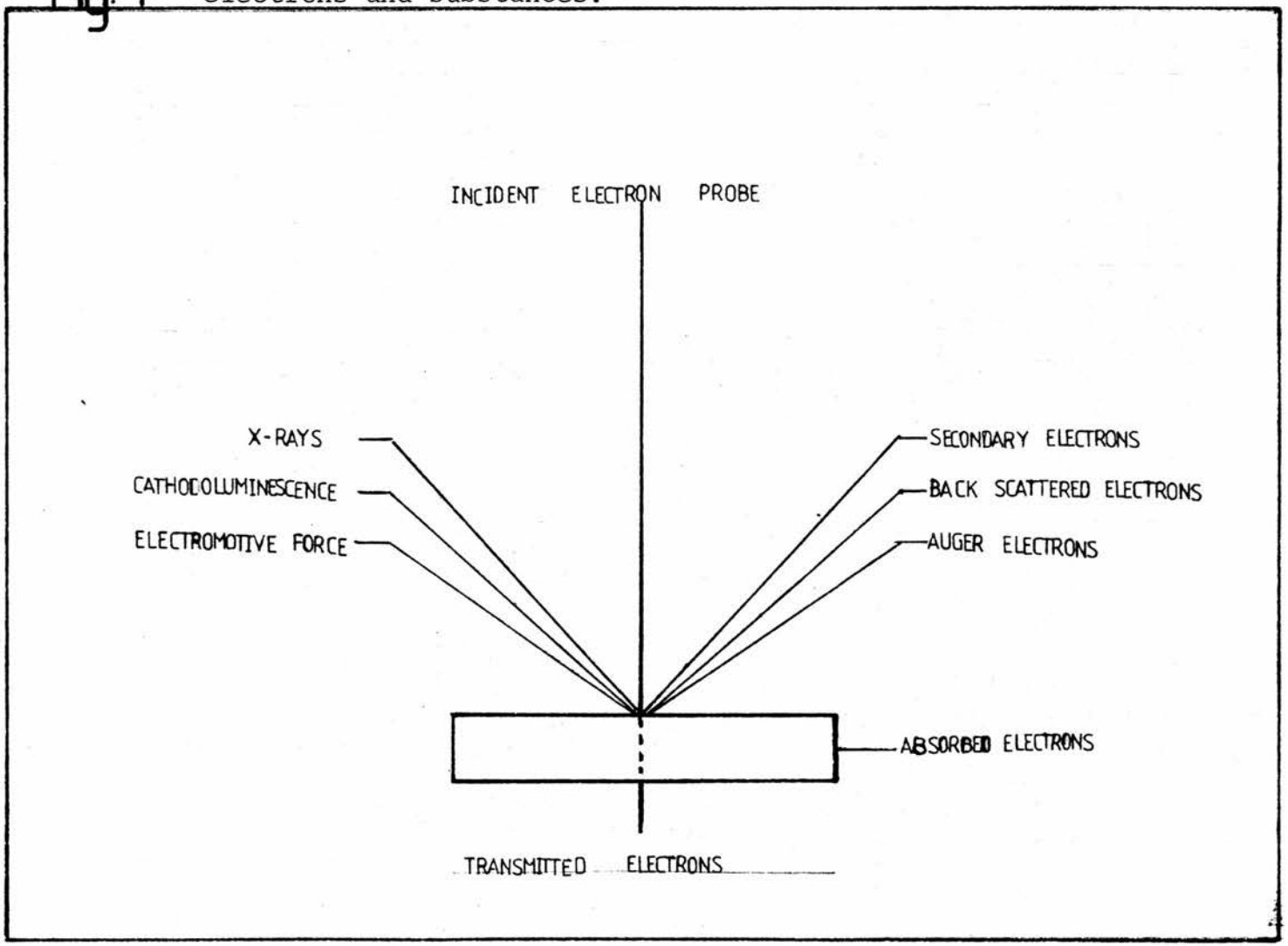
ZOO MAMMALS

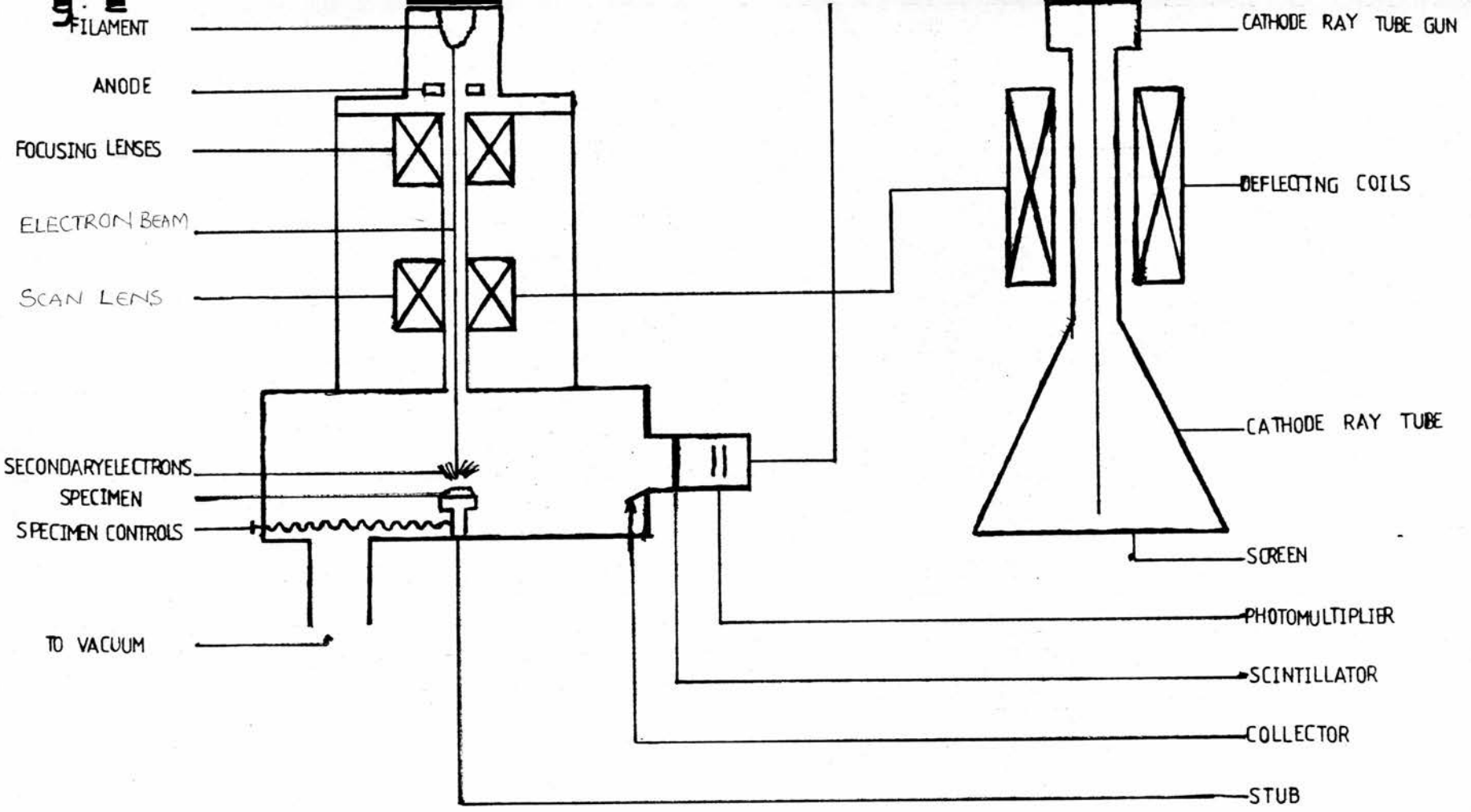
H1

Table 12

					(4.76)	(5.71)	(8.70)	(17.65)		
Hyaena	13	May-Grün wald's Giemsa	200	4.0	21.0	35.0	23.0	17.0	8	4.0
						(4.55)	(3.03)	(20.0)	(27,27)	
LM1 Langur Monkey Table 13		Unknown	200	3.0	22.0	33.0	20.0	22.0	12	6.0

Fig. 1 Kinds of signals produced by the interactions between incident electrons and substances.





Structure and formation of image on the Scanning Electron Microscope.

Fig. 3 Graph to show variation of drumsticks in the thirteen groups.

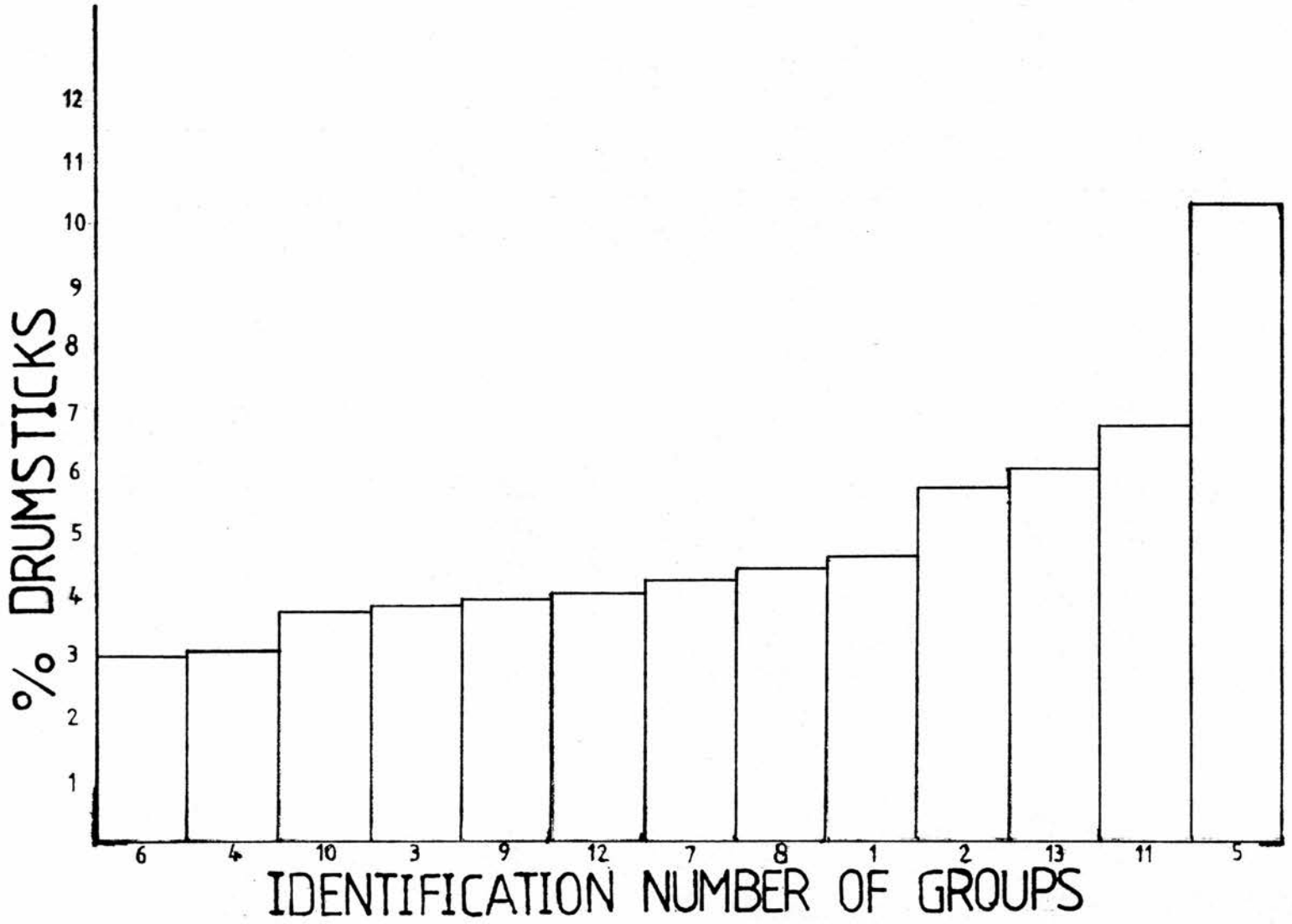


Fig. 4 ... of the polymorphonuclear leucocyte and the (%) of drumsticks.

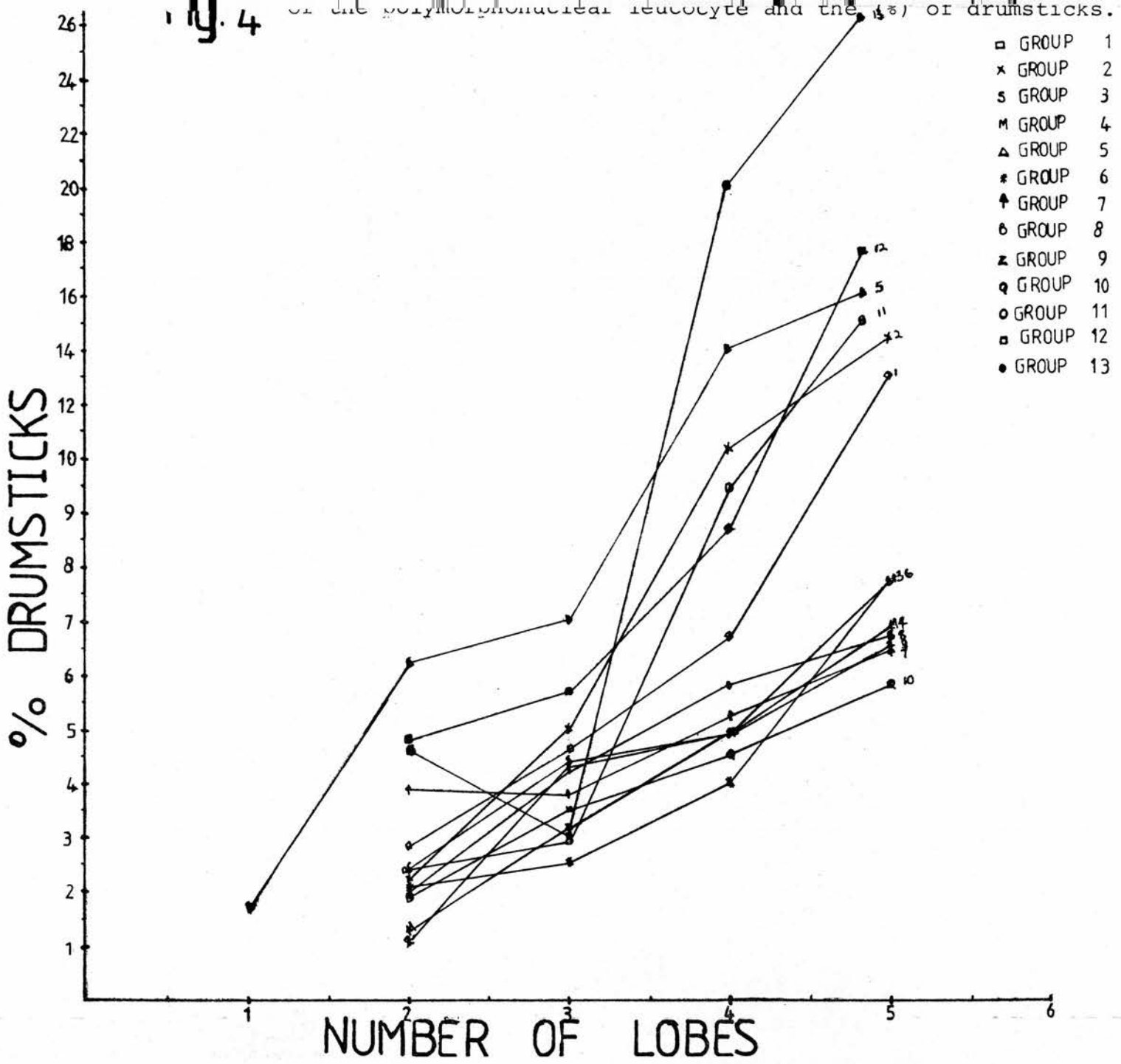


Fig. 5

Graph to show incidence of drumsticks in five normal women throughout one menstrual cycle.

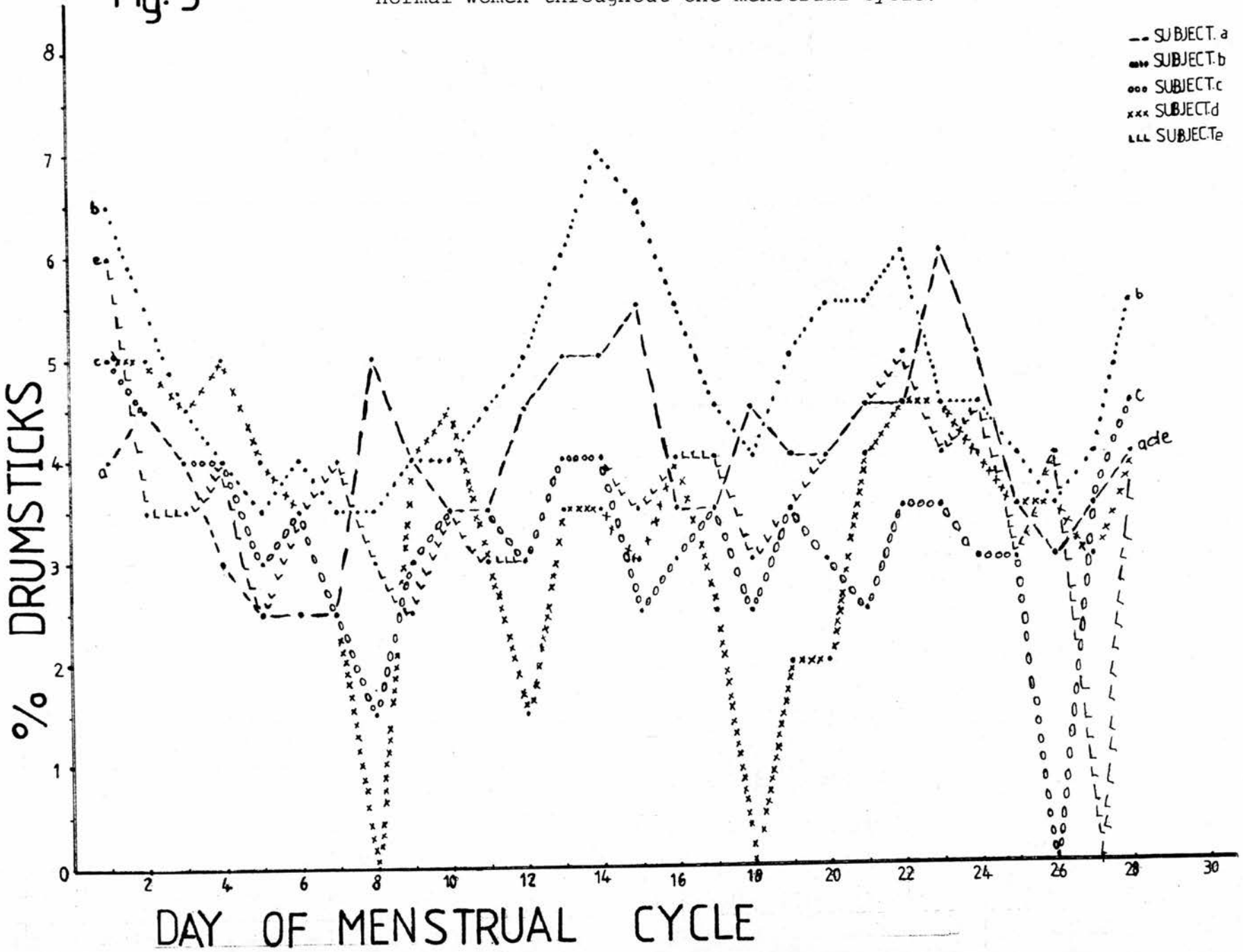
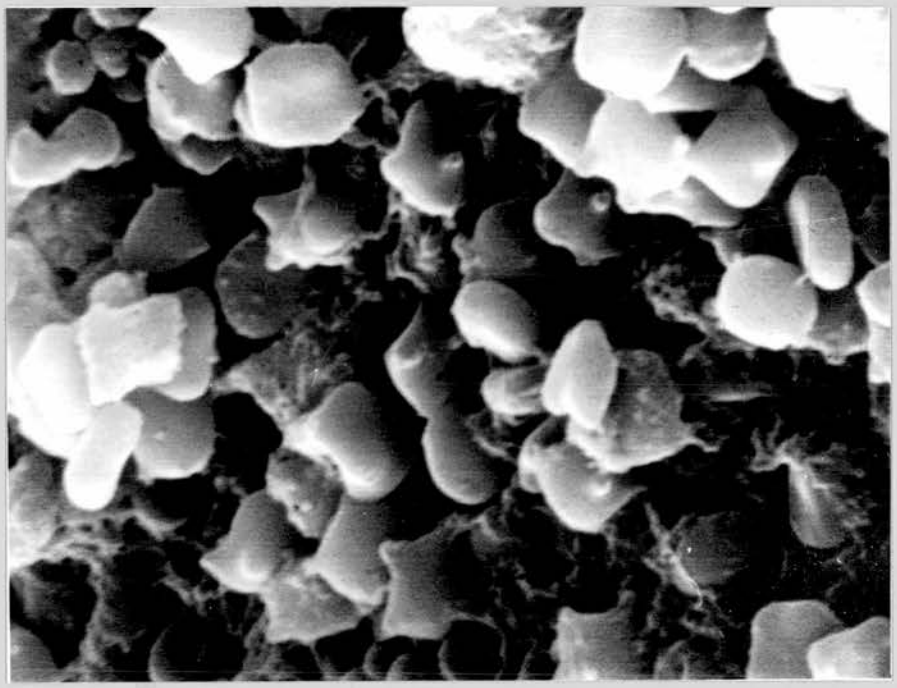
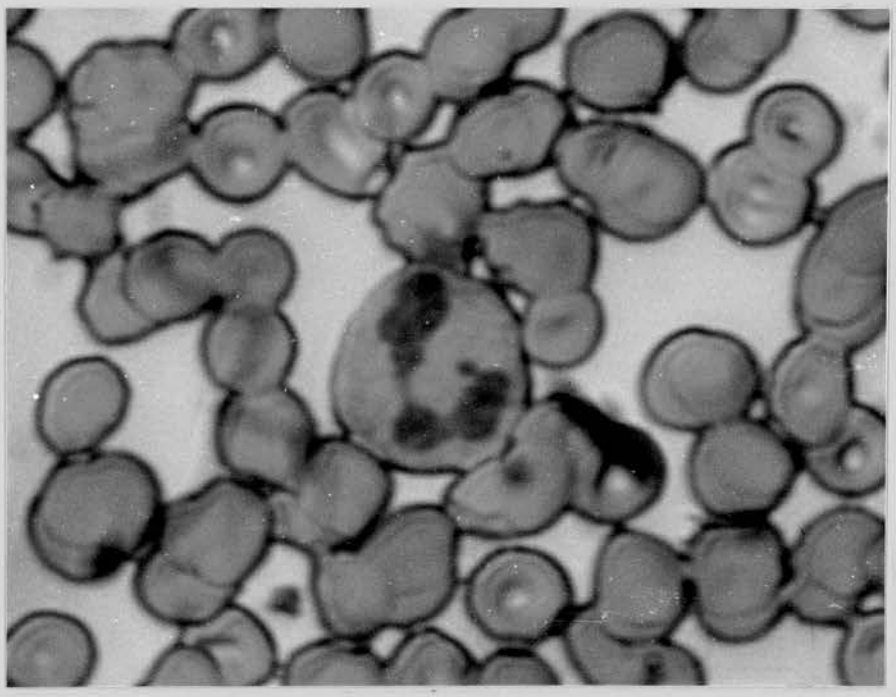


Fig. 2a

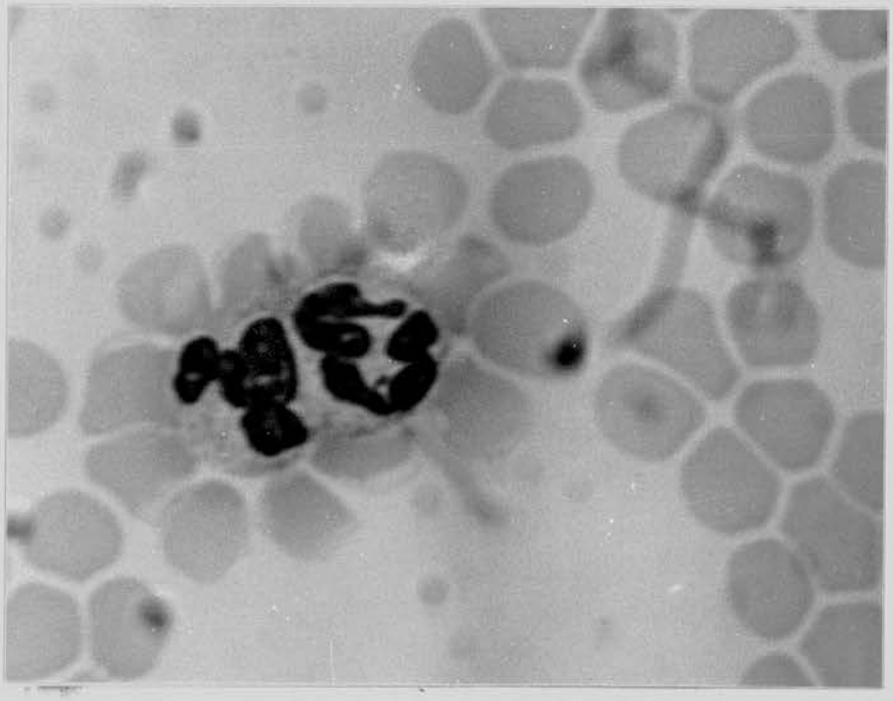


2(a) WHITE AND RED BLOOD CELLS AS SEEN UNDER THE SCANNING ELECTRON MICROSCOPE

Fig. 6a & b

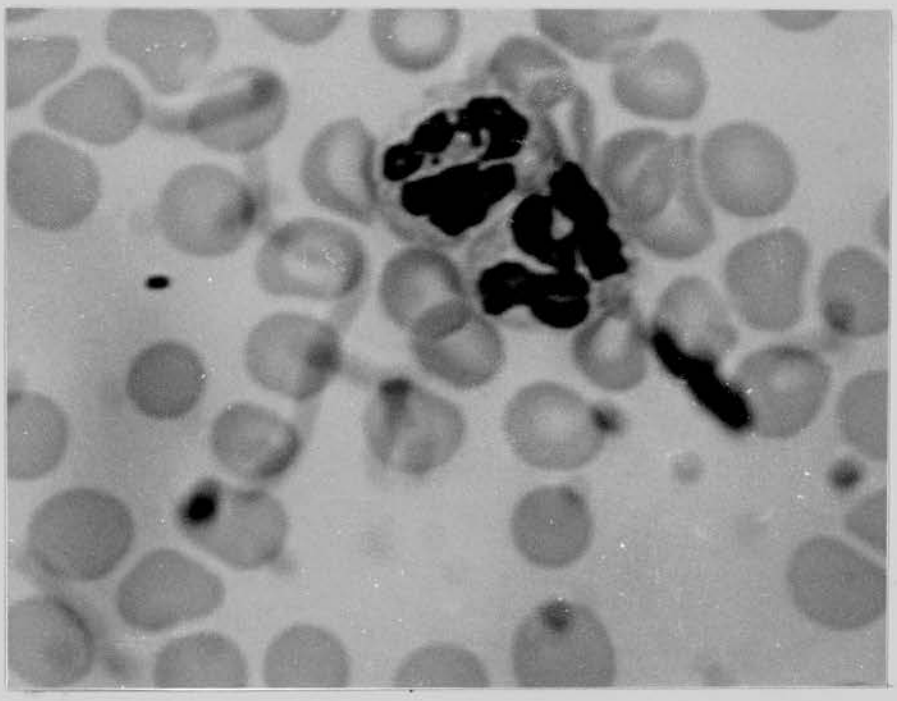


6(a) A NORMAL FEMALE THREE LOBED NEUTROPHIL SHOWING DRUMSTICK

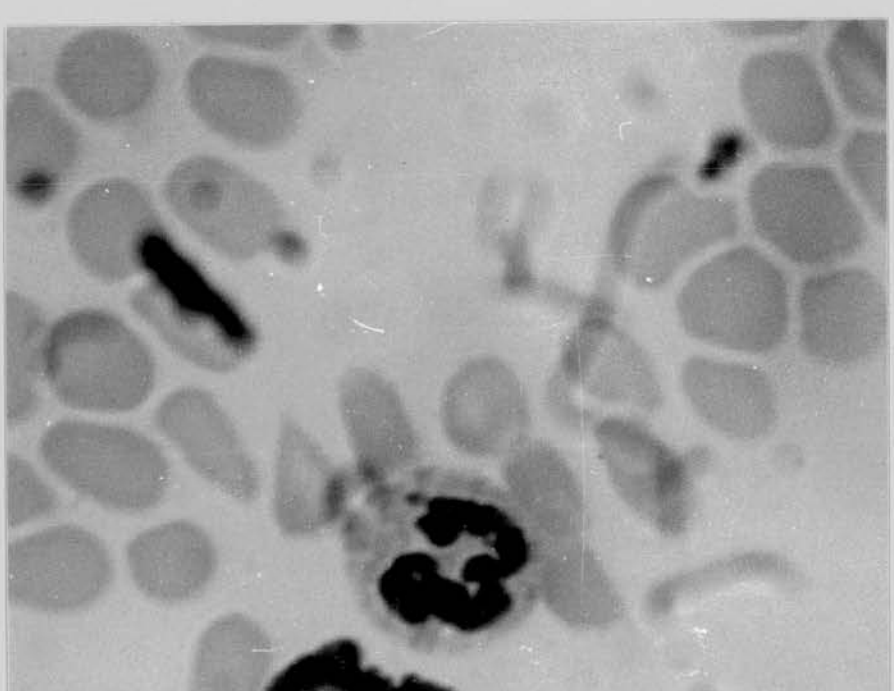


(b) A FEMALE PATIENT WITH MEGALOBLASTIC ANAEMIA SHOWING DRUMSTICK

Fig. 7a & b

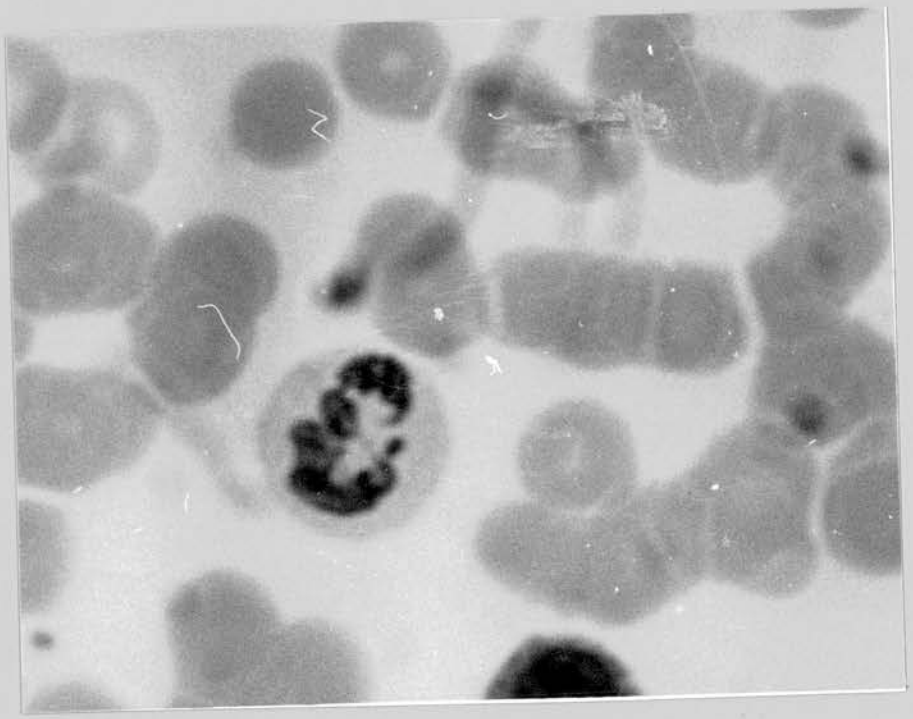


7(a) PIG DRUMSTICK

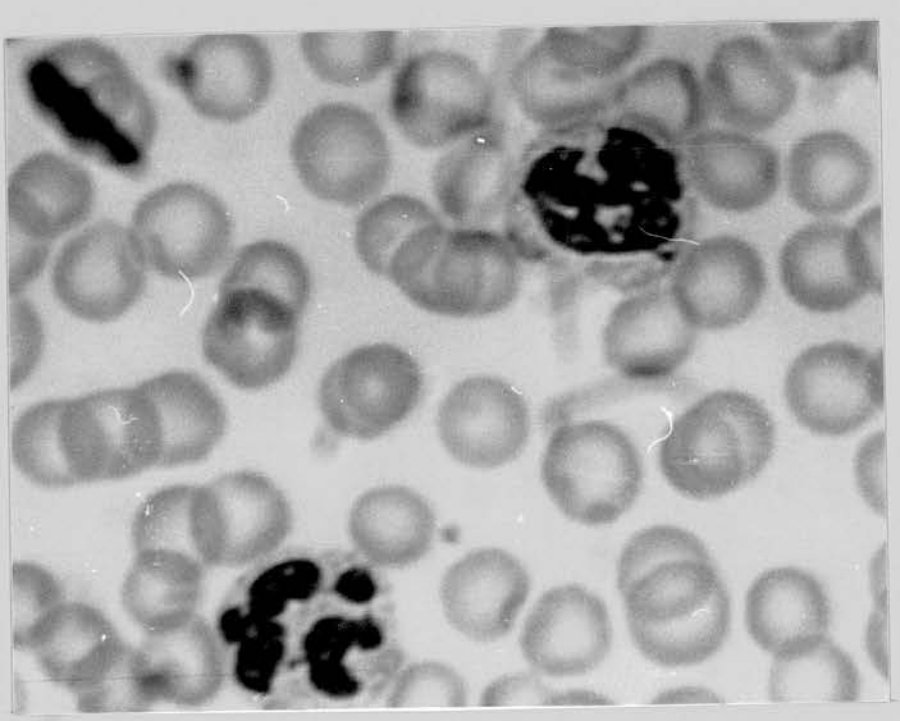


(b) SHEEP DRUMSTICK

Fig. 8a & b

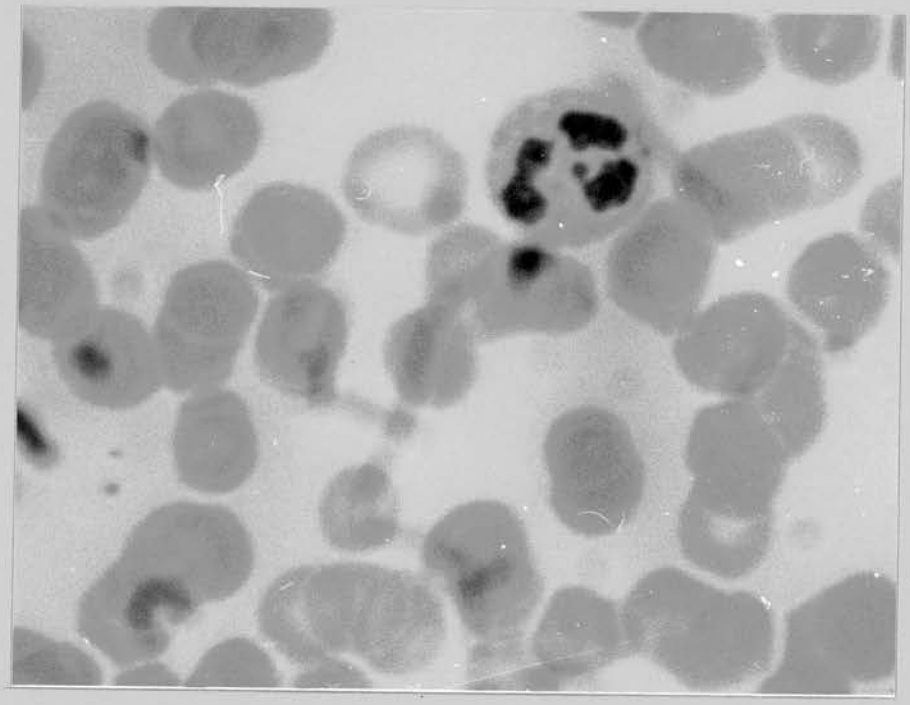


8(a) CATTLE DRUMSTICK

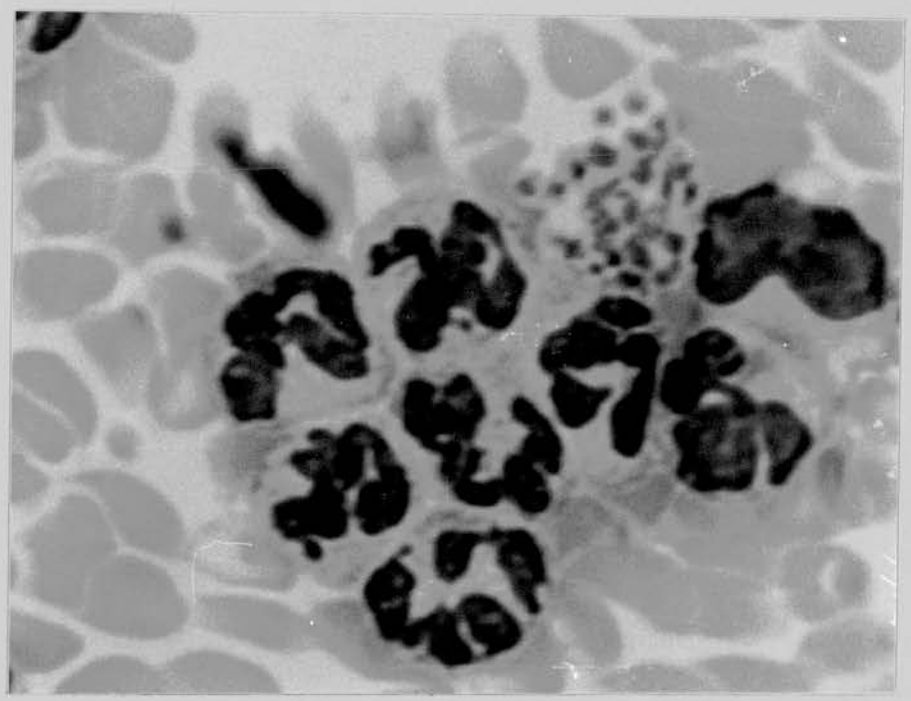


(b) DOG DRUMSTICK

Fig. 9a & b



9(a) CAT DRUMSTICK



(b) MONKEY DRUMSTICKS WITH SOME SESSILE NODULES AND ROD SHAPED APPENDAGE

DISCUSSION

This comparative study of the incidence of Barr Bodies in mammalian blood was investigated in the following groups :-

1. New-born babies and pre-pubertal girls;
2. Pregnant women;
3. Pre-menopausal women and the influence of the menstrual cycle;
4. Post-menopausal women;
5. The megaloblastic anaemias;
6. Cows;
7. Sheep;
8. Pigs;
9. Cats;
10. Dogs;
11. Monkeys (stub-tailed Macaques);
12. Other exotic animals - hyena (1case);
13. Langur Monkey (1case).

The mean incidence of drumsticks was worked out for each group followed by an analysis of the variance from which the standard deviation was worked out. In groups 12 and 13, the mean, variance and standard deviation was not calculated because only one sample from each was available.

In calculating the total white cell count, errors can be introduced through dilution of blood with the white cell diluting fluid. This was avoided by doing a double count for every sample. It was not possible to do the total white cell count for every group because of problems encountered.

1. Pet owners refused to allow more than minimal interference;
2. Over-reaction prevented more than minimal interference, particularly in the monkeys examined;

3. Reluctance by some patients to submit to more than a minimal test (blood film) by a non-medical researcher.

In the graph showing the incidence of drumsticks throughout the menstrual cycle, it would have been possible to show the mean incidence for the five girls to avoid confusion in tracing individual points on the sheet. I thought it was not wise, since I will not be showing the raw data obtained. This was the reason for using different signs rather than straight lines. Some of the points were co-incident on some days. For the Arneth count, it was observed in most of the animals, some humans and patients with pathological conditions, that they were cells with six or seven lobes. It would have been possible to extend the groupings up to seven, since Cooke modified the Complex Classification of Arneth, who designated as many as fifteen different nuclear types, into five. We adopted his method, thus making it easier to perform the lobe count. This method also has a definite value in diagnosis, prognosis and treatment of patients. Granulocytes in which the nucleus is either unlobed or lobed and the lobes joined by definite band of chromatin belong to group one; if the materials joining the lobes is a single or double filament and two complete lobes present, it belongs to group 2. Group 3 includes those with two or four filaments with three lobes. Group 4 three or six filaments with four lobes and Group 5 four or eight with five or more lobes. This approach made it unnecessary to look at monocytes and lymphocytes, although it has been published that drumsticks can be identified in these cells (26; 27; 73; 74). In order to interpret the results obtained in this project one must have a clear idea of what the drumstick or sex chromatin is and how it is related to X Chromosomes. This, apart from what happens under pathological conditions, must also be taken into account.

It appears that sex chromatin/drumsticks are formed by one X Chromosome (68; 69). Ohno's findings form the basis of the Lyon Hypothesis (48). One of the two X Chromosomes in the cell of a normal female mammal is genetically inactivated. The inactive X can be either maternal or paternal one in different cells of the animal. The inactivation occurs early in embroyonic development and once it has occurred, remains fixed throughout the development of each cell line. The inactivation which occurs early in embroyonic stage involves the development of precocius condensation during prophase and formation of a peripherally situated heteropyknotic body in the resting nuclei. Delayed replication of DNA during the Synthetic S - Stage of interphase and the switching off of the genetic loci or some of them in the transformed X. Grinberg et al (32) in his studies showed a late replicating pattern of DNA. They showed that the sex chromatin is formed by a greater part of the late replicating X Chromosome by using radioactive labelling. Similar investigations were carried out on drumsticks of the polymorphonuclear leucocytes. They found that its nucleus was composed of two blocks of chromosomes which replicate over two different portions of the S-Stage. One block constitutes the bulk of the nucleus, while the small asynchronus material composes the drumsticks, which means that the drumstick is made up of the late replicating X Chromosome. Park, W.W. (71) tried to establish at what stage of development in tissues sex chromatin appears and its distribution at early stages of development. The human and Macaque embryos used in his studies were aged between 36 hours to 24 days and 9-34 days respectively. He found that in humans, sex chromatin is seen at the approximate age of 12 days in the trophoblast and in the embryo itself at approximately 16 days. In Macaques it was seen very

occasionally from the tenth day onwards in the trophoblast and in greater number in the embryo itself at the nineteenth day. He concluded this study by suggesting that sex chromatin appears at different times or with different rates in the different tissues of the embryo.

The Study of Sex Chromatin has passed through the descriptive into the analytical phase. The descriptive phase consisted of the presence of sex chromatin as a female characteristic in the nuclei of various animals; its size, shape, position in the nucleus (6; 8; 12; 19; 28; 30; 56), and its staining characteristic (25). The analytical phase began with the demonstration by Ohno et al (9; 33A; 68; 69) and others of the single X origin of the sex chromatin. It has continued with the demonstration of late replication of the heteropyknotic X chromosome that forms the sex chromatin during interphase, together with the importance of genetic implications of heteropyknosis and genetic inertness of one of the two X chromosomes of the female somatic cells (32; 48). Seven types of interphase nuclei with respect to the sex chromatin patterns are known (10) all but two are based on differences in the number of X Chromosomes. Two important factors are established concerning the behaviour of sex chromosomes during interphase. The Y-Chromosome does not produce a recognizable chromocentre and that the presence or absence has no bearing in X-Chromosomes. The number of sex chromatin masses equals the number of X-Chromosome minus the number of haploid sets of autosomes divided by two. Thus for diploid or near diploid cells the number of sex chromatin masses is one less than the number of X Chromosomes.

In individuals whose cells contain only one X Chromosome, the nuclei are sex chromatin negative. The explanation of chromatin negative nuclei in the majority of females with Turner's Syndrome (64)

was one of the first demonstrations of aneuploidy as a cause of congenital malformation in man. They are shown to have a complement of 45 chromosomes. Interphase nuclei are consistently chromatin negative, not only in normal male but also in XY individuals with various anomalies of the gen talia; these include a minority of Klinefelters Subjects (31). Somatic cells are singly chromatin positive when they contain XX sex chromosome complex in normal females and in females with abnormalities of the genitalia that are caused by gene mutation. The same relationship applies where there are certain developmental errors of the reproductive system. Chromosome analysis reveals that only XX cells of the buccal smears are typically chromatin positive (10). There are exceptions in this rule; some researchers found XX cells in cultures from various sources in a chromatin positive male with clinical traits of Klinefelter's Syndrome (13; 31; 64). Single X heteropyknosis is not altered in any way when a Y Chromosome is added to the two X's. XXY is the most common sex chromosome abnormality and was first discovered in man (13; 31; 64). The presence of a proportion of cells with two sex chromatin masses is the rule when the complement contains three X chromosomes and this is not affected by the addition of one or two Y chromosomes.

The development of clinical sex chromatin test has lead to the understanding of sex anomalies of nuclear sexual dimorphism of somatic cells (62). The use of sex chromatin tests on patients resulted in a reconsideration of sex differentiation in embryonic development (5) and of the embroyogenesis of certain congenital errors of sex development (31; 64). It has also become useful as a clinical aid to differential diagnosis of patients with ambiguous sexual development. Cytogenetically, the test is used as a guide to the number of X chromosomes in patients'

somatic cells (9; 10). The recognition of sexual dimorphism in cell nuclei of various mammals suggested several areas of research that might benefit from such a cytological "tool". Nucleoprotein synthesis (15) Bovine freemantism (63); Sex differentiation (5; 46) and Carcinogenesis (80).

Davidson et al (26; 27) developed a method sexing leucocytes in blood films; the validity of the technique has been confirmed repeatedly (21; 53; 66; 73), but the interpretation of a blood film is more tedious than interpretation of tissue cells; buccal smears etc. This makes it impossible to expect that the same results could be expected in tissues. The reason why drumsticks are not present in all the cells could be linked to their variation under different conditions. It might be possible that in different metabolic conditions of the cell that heterochromatin is converted to euchromatin, when no drumstick will be seen in that cell. The lower ratio of drumsticks in blood cells than sex chromatin masses in other tissues could be related to the fact that white blood cells have a very short life and thus a fast turnover; also unless actively taking part in the immune reaction, they have a much lower metabolic rate than other somatic cells. This could result in a different ratio of sex chromatin to drumsticks.

Extrusion of drumsticks from the nuclei has been reported to be certainly linked to the process of nuclear lobing; severe shifts to the right of the Arneth Count increase the number of drumsticks, while shifts to the left result in decrease in the number (16; 26; 27; 32). The frequency of drumsticks also increases in inherited oversegmentation of neutrophils, in pregnancy and at birth, especially in premature babies. No convincing explanation has been given for this increase. Apart from inherited oversegmentation of neutrophils, others could be caused by anaemia. This can be defined as a state in which the quantity or quality of the circulating red cells is reduced below normal level.

Broadly speaking anaemias can be divided into two; Primary and Secondary. These two groups can be sub-divided into four groups - Dyshaemopoietic anaemias due to inefficient blood production; (ii) Haemolytic anaemias due to excessive blood destruction; (iii) Haemorrhagic anaemias due to extravascular blood loss and (iv) Anaemias of unknown cause. Pregnancy causes some form of anaemia and is due to strain on iron metabolism; three factors appear to operate in causing anaemia under pregnancy. The foetus obtains from the mother large stores of iron and if twins the demand becomes higher. If the intake of the mother is small the maternal store is exhausted quickly . There may be a temporary achlorhydria due to derangement of gastric function during pregnancy. These factors may operate singly or in combination. Excess blood loss during menses could also lead to anaemia. In almost all cases of anaemia, it has been found that they are due to nutritional deficiencies (Iron, folate and Vitamin B_{12} (23A; 23B; 23C)). An inadequate dietary folate intake is a major factor in the production of megaloblastic anaemia in association with other factors, such as chronic haemolysis, anticonvulsant drugs for control of epilepsy and alcoholism. Deprivation of dietary folate leads to megaloblastic anaemia in all animals studied so far, 23B; 47. Oral contraceptives have been reported to cause megaloblastic anaemia. Both iron, folate and Vitamin B_{12} deficiencies are associated with increased frequency of hypersegmentation of neutrophils.

In my experiments, I found it impossible to identify drumsticks under the scanning electron microscope (SEM). This observation must have been due to the character of the SEM which is mainly used for the study of the surface morphology of objects looked at, and in the case of the buffy coat preparations, I was unable to see the nucleus where drumsticks are attached, Fig. 2a. My observations might have also been

affected by the processes the cells went through, i.e. centrifugation, pre and post fixation, dehydration, critical point drying and coating with gold alloy. Lawler et al (42A) prepared buffy coat samples for examination under the transmission electron microscope but were unable to identify drumsticks. This they attributed to thinness of sections and their inability from a single section to recognise such structures.

I found the incidence of drumsticks in all samples to be within the normal range reported by Briggs (18); Carpentier (21) and Davidson et al (26:27). Davidson et al gave a range of 1.02% - 16.66% while I had a range of 3% - 10.3%; however the highest incidence of drumsticks in my experiments was observed in women with pathological conditions. This confirms Davidson et al (26:27) and Clark et al (23D) results, that drumsticks increase with number of lobes and under pathological conditions.

In the investigation of the relationship between the number of lobes of the nucleus of the neutrophil and the percentage of drumsticks, a general pattern can be seen from the result sheets. Drumsticks are not seen on immature stab cells (16:27) and the percentage on the two and three lobed cells were lower than the ones found in the four and five lobed cells. It appears that as the number of lobes increase so does the likelihood of drumsticks forming and since the number of lobes indicates age and/or a pathological condition (27; 42A; 47), it appears that it is here the so called "Rejection Phenomenon" acts.

In my experiments to determine the frequency of drumsticks in normal pre-pubertal girls, whose ages ranged between 30 minutes and 12 years; the average incidence was found to be 4.63% $\pm$ 1.63. This is within the normal range. The Arneth Count was normal. It was not possible to check the incidence on premature babies as none were delivered while I was there.

In pregnant women, the average incidence was 5.71 ± 0.69; this is within the normal range. There was a slight shift to the right in the Arneth Count which confirms that pregnancy could induce anaemia.

Nineteen women aged between 67 and 95 years (post-menopausal subjects) were examined. The average incidence was 3.11% ± 0.98; this again is within the normal range; maybe the incidence would have been higher if the women had not been treated for anaemia. The Arneth Count was normal.

When the variation in the menstrual cycle was investigated in five normal girls, aged between 21-29 years, it was observed that subject (a) had three peaks in the incidence drumsticks. The first was on the first day of the menses at 4%; the second on the 15th day at 5.5% and the third on the 23rd day at 6%. In subject (b) there were also three peaks; first on the first day at 6.5%; second on the 14th day at 7% and third on the 22nd at 6%. For subject (c) two peaks were observed on the first and 14th day at 5% and 4% respectively. In subject (d) there was only one peak on the first day at 5% and in subject (e) there were two peaks on the first and 22nd day at 6% and 5%. These peaks correspond with the time of ovulation and the luteal phase of the cycle in most of the subjects as has been reported by most research workers whose work was done on tissues and buccal smears (16; 20; 34; 50; 71; 78). The rise in drumsticks is in parallel with an increase in oestrogen level (1) and the following decrease could be in parallel with the increase in progesterone which drops off around the 22nd day when another peak is seen. Throughout the luteal phase, the oestrogen level is high when the progesteron decreases ; this could be a reason why there is a peak on the 22nd day. From my observations it would appear that an increase in oestrogen secretion causes an increase in the number of drumsticks and increase in progesterone secretion causes a decrease in drumstick (77). I would personally think that since menstrual cycle goes on until the menopause age is attained, there

should be a time when drumstick frequency becomes more or less constant, thereby agreeing with Aboli et al findings (1). This could be the reason why Dolan (17; 29) found no relation between sex chromatin frequency and female sex hormones. Excess blood loss during menses could cause anaemia which could be a reason for increased frequency of drumsticks. This same subjects were used for the investigation of drumstick frequency in pre-menopausal women. The average incidence was 3.48% ; this falls within the normal range and the Arneth count was normal.

In the investigation of drumstick incidence in patients with pathological conditions, mainly anaemias; the average incidence was 10.3% $\pm$ 1.47; there was a severe shift to the right of the Arneth Count and most of the cells with drumsticks had four or more lobes.

In all animals examined to investigate drumstick incidence, the average incidence in cattle was 3.0% $\pm$ 1.13; this was the lowest compared to others and confirms some reports (27). In sheep it was 4.23% $\pm$ 0.95; Pigs 4.43% $\pm$ 1.17; Cats 3.66% $\pm$ .77; Dogs 3.93% $\pm$ 0.83; Monkeys 6.7% $\pm$ 2.67. Hyaena 4.0% and Langur monkey 6.0%. The incidence in the above animals fall within the normal range. There was a slight to severe shift to the right of the Arneth Count. The Arneth Count in man was used as a standard since I know nothing about the Arneth in animals. It would appear that the results obtained from the Arneth count in animals are normal for them. This shift could also be due to iron, folate and Vitamin B_{12} deficiencies in the animal feed.

In conclusion, it appears from my results that various factors influence the incidence of drumsticks in peripheral blood. The range given as normal to me is too high; it might have been between 1-6%. Given enough time it would have been possible to carry out a detailed investigation into the incidence of drumsticks under different conditions and maybe say if the incidence have a significant purpose

other than genetic sex differentiation. Its current uses in Psychosexual differentiation (54); Sex Chromatin and Medicolegal Problems (65): Sex Chromatin and antenatal sex diagnosis (75; 75A,1), would be worthy of a more detailed study.

BIBLIOGRAPHY

1. ABOLI, S.H.M. et al 1978. Effect of Oestrogen on the incidence of Drumstick in the rabbit neutrophils. Indian Journal of Physiology and Pharmacology. Vol 22(4) PP383.

2. ASHLEY, D.J.B., 1957. Occurrence of sex chromatin in the cells of the Blood and Bone Marrow in Man. Nature (Lond.) Vol.179 pp.969-970.

3. ASHLEY, D.J.B., 1958. Discrepancies in the diagnosis of Genetic Sex by leucocyte morphology. Lancet Original Articles, pp.240.

4. ASHLEY, D.J.B., 1959. Nuclear Sex in Species showing male homagamely. Anat. Rec. Vol.135 pp.115.

5. AUSTIN, C.R., 1966. Sex chromatin in Embroyonic and foetal tissues. The sex chromatin. Chap. 15, pp.241-253.

6. BARR, M.L. et al, 1949. A morphological distinction between neurons of the male and female and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature (Lond.) Vol.163, pp.676.

7. BARR, M.L. et al, 1951. The behaviour of nuclear structures during depletion and restoration of Niss l material in motor neuron. J. Anat. (Lond.) Vol.87, pp.171.

8. BARR, M.L. et al, 1952. A sex difference in the morphology of metabolic nuclei in somatic cells of the Cat. Anatomical Records. Vol. 112(4), pp.709.

9. BARR, M.L. et al, 1957. Chromosomes, sex chromatin and cancer. CANADA CANCER CONF. 2, pp.3.

10. BARR, M.L., 1966. Correlation between sex chromatin patterns and sex chromosome complexes in man. The Sex Chromatin Chap.7, pp.129-153.

11. BASU, P.K., 1966. Sex Chromatin in Transplanted Tissues. The Sex Chromatin, Chap.16, pp.255-262.

12. BEATH, M.M., et al, 1962. The Sex Chromatin in interphase nuclei in Ptatyrrhine monkeys. Cytologia (Tokyo) Vol.27, pp.1.

13. BENIRSCHKE, K., 1966. Sex Chromatin and developmental abnormalities. The Sex Chromatin, Chap.25, pp.444-451.

14. BERTRAM, E.G., 1949. Cytological Changes in motor nerve cells following prolonged stimulation of their axons. Anat. Rec. Vol.103, pp.567.

15. BERTRAM, E.G., 1966. Behaviour of the Sex Chromatin during altered states of Cell metabolism. The Sex Chromatin, Chap.8, pp.162-172.

16. BILLINGTON, P.J., 1980. A review of the literature on Drumstick of the polymorphonuclear leucocytes with some original observation. B.Sc. Honours Thesis, University of St. Andrews.

17. BRAINERD, T. et al, 1965. Absence of correlation between Sex Chromatin incidence and menstrual cycle. Acton Cytologica, Vol.9(6), pp.440-441.

18. BRIGGS, D.K. et al, 1956. Sex difference by leucocyte morphology. J. Clin. Endocr. Vol.16, pp.1163.

19. BRUM, N. et al, 1959. A study on Sex Chromatin. Tex Rcp Bio Med. Vol.17, pp.73.

20. CARATZALI, A., 1963. Sex appendages; Arneth formula and menstrual cycle. Cytogenetics, Vol.2, pp.15.

21. CARPENTIER, P.J. et al, 1957. The Sex linked difference in Rabbit Neutrophils. Nature (Lond.) Vol.180, pp.554.

22. CARPENTIER, P.J. 1966. Sex Chromatin in smears from the reproductive and urinary tracks. The Sex Chromatin, Chap.10, pp.186-200.

23. CASTRO, N.M. De et al, 1959. Sex Chromatin (Chromoleme) in the Purkinje Cells of some mammals. Nature (Lond.) Vol.182, pp.293.

23A. CHANARIN, I., 1979. Effects of Gastric Surgery. The Megaloblastic Anaemias, pp.387.

23B. CHANARIN, I., 1979. Nutritional folate deficiency. The Megaloblastic anaemias. Chap.26, pp.458.

23C. CHANARIN, I., 1979. Megaloblastic anaemia associated with Pregnancy. The Megaloblastic Anaemias, Chap.27, pp.466-490.

23D. CLARK, et al, 1970. Myeloproliferative Disorders of Animals and man. U.S. atomic energy commission, Division of Technical Information.

24. COIDAN, R.S., 1952. The paranucleolar bodies in spinal neurons of mammals. J. Comp. Neurol. Vol.97, pp.61.

25. CULLING, C.F.A., 1966. Staining Affinities and Cytochemical properties of the Sex Chromatin. The Sex Chromatin, Chap.5, pp.91-111.

26. DAVIDSON, W.M. et al, 1954. A morphological sex difference in the polymorphonuclear neutrophils leucocytes. Brit. Med. J. Vol.3, pp.6.

27. DAVIDSON, W.M., 1966. Sexual dimorphism in nuclei of polymorphonuclear leucocytes in various animals. The Sex Chromatin, Chap.3, pp.59-71.

28. DIXON, A.D. et al, 1957. Sex determination of human tissues from cell morphology. J. Foren Med. Vol.4, pp.11.

29. DOLAN, Sister Bernard Eileen, 1968. Sex Chromatin incidence and the Human menstrual cycle. Acta. Cytologica. Vol.12(2), pp.128-131.

30. EMERY, J.L. et al, 1954. Observations on the female sex chromatin in human Epidermis and on the value of skin biopsy in determining sex. J. Path. Bact. Vol.68, pp.17.

31. FERGUSON-SMITH, M.A., 1966. Sex Chromatin; Klinefelter's Syndrome and mental deficiency. The Sex Chromatin. Chap.18, pp.277-312.

32. GRINBERG, M.A. et al, 1966. Investigation with tritiated thymidine of the relationship between sex chromosomes; sex chromatin and drumsticks in the cells of the female nine banded Armadillo. Dasy pus Nove micinctus. Cytogenetics. 5. pp.64-74.

33. GRAHAM et al, 1977. A sex difference in the morphology of metabolic nuclei in somatic cell of the Cat. Anat. Rec. Vol.112(4), pp.709.

33A. GRUMBACK, M.M. et al, 1962. Sex chromatin and the sex chromosomes on the Origin of Sex Chromatin from a single X chromosomes. Acta. Cytol. (Philad) Vol.16, pp.46.

34. HAGY, G.W. et al, 1962. Variation of Sex Chromatin in human oral mucosa during the menstrual cycle. Acta Cytologica Vol.16(4), pp.314-3

35. HAMERTON, J.L. et al, 1961. The Somatic Chromosomes of the gorilla. Nature (Lond) Vol.192, pp.225.

36. HAMERTON, J.L. et al, 1963. The Somatic Chromosomes of the Hominodea. Cytogenetics, Vol.2, pp.240.

37. HAY, J.C. et al, 1961. The Sex Chromatin in various mammals. Acta. Anat. Rec. Vol.136, pp.317.

38. HEITZ, E., 1963. Die Somatishe Heteropyknose bei Drosophila melanogaster und ihre genetische bedeating. Z. Zalforsch Abt. Histochem. Vol.20, pp.237.

39. HENKING, H., 1891. Untersuchungen uber die ersten Enlwecklungs vorg ange in den Eiern der Iqsekton 11 riber Spermatogenese und deren Baziehung Zur Eientwicklung bei Pyrrhocon's Apterus. L.Z. Wiss Zool Vol.51, pp.685.

40. HOPKINS, T.F. et al, 1959. The demonstration of Sexual dimorphism in liver cells of the rodent. Exp. Cell. Res. Vol.18, pp.178.

41. HOSHINO, T. et al, 1959. Sex Chromatin in domestic animals Tohoku J. Agric. Res. Vol.9, pp.113.

42. JAMES, J., 1960. Observations on the So called Sex Chromatin Z. Zellforsch Vol.51, pp.597.

42A. LAWLER, S.D. et al, 1966. Marron dysplasia with C trisomy and anomalies of the granulocyte. J. Clin. Path. Vol.214, pp.215-219.

43. KUNGER, H.P., 1957. The Sex Chromatin in fetal and maternal portion in the human placenta. Acta. Anat.(Basel) Vol.30, pp.371.

44. KLINGER, H.P., 1963. The Somatic Chromosomes of some primates. Cytogenetics, Vol.2, pp.140.

45. KLINGER, H.P., 1966. Morphological Characteristics of the Sex Chromatin. The Sex Chromatin, Chap.4, pp.76-88.

46. LENNOX, B., 1966. The Sex Chromatin and Hermaphroditism. The sex chromatin, Chap.22, pp.387-402.

47. LINDENBAUM, J. et al, 1980. Megaloblastic Anaemia and Neutrophil hypersegmentation. Short Communication Brit. J. Haematology, Vol.44, pp.511-513.

48. LYON, M.F., 1966. Sex Chromatin gene action in the X Chromosome of mammals. The Sex Chromatin, Chap.21, pp.370-381.

49. MACLEAN, N., 1962. The drumstick of polymorphonuclear leucocytes in Sex Chromosome abnormalities. Lancet 1.1154.

50. MACLEAN, N., 1966. Sex Chromatin Surveys in Newborn babies. The Sex Chromatin, Chap.11, pp.202-209.

51. MARIA, S. Blanco de del Campo et al, 1965. Fluctuations of Sex Chromatin during menstrual cycle. Acta. Cytologica, Vol.9(3), pp.251-256.

52. MILES, C.P., 1966. The Sex Chromatin in cultured cells. The Sex Chromatin, Chap.12, pp.211-218.

53. MITTWOCH, U., 1956. Some observations on the incidence of drumstick in polymorphonuclear neutrophils of females. Acta. Genet. (Basel) Vol.6, pp.263.

54. MONEY, J., 1966. The Sex Chromatin and Psychosexual differentiation. The Sex Chromatin, Chap.24, pp.434-442.

55. MOORE, K.L. et al, 1953. Morphology of the nerve cell nucleus in mammals with Special reference to the Sex Chromatin. J. Comp. Neurol. Vol.98, pp.213.

56. MOORE, K.L. et al, 1954. Nuclear Morphology according to sex in Human tissues. Acta. Anat. (Basel) Vol.21, pp.197.

57. MOORE, K.L. et al, 1955. Smears from the Oral mucosa in the detection of Chromosomal Sex. Lancet Vol.2, pp.57.

58. MOORE, K.L. et al, 1958. Sex Chromatin in mammalian cells. Anat. Rec. Vol.130, pp.431.

59. MOORE, K.L., 1966. Introduction to the Sex Chromatin. The Sex Chromatin pp.1-5.

59A. MOORE, K.L., 1961. The Sex Chromatin; its discovery and variation in the animal Kingdom. Acta. Cytol. (Philad) Vol.6, pp.1.

60. MOORE, K.L., 1966. The discovery of the Sex Chromatin. The Sex Chromatin, Chap.1, pp.7-15.

61. MOORE, K.L., 1966. Sex Chromatin Patterns in various animals. The Sex Chromatin, Chap.2, pp.16-55.

62. MOORE, K.L., 1966. The development of Clinical Sex Chromatin Test. The Sex Chromatin, Chap.9, pp.173-183.

63. MOORE, K.L., The Sex Chromatin of freemartins and other animals, intersexes. The Sex Chromatin, Chap.14, pp.229-238.

64. MOORE, K.L. Sex Chromatin and Gonadal Dysgenesis. The Sex Chromatin, Chap.19, pp.316-337.

65. MOORE, K.L., 1966. Sex Chromatin and Medicolegal Problems. The Sex Chromatin, Chap.26, pp 453-465.

66. MURTHY, M.S.N. et al, 1958. The occurence of Sex Chromatin in white blood cells of young adults. American J. Clin. Path. Vol.30, pp.216.

67. MYLLE, M. et al, 1954. Sex Chromatin in neurons of human frontal cortex and sympathetic ganglion. Anat. Rec. Vol.118, pp.402.

68. OHNO, S. et al, 1959. Formation of the Sex Chromatin by a Single X Chromosome in liver cells of Rattus norvegicus. Exp. Cell. Res. Vol.18, pp.45.

69. OHNO, S., 1966. Single X derivation of Sex Chromatin. The Sex Chromatin, Chap.6, pp.113-126.

70. PAINTAL, I.S. et al, 1974. Fluctuation in sex chromatin during various phases of the menstrual cycle. Indian J. Physiol & Pharm Vol.18(1), pp.60-62.

71. PARK, W.W., 1957. The occurence of Sex Chromatin in early human and Macaque embroys. J. Anat (Lond) Vol.91, pp.369.

72. POLANI, P.E., 1966. Sex Chromatin; Sex Chromosomes and Sex linked characters. The Sex Chromatin, Chap.20, pp.342-364.

73. PORTER, K.A., 1957. The sex difference in morphology in Neutrophils and Eosinophilic granulocytes. Dan. Med. Bull. Vol.2, pp.190.

74. RISS, P. et al, 1957. A sex difference in the Chromatin Structure of the peripheral blood lymphocytes. Nature (Lond) Vol.179, pp.785.

75. RISS, P. et al, 1966. Sex Chromatin and antenatal sex diagnosis. The Sex Chromatin, Chap.13, pp.220-227.

75A. RUSSELL, L.B., 1961. Genetics of mammalian Sex Chromosomes. SCIENCE, Vol.133, pp.1795.

75A1. SACHS, et al, 1956. Possibility of pre-natal diagnosis of sex in cattle and sheep. Nature (Lond) Vol.178, pp.1402.

76. SCHULTZ, M.G. et al, 1962. Male Pseudohermaphroditism diagnosed with the aid of Sex Chromatin Technique. J. Amer. Vet Med. Ass. Vol.140,pp.241.

77. SPASOR SPAS. A. et al, 1967. Sex Chromatin and Sex hormones. Amer J. Obst. and Gyno. Vol.97(5), pp.714-718.

78. SPASOR SPAS. A., 1968. Sex Chromatin pattern and menstrual cycle. Acta. Cytologica, Vol.12(2), pp131-136.

79. STEVENSON, A.C., 1966. Sex Chromatin and the Sex ratio in Man. The Sex Chromatin, Chap.17, pp.263-275.

80. TAVARES. A.S., 1966. Sex Chromatin in Tumors. The Sex Chromatin, Chap.23, pp.405-428.