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Sex Chromatin*

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The term 'sex chromatin', in present-day usage, denotes a body in the nuclei of cells, which indicates the sex of the organism from which the cell originated. The sex chromatin body is present in the nucleus only during interphase and disappears during mitosis. The term 'sex-chromatin' had previously been used in a different sense by Wilson (1925) to describe the (hypothetical) part of the sex chromosomes that acted as a sex differentiator in development.

Although the subject of sex chromatin has become a part of human genetics only within the last decade or so, it has attracted very considerable interest and today provides one of the most active growing points. One reason for this is practical. It has been estimated that about 2 or 3 persons per 1,000 suffer from an abnormality of their sexual development (Overzier, 1963), and it appears that the incidence may be even higher at birth (see p. 70). The sex chromatin test provides a simple and often indispensable aid in the diagnosis of such patients. Moreover, the technique has made it possible to screen large populations and thus obtain estimates regarding the incidence of these abnormalities. On a more theoretical level, the origin of the sex chromatin and its relation to the sex chromosomes has given rise to speculations, which are further stimulating experimental work, as to the way in which the X chromosome functions, both in sex determination and in general.

Some Landmarks in the Development of the Sex Chromatin Concept

The discovery of sex chromatin is generally placed in 1949, when Barr and Bertram, in London, Ontario, described a morphological distinction between neurones of male and female cats. The sex difference consisted of a small body which was present in the nuclei of nerve cells from female cats but was absent in those of males (Fig. 1). The

body, which stained with nuclear dyes, was intimately associated with the nucleolus and was at first called a 'nucleolar satellite'. This term became inappropriate when Barr (1951) found that in neuroglial cells of the cat the sex differentiating body lies against the inner side of the nuclear membrane. Henceforth, the term 'sex chromatin' was employed. The finding of the sex chromatin in the cat was an unexpected result of experimental neurocytology. Barr and Bertram (1949) wrote: 'It appears not to be generally known that the sex of a somatic cell as highly differentiated as a neurone may be detected with no more elaborate equipment than a compound microscope following staining of the tissue with the routine Nissl method'. Sections of brain, spinal cord, or sympathetic ganglia could now be readily attributed to the correct sex of the animal from which they originated.

A few years before the appearance of the paper by Barr and Bertram, S. G. Smith (1944, 1945a, b), in Ottawa, Ontario, found that in cells of the larvae of the spruce budworm, *Choristoneura (Archips) fumiferana* (Clem.), a darkly staining body was present in the nuclei of the female and was absent from the male (Fig. 2). This body could be used for sexing larvae prior to the development of the gonads, and it was suggested that this method might be applied to other organisms, including mammals. These findings are of importance not only because they provide the first indication that a nuclear body present in interphase can be used as an indicator of sex, but also because the spruce budworm is a member of the Lepidoptera, and in this order the female is the heterogametic sex. A female spruce budworm has only one X chromosome, and Smith postulated that the single X remained condensed (heteropycnotic) in interphase nuclei, in contrast to the two X chromosomes of the male, which became invisible. Thus, the situation appears to be precisely the opposite from that found in mammals. Sex chromatin in heterogametic females will be further discussed later (p. 68).

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FIG. 1. Sex difference in neurones of the cat. (a) 'nucleolar satellite' adjacent to nucleolus in female; (b) no nucleolar satellite in male. (By courtesy of Professor M. L. Barr.)

FIG. 2. Sex difference in the spruce budworm, *Choristoneura (Archips) fumiferana* (Clem.). (a) cells from female; (b) cells from male. (By courtesy of Dr. S. G. Smith.)

Some still earlier works, which are relevant to the problem of sex chromosomes in interphase nuclei, are those of Geitler (1937, 1938, 1939), who worked on insects belonging to the bugs (Hemiptera) and flies (Diptera), in both of which the female is homogametic and the male heterogametic. Geitler found that in somatic interphase nuclei the X chromosome was heteropycnotic in both sexes, i.e. the female had two heteropycnotic bodies and the male one. This situation seems to differ from that both in spruce budworms and in cats. The differences in these findings have not yet been resolved, and it is clear that for the time being any theories based on data from the sex chromatin of mammals will have to be confined to this class.

In 1952, Graham and Barr were able to show that in the cat sex chromatin could be demonstrated in tissues other than neurones. The tissues examined included smooth, skeletal, and cardiac muscle, cartilage, epidermis, endothelium, gastric epithelium, liver, pancreas, adenohypophysis, thyroid, adrenal, cortex and medulla, interstitial cells of ovary and testis, kidney, uterine epithelium, and prostate. Sex chromatin was found in all tissues except in liver and pancreatic acinar cells. The sex chromatin was present in one-half to two-thirds of the cells from female cats and only very rarely in those from male cats.

Just a year after the discovery of sex chromatin in the cat, a sex difference was shown to exist in human sympathetic ganglion cells (Barr, Bertram, and Lindsay, 1950; Bertram, Bertram, and Lindsay, 1950). Sex chromatin became part of human genetics in 1953 when Moore, Graham, and Barr showed that sex chromatin could be demonstrated in preparations of skin biopsies, including that of a hermaphrodite. Cells in the stratum spinosum are particularly favourable in showing the sex chromatin. In an intensive survey of human tissues, Moore and Barr (1954) found sex chromatin in all the cell types examined, which included cartilage and adrenal cortex, smooth muscle, bladder, and thyroid cells. The material was mainly derived from necropsies.

As a result of using the technique of sexing skin biopsies, it was shown that most patients with Turner's syndrome lacked sex chromatin (Polani, Hunter, and Lennox, 1954; Wilkins, Grumbach, and van Wyk, 1954). In 1955 the sex chromatin test became further simplified when it was found that the presence or absence of sex chromatin could be demonstrated in cells scraped off the buccal mucosa (Marberger, Boccabella, and Nelson, 1955; Moore and Barr, 1955). Yet another finding of great potential importance was the discovery that a sex

difference could be demonstrated in the polymorphonuclear leucocytes of peripheral blood (Davidson and Smith, 1954). Techniques for demonstrating sex chromatin in vaginal smears were described by Carpentier, Stolte, and Visschers (1956) and Guard (1959).

The development of these new techniques provided an enormous impetus to sex chromatin studies, and in 1956 at least 6 groups of workers were able to show that sex chromatin was present in a majority of patients with Klinefelter's syndrome (Riis, Johnsen, and Mosbech, 1956; Jackson, Shapiro, Uys, and Hoffenberg, 1956; Plunkett and Barr, 1956; Bradbury, Bunge, and Buccabella, 1956; Nelson, 1956; Sohval, Gabrilove, Gaines, and Soffer, 1956).

When techniques for studying human chromosomes became available from 1959 onwards, a striking correlation was revealed between the sex chromatin status and the number of X chromosomes in an individual. The presence of sex chromatin was found to be associated with the presence of two X chromosomes (or, more accurately, 16 chromosomes in the 6-12 group, since these cannot be distinguished unequivocally from the X on morphological grounds), while the absence of sex chromatin was found to be associated with the presence of a single X chromosome (15 chromosomes in the 6-12 group) (Barr and Carr, 1960; Stewart, 1960). Thus, sex chromatin studies have become indispensable in the elucidation of abnormalities in sexual development, for the following reasons: (1) The simplicity and speed of the techniques allows one to forecast the number of X chromosomes present in patients suspected of an abnormality of the sex chromosomes; (2) since X chromosomes in metaphase cannot be recognized unequivocally, the sex chromatin provides a check as to the number of X chromosomes present; (3) the method can be used in surveys of large groups of people.

The early assumption that the drumsticks of the polymorphonuclear leucocytes are equivalent to the sex chromatin bodies of epithelial and other tissue cells has been substantiated by later work (see p. 66). Therefore, the term 'sex chromatin' now includes drumsticks as well as sex chromatin bodies in cells other than leucocytes. The latter are now often called 'Barr bodies'.

Barr Bodies: General Properties

A Barr body is a small well-defined body which stains intensely with nuclear dyes (Fig. 3-5). It is present in a large proportion of nuclei of female origin and absent in male nuclei. The size of a Barr body is about $1\ \mu$ in diameter. The average size has

FIG. 3

FIG. 3. Sex chromatin in buccal mucosa cell of a woman. (Cresyl fast violet. $\times 2,200$.)

FIG. 4

FIG. 4 and 5. Sex chromatin in fibroblasts cultured from skin of female donor. (Thionin. $\times 2,200$.)

FIG. 5

been estimated as $0.7 \times 1.2 \mu$, both in nuclei of buccal mucosa and in sections of several human tissues (Moore and Barr, 1954, 1955). Interphase nuclei are rarely quite homogeneous, and they may contain a number of more or less darkly staining bodies, which are known as chromocentres (Fig. 5). Such non-specific chromocentres are generally smaller than Barr bodies and have a less well-defined outline. In cases where a small percentage of Barr bodies have been reported in male nuclei, this is almost certainly due to non-sex-specific chromocentres, which are sufficiently similar to Barr bodies to have been mistaken for them.

Barr bodies are most commonly situated at the periphery of the nucleus (Fig. 3-5). However, a minority of Barr bodies are found in other parts of the nucleus, and many of these are situated in proximity of a nucleolus (Fig. 6). Klinger (1957) found that in amnion and connective tissue, 61.8% of the Barr bodies were at the nuclear periphery, 23.2% were lying against a nucleolus, and 9.2% were apparently free in the cytoplasm (although it cannot be excluded that a Barr body, which is seen in the centre of a nuclear profile is not, in fact, lying against the nuclear membrane in the three-dimensional nucleus).

Barr bodies have one of several distinct shapes. Many appear to be plano-convex or wedge-shaped, with the plane side resting against the nuclear membrane and the convex part pointing towards the cytoplasm. Barr bodies in the centre of the nucleus appear to be rectangular, and some rectangular Barr bodies may also be observed at the periphery of the nucleus. Some Barr bodies,

particularly the rectangular ones, appear to be bipartite, but, as pointed out by Klinger (1958), the distance separating the 2 components is near the limit of the resolving power of the light microscope.

Some investigators, when scoring Barr bodies, include only those that are situated at the periphery of the nuclear membrane. This practice almost certainly underestimates the true incidence of the Barr bodies. On the other hand, since it is sometimes more difficult to distinguish central Barr bodies from non-specific chromocentres (Miles, 1960), it may be wise to exclude all centrally placed bodies

FIG. 6. Sex chromatin adjacent to nucleolus in fibroblast of female origin. (Thionin, $\times 2,200$.) (By courtesy of the Editor of *Cytogenetics*.)

if preparations have to be used that are not of the highest technical standard. This happens not infrequently in preparations of buccal mucosa which may contain a high proportion of moribund cells. Since the majority of Barr bodies are at the periphery of the nucleus, whichever method of scoring is used should not affect the end-result of whether sex chromatin is present or absent. It goes without saying that the criteria used in scoring the Barr bodies should be explicitly stated.

Barr bodies can be demonstrated particularly well in cells grown in culture. Thus Serr, Ferguson-Smith, Lennox, and Paul (1958) found sex chromatin in 90% of the cells grown from human thyroid, while Fraccaro and Lindsten (1959), who cultured a large number of organs from human foetuses, reported incidences of sex chromatin varying between 34% and 88% in the cultures originating from female foetuses. Miles (1960) found a negative correlation between the incidence of Barr bodies and the frequency of mitoses in cultured amnion cells, and this relation was confirmed by Therkelsen and Petersen (1962) in fibroblast cultures originating from bone marrow and embryonic skin. A technique for demonstrating sex chromatin in tissue culture has been described by Ross (1962). Sex chromatin may no longer be visible in tissue culture if large-scale chromosomal changes have taken place, i.e. the cells are 'transformed' (Orsi and Ritter, 1958; Miles, 1959). Apart from this, cultured cells are particularly favourable for the study of sex chromatin, since the cells are present in a single layer and the nuclei flatten themselves on the glass of the cover slip. For the same reasons, excellent preparations of sex chromatin may also be obtained from whole mounts of embryonic membranes, as demonstrated by Klinger (1957) and Klinger and Schwarzacher (1962).

Barr bodies can be stained with a large number of nuclear dyes (for a review see Barr, 1963). Staining techniques in common use employ cresyl echt violet (Moore and Barr, 1955), or thionin (Klinger and Ludwig, 1957), or haematoxylin for sectioned material, such as skin biopsies (Barr, 1955). The fact that Barr bodies will stain by the Feulgen technique following acid hydrolysis, and have an affinity for methyl green rather than pyronin (Lindsay and Barr, 1955), indicate that they are composed of material similar to that of the chromosomes and contain DNA.

Barr bodies can also be seen in unstained and in living cells by means of phase-contrast microscopy. This was described by James (1960), who observed sex chromatin in living cells cultured from a female

cat, and by Miles (1960) who cultured cells from human amnion. DeMars (1962), using fibroblast cultures obtained from human skin biopsies, observed the sex chromatin in the same cells both in the living condition and after fixation and Feulgen staining. The sex chromatin after staining was found to be in the same position where it had been in the living cell. Schwarzacher (1963) reported that in living human cells grown *in vitro*, sex chromatin is visible in 46% of the nuclei, whereas after fixation and staining it can be seen in about 90% of the cells. In the remaining 10% of the nuclei sex chromatin could not be seen by any method.

There is as yet no conclusive explanation as to why the sex chromatin does not appear to be present in all the cells of female origin. Mittwoch (1963, 1964b), as a result of measuring nuclear areas in short-term human fibroblast cultures, found that nuclei containing a Barr body were on an average about 6% smaller than the nuclei in which no Barr body can be seen. The smaller size of nuclei containing a Barr body might be due to two reasons: (1) the condensation of one X chromosome to form a Barr body (see p. 58), and (2) the possibility that the Barr bodies disappear at some stage during DNA synthesis, when the nucleus might be expected to reach its maximum size. The latter possibility would be in agreement with the observation of an inverse relation between the incidence of sex chromatin and the rate of mitosis.

Barr bodies can be seen in female embryos, but not in the earliest ones. Glenister (1956) examined 22 male and 12 female human foetuses, of 18–150 mm. crown-rump lengths: in all of them, the presence or absence of sex chromatin corresponded to the histological state of the gonads. In the female embryos, 30–50% of the nuclei had sex chromatin. Klinger (1957) found that in 21 embryos the sex chromatin state was in accordance with the external genitalia and the morphology of the gonads; of 3 further embryos, which were too young to be sexed in this way, 1 contained sex chromatin and 2 lacked it. Park (1957) studied serial sections from 33 human and 18 macaque embryos, belonging to the Carnegie Institute, Washington. The ages of the human embryos ranged between 36 hours and 24 days (the youngest was in the two-cell stage), and the macaque embryos were between 9 and 34 days. In the human embryos, sex chromatin was first seen in the trophoblast at about the twelfth day of gestation and in the embryo itself after about 16 days. In the macaque embryos, sex chromatin began to be seen on the tenth day in the trophoblast, while larger numbers of Barr bodies appeared in the

embryo on the nineteenth day. Other embryos of equal age had no sex chromatin. These results are in accordance with observations on embryos of the cat, made by Graham (1954a, b) and by Austin and Amoroso (1957) who suggested that the absence of sex chromatin in early embryos was connected with the large size of their cell nuclei.

Sex chromatin findings in tumour cells have recently been reviewed by Lennox (1963). In the majority of tumours, the sex chromatin status appears to be the same as that of the host. However, the teratomata seem to offer an interesting exception, as was first shown by Hunter and Lennox (1954). All the teratomata in females contain sex chromatin, and sex chromatin is also found in the teratomata of half the males, while the other half lack it. Lennox explains this on the assumption that teratomata arise by the fusion of haploid cells; thus, two haploid male cells, each containing an X chromosome, could give rise to a cell with two X chromosomes, which would account for the Barr body. On the other hand, Taylor (1963a), who reported aberrant sex chromatin findings in a proportion of embryonic teratomata and some other embryonic tumours suggests that the most likely explanation is the presence of an abnormal sex chromosome constitution, such as XO, XXY, etc.

Atkin (1960), working on uterine and other tumours in women, found that when sex chromatin was present, those with a near diploid number of chromosomes had 1 sex chromatin body in the majority of nuclei, while tumours with a near-tetraploid number of chromosomes had 2 sex chromatin bodies in most nuclei. The significance of this finding will be discussed in the following section.

Numbers of Barr Bodies per Nucleus

In general, the maximum number of Barr bodies per nucleus in any organism or tissue is either 0 or 1, corresponding to a karyotype containing one or two X chromosomes respectively. However, cells with multiple Barr bodies may be encountered, and this invariably means that more than two X chromosomes are present in the karyotype. The excess of X chromosomes may be due either to aneuploidy (e.g. if three X chromosomes are present in a diploid nucleus) or to polyploidy (e.g. as a result of doubling a female chromosome complement, resulting in a tetraploid nucleus with four X chromosomes).

A female patient with three X chromosomes was described by Jacobs, Baikie, Court Brown, MacGregor, Maclean, and Harnden (1959). On examining the buccal mucosa it was found that 14% of the nuclei contained two Barr bodies, the remainder

FIG. 7. Two Barr bodies in buccal mucosa cell of triple X female. (Cresyl fast violet, $\times 2,200$.)

having either one or none. Double Barr bodies are an invariable finding in these patients (Johnston, Ferguson-Smith, Handmaker, Jones, and Jones, 1961). Such cells can be well demonstrated in buccal mucosa or cultured fibroblasts employed for chromosome analysis (Fig. 7 and 8).

Carr, Barr, and Plunkett (1961) described 2 patients with 4 X chromosomes, and these had a proportion of cells with 3 Barr bodies in their buccal mucosa. Finally, Kesaree and Woolley (1963) described a female patient with five X chromosomes, and she had 4 Barr bodies in many cells of the buccal mucosa.

The Y chromosome appears to have no effect on the formation of Barr bodies, even though it results in a male phenotype. All patients with 2 or more X chromosomes in addition to a Y carry the stigmata of Klinefelter's syndrome. Barr, Shaver, Carr, and Plunkett (1959) described 3 such patients, in whom cells with 2 Barr bodies were found and all

FIG. 8. Two Barr bodies in fibroblast nucleus of triple X female. (Thionin, $\times 2,200$.)

FIG. 9. Large fibroblast cell, presumed to be at least tetraploid with 2 Barr bodies. (Thionin, $\times 2,200$.)

FIG. 10. Large fibroblast cell, presumed to be at least octoploid, with 4 Barr bodies. (Thionin, $\times 2,200$.)

were found to have an XXXY sex chromosome constitution. Two further male patients with double Barr bodies and XXXY sex chromosome constitution were described by Ferguson-Smith, Johnston, and Handmaker (1960). A patient with XXXXY sex chromosomes was first described by Fraccaro, Kaijser, and Lindsten, (1960b), Fraccaro and Lindsten (1960), and Fraccaro, Klinger, and Schutt (1962). By now, more than 12 such patients are known (Atkins, Böök, Gustavson, Hansson, and Hjelm, 1963); all have cells with 3 Barr bodies in their buccal mucosae.

It is clear from the sex chromatin findings in these patients, as well as in normal male and female subjects, that in diploid cells the maximum number of Barr bodies is one less than the number of X chromosomes. That polyploidy also affects the formation of Barr bodies became apparent from the work of Reitalu (1957) who observed that in liver obtained from female embryos, some of the larger cells which were assumed to be tetraploid contained 2 sex chromatin masses; from Klinger and Schwarzscher (1960) who found 2 Barr bodies in cells from human amnion, in which DNA measurements indicated the presence of a large proportion of polyploid cells; and from the finding of Atkin (1960) that tumours of female origin with a near-tetraploid number of chromosomes had two sex chromatin bodies in many nuclei.

In order to explain the relation between the number of Barr bodies, the number of X chromosomes, and the degree of ploidy of the cell, Harnden (1961) proposed the formula,

$$B = X - P/2,$$

where B = number of Barr bodies, X = number of X chromosomes, and P = degree of ploidy.

This formula fits in well with observation, providing (1) B is taken as the maximum number (which is not present in all cells) and (2) the ploidy number is even. Thus, in diploid cells the maximum number of Barr bodies is one less than the number of X chromosomes, in tetraploid cells it is two less than the number of X chromosomes, while in octoploid cells it is four less than the number of X chromosomes (Fig. 9 and 10). However, the formula cannot be meaningfully applied to cells with uneven ploidy numbers, for in such cases the result would be in half Barr bodies, which is not a reasonable answer.

There are 6 reports on the presence of Barr bodies in triploid cells. The first is by Böök and Santesson (1961) who described a patient with triploid and diploid cells. The triploid cells had XXY sex chromosomes, and no sex chromatin was found in any of the tissues. Similarly, Carr (1963)

found no sex chromatin in triploid cells with XXY chromosomes, derived from an aborted foetus, while Ferrier, Ferrier, Stalder, Bühler, Bamatter, and Klein (1964) found no sex chromatin in a patient who was a mosaic with diploid/triploid cells with XXY sex chromosomes; in the latter case, however, the proportion of triploid cells was less than 10%. By contrast, in a similar culture derived from an XXY triploid foetus, Mittwoch and Delhanty (1961) found sex chromatin to be present. Atkin and Klinger (1962) observed that in a culture grown from a hydatidiform mole, in which the modal number of chromosomes was 69, a considerable number of nuclei had 2 Barr bodies. Similar results were obtained by Mittwoch, Atkin, and Ellis (1963) in fibroblast cultures obtained from a diploid/triploid female patient. The diploid cells had XX, and the triploid cells XXX sex chromosomes. Double Barr bodies were found in 6% of the cells, and DNA measurements made on individual interphase nuclei, using an integrating microdensitometer, indicated that some of these double Barr bodies were present in triploid cells, the rest being in more highly polyploid cells.

No explanation can as yet be advanced as to the reason for these apparently conflicting reports. Nevertheless it may be concluded that, under certain conditions, triploid nuclei may have the same maximum number of Barr bodies as diploid ones.

Barr Bodies of Abnormal Size

Although the majority of patients with Turner's syndrome have an XO sex chromosome constitution, a large number of cases have now been described where a second X chromosome was present but was structurally abnormal. The first such patient to be described (Jacobs, Harnden, Court Brown, Goldstein, Close, MacGregor, Maclean, and Strong, 1960) had an X chromosome from which a part of the long arm had been deleted, and this patient was noted to have Barr bodies that were smaller than usual. Small Barr bodies were also noted in another patient who had a deletion of the short arm of the X chromosome (Jacobs, Harnden, Buckton, Court Brown, King, McBride, MacGregor, and Maclean, 1961). A relatively frequent abnormality of the X chromosome is the so-called presumptive iso-chromosome for the long arm of the X, which was first described by Fraccaro, Ikkos, Lindsten, Luft, and Kaijser (1960a). This chromosome is larger than the normal X, and all such patients have sex chromatin that appears to be larger than normal (Lindsten, Fraccaro, Ikkos, Kaijser, Klinger, and Luft, 1963). This impression

was confirmed by measurements of the area of the sex chromatin in patients with iso-X chromosomes, and in normal women (Lindsten, 1963), which showed that the area of the sex chromatin in the patients with iso-X chromosomes was significantly larger than in the normal women. DNA measurements on the Barr bodies were also undertaken and indicated that the Barr bodies in iso-X patients contained on an average more DNA than those in normal women, while the Barr bodies in a patient with a deleted X chromosome contained less DNA than those of the normal women (Lindsten, 1963).

Late Replicating X Chromosomes

The findings on Barr bodies in normal women and in patients with numerically or structurally abnormal X chromosomes have recently found striking confirmation as a result of experiments involving autoradiography. It was shown by Taylor, Woods, and Hughes in 1957 that if growing cells were exposed to a solution of thymidine labelled with tritium for a brief period during DNA synthesis, the radioactive substance would be taken up by the chromosomes and could be revealed by applying a radio-sensitive film. Using this technique, Taylor (1960) has shown that in the Chinese hamster not all chromosomes take up thymidine and duplicate their DNA at the same time. When tritiated thymidine is applied to cultures of human cells just before the end of the period during which DNA is synthesized, most of the chromosomes have almost completed their DNA synthesis; however, autoradiography reveals that in cells from normal females one chromosome in the 6-12 is heavily labelled (Grumbach and Morishima, 1962; German, 1962a, b; Gilbert, Muldal, Lajtha, and Rowley, 1962; Morishima, Grumbach, and Taylor, 1962; Moorhead and Defendi, 1963) (Fig. 11). Since this chromosome does not occur in normal males, the chromosome which duplicates its DNA later is assumed to be the second X chromosome. In patients with 3 X chromosomes, 2 such late replicating chromosomes are found in many cells (Grumbach, Morishima, and Taylor, 1963; Giannelli, 1963) and in patients with XXXXY sex chromosomes, 3 X chromosomes are late labelling (Rowley, Muldal, Gilbert, Lajtha, Lindsten, Fraccaro, and Kaijser, 1963; Giannelli, 1963) (Fig. 12); while in a female infant with 5 X chromosomes, Grumbach *et al.* (1963) found 4 late replicating X chromosomes! It is apparent that the number of late-labelling X chromosomes is, like that of Barr bodies, one less than the total number of X chromosomes, and there can be little doubt that these late-labelling chromosomes do, in fact,

FIG. 11. Metaphase chromosomes of a human female, following application of radioactive thymidine and autoradiography. The late replicating chromosome is assumed to be one of the X chromosomes. (By courtesy of Dr S. Muldahl.)

form the Barr bodies. This conclusion is further strengthened as a result of autoradiographic studies on structurally abnormal X chromosomes. In patients with iso-X chromosomes, this chromosome, and not the normal X chromosome, is found to be labelled with the isotope (Muldal, Gilbert, Lajtha, Lindsten, Rowley, and Fraccaro, 1963; Giannelli, 1963); in a patient who had one X chromosome in the form of a ring, this ring chromosome was late labelling (Fig. 13; Lindsten, 1963); and in a patient with a deleted X chromosome, the deleted chromosome was late labelling (London, Kemp, Ellis, and Mittwoch, 1964). These findings explain the fact that the Barr bodies associated with these abnormal chromosomes are of abnormal size.

Also using tritiated thymidine, Bishop and Bishop (1963) succeeded in labelling the Barr body in the interphase nucleus. After removing the grains, the most heavily labelled area of the cell was found to correspond to the Barr body in the same cell.

The Origin of the Barr Body

The findings just described have greatly clarified our ideas as to the origin of the Barr body. The older hypothesis that a Barr body was formed from both members of a pair of X chromosomes (Moore and Barr, 1953) became inadequate when the numerical relation between Barr bodies and X chromosomes was discovered, and was replaced by

the idea, first formulated by Ohno, Kaplan, and Kinosita (1959), that the Barr body was formed from a single X chromosome. The observation underlying this idea was that during prophase of mitosis in liver cells of the rat, one chromosome in the female appeared to be more condensed than the rest and that such a condensed chromosome was not seen in the male. Tetraploid cells in the female appeared to have two such chromosomes. The numerical relation between Barr bodies and X chromosomes, as well as knowledge gained from autoradiographic studies, leave no reasonable doubt that it originates from a single X chromosome which is highly condensed during interphase, while the rest of the chromosomes are extended. The other X chromosome in females, as well as the single X chromosome in males, is similarly extended. Thus, the behaviour of the X chromosome in somatic cells is exactly opposite to that which it shows in germ cells for, as has been known for a long time, the single X chromosome of insects becomes condensed (heteropycnotic) during the later spermatogonial divisions, and particularly during the prophase of meiosis in the spermatocyte, whereas the paired chromosomes of the female remain uncondensed (see Wilson,

1925; Schrader, 1928). Conspicuous heteropycnosis of the sex chromosomes in mammalian spermatocytes was also observed (Minouchi, 1928). Recently this contrary behaviour of the X chromosomes in germ and somatic cells of males and females has been found to apply also to man (Ohno and Makino, 1961; Ohno, Makino, Kaplan, and Kinosita, 1961): the single X chromosome of the male becomes heteropycnotic in the spermatocyte, whereas both X chromosomes in the oocyte remain isopycnotic; while in somatic cells, one of the X chromosomes of the female becomes heteropycnotic and the X chromosome of the male does not.

Chromosomes, or parts of chromosomes, whose cycle of condensation differs from that of the majority of the chromosomes are said to exhibit 'heteropycnosis' (Guthertz, 1907). Heteropycnosis is said to be positive if the chromosomes are more condensed, and negative if they are less condensed, than the rest. A Barr body, therefore, is the result of positive heteropycnosis of a single X chromosome during interphase. Chromosomes or parts of chromosomes exhibiting heteropycnosis are often said to consist of 'heterochromatin' (Heitz, 1928).

The remains of a Barr body can often be seen

FIG. 12. Metaphase chromosomes of a patient with XXXXY sex chromosomes. (a) before applying radio-sensitive film, (b) after applying radio-sensitive film. (By courtesy of Drs B. B. Mukherjee, O. J. Miller, S. Bader, and W. R. Breg, and the Editor of the *Scientific American* (1963), 209, 54.)

FIG. 13. Autoradiography in patients with abnormal X chromosomes: (a) autoradiograph of a metaphase cell containing the ring X chromosome; (b) the same cell after removal of the grains; (c) autoradiograph of metaphase cell containing an X chromosome with a deletion of the short arm; (d) similar cell containing an X chromosome with a possible deletion of the long arm. (By courtesy of Drs M. Fraccaro and J. Lindsten, and the Editor of *Proceedings of the Symposium of Cytogenetics of Cells in Culture*, Academic Press.)

during the prophase of mitosis (Fig. 14). As prophase proceeds, heteropycnosis of the X chromosome disappears and at metaphase the two X chromosomes are indistinguishable from each other, nor, indeed, can they be distinguished with certainty from autosomes 6–12. It is possible that in the daughter nuclei one of the X chromosomes may become heteropycnotic again during telophase, since a structure

resembling a Barr body may sometimes be seen in such nuclei (Fig. 15); though the fact that telophase nuclei contain many chromocentres still leaves some doubt on this point.

There is now considerable evidence that during mitotic metaphase the chromosomes do not distribute themselves at random within the cell, but that certain chromosomes tend to be found in character-

FIG. 14. Fibroblast nucleus in prophase showing remains of Barr body. (Thionin, $\times 2,200$.)

istic positions (Schneiderman and Smith, 1962; Miller, Breg, Mukherjee, Gamble, and Christakos, 1963a; Miller, Mukherjee, Breg, and Gamble, 1963b; Barton, David, and Merrington, 1963; Merrington and Penrose, 1964). In ordinary

FIG. 15. Fibroblast nuclei in telophase, showing possible re-formation of Barr bodies. (Thionin, $\times 2,200$.)

preparations, the X chromosomes cannot be distinguished with certainty, but the Barr body forming X chromosome is clearly labelled in cells treated with radioactive thymidine. In such preparations it is found that this chromosome is situated at the periphery of the nucleus in the majority of the cells. This observation doubtless throws some light on the hitherto puzzling fact (Miles, 1961) that the Barr body appears to be preferentially situated at this site.

The conclusion that the formation of a Barr body is a function intrinsic in the X chromosome constitution is entirely consistent with certain other observations made over the past years. For instance, the sex chromatin of animals is not affected by castration (Moore and Barr, 1953); the membranes of male fetuses do not contain sex chromatin in spite of their maternal environment (Klinger, 1957); and in organisms that are mosaics with respect to their X chromosome constitution, the sex chromatin occurs only in tissues containing at least two X chromosomes. A particularly striking example of the last phenomenon was furnished by Klinger and Schwarzacher (1962) who described a foetus in which patches of cells containing sex chromatin and an XXY sex chromosome constitution alternated with areas that lacked sex chromatin and had an XY constitution.

The fact that sex chromatin is intrinsic in the X chromosome constitution does not, of course, imply that its formation is entirely uninfluenced by external factors. The observation that the incidence of Barr bodies is low immediately after birth (Smith, Marden, McDonald, and Speckhard, 1962; Taylor, 1963b;) may suggest such a possibility. Sohval and Casselman (1961) found that the effect of a number of antibiotics was to reduce the size of Barr bodies without altering the incidence. Further work on the effect of external agents on Barr body formation would certainly be of value.

Drumsticks: General Characteristics and Incidence

Following the discovery of the sex chromatin in a large variety of cells, Davidson and Smith (1954) searched for a similar sex difference among the leucocytes of the peripheral blood and found one among the granulocytes. The sex specific body which is present in the blood films from females but not from males consists of a head, about 1.5μ in diameter, which is attached to the body of the nucleus by means of a filament of varying length (Fig. 16). This appendage is commonly called a 'drumstick', though some other names such as 'sex nodules' have sometimes been given to it. The

FIG. 16a

FIG. 16b

FIG. 17a

FIG. 17b

FIG. 17c

FIG. 18a

FIG. 18b

FIG. 16, a, b. Polymorphonuclear neutrophil leucocytes from a female, each showing a drumstick. (May-Grünwald-Giemsa, $\times 2,200$.)

FIG. 17. Non-specific appendages of the nuclei of polymorphonuclear leucocytes, which must be distinguished from drumsticks. (a) and (b) Small appendages, shaped like a drumstick but smaller and sometimes less intensely stained; (c) small lobe, distinguished from a drumstick by irregular outlines and contours, in addition to larger size.

FIG. 18 a, b. Polymorphonuclear leucocytes from females, showing a 'sessile nodule'. (May-Grünwald-Giemsa, $\times 2,200$.)

occurrence and sex specificity of drumsticks was soon confirmed by numerous investigators (e.g. Wiedemann, Romatowski, and Tolksdorf, 1956; Lüers, 1956; and see Davidson and Smith, 1963).

Like the Barr bodies, drumsticks are stained by nuclear dyes, including basic fuchsin (Feulgen). Blood films stained with Romanowsky stains, such as the May-Grünwald-Giemsa technique, are suitable for the demonstration of drumsticks; but for a proper evaluation a high technical standard of preparation is necessary. For this reason blood films made by the cover slip technique (Wintrobe, 1961) are preferable to those made on slides, since in addition to ensuring a more thorough distribution of the different cell types, the cover slip method results in better cytological preservation.

Drumsticks may be demonstrated on all types of polymorphonuclear leucocytes, but for practical purposes only those present on neutrophils are usually considered.

The average incidence of drumsticks is about 1 per 38 neutrophils in the blood films from females, and they are absent in males. However, the nuclei of neutrophils may show a variety of appendages that are not sex specific, and care must be taken to distinguish these from true drumsticks (Fig. 17, see also Davidson and Smith, 1963). These non-specific appendages are distinguished from drumsticks by (1) their size and shape, and/or (2) their less intense staining (drumsticks, like Barr bodies, are highly condensed deeply staining bodies). The majority of such non-specific appendages (small clubs) are smaller than drumsticks (less than $1\ \mu$ in diameter); they may be intensely stained, and in such cases can be distinguished from true drumsticks only by size (but see p. 66). More rarely, an appendage of the size of a drumstick may have an unstained centre ('tennis racket', Davidson and Smith, 1954), or an apparently larger appendage shows uneven staining, and generally also a more or less uneven outline (Fig. 17c, 'minor lobe', Davidson and Smith, 1954). Minor lobes may be connected to the rest of the nucleus by one or two filaments.

In practice, no doubt, an occasional structure of this type may look sufficiently similar to a drumstick to be scored as such. Mittwoch (1961) scored 5 drumsticks among 15,000 neutrophils from males and suggested that an error of this order of magnitude was unavoidable in the method of scoring drumsticks. This agrees with the opinion by Sohval (1963) that the finding of a single drumstick in less than a 1,000 cells has no certain diagnostic value.

In addition to drumsticks proper, in which the head is attached to the rest of the nucleus by a single thin filament, a proportion of neutrophils of female

origin generally contain another type of appendage, consisting of a head which is attached more or less directly to the nucleus (Fig. 18). Such a structure is known as a sessile nodule (according to Kosenow and Scupin, 1956, drumsticks are called 'type A' and sessile nodules 'type B' appendages). A sessile nodule may be regarded as an incompletely developed drumstick; and varying degrees of development may be observed. Although sex specific, sessile nodules are more easily confused with non-specific appendages than are drumsticks, and the error inherent in their determination is greater. From a practical point of view, sessiles are of value mainly in corroborating the evidence obtained from drumstick data. The 2 types of appendages should be scored separately.

The incidence of drumsticks in individual women varies very considerably. Davidson and Smith (1954) found that among 125 blood films from women, the incidence of drumsticks varied between 1 per 98 neutrophils (1%) to 1 per 6 neutrophils (17%), with an average of 1 per 38 cells (2.9%). These frequencies were arrived at by enumerating the number of neutrophils containing 6 drumsticks, and it should be noted that this method results in a somewhat higher frequency of drumsticks (since the last cell always contains a drumstick) than if a constant number of cells is counted; this discrepancy is more marked if the incidence of drumsticks is low. Following Davidson and Smith, it has become an accepted rule among many investigators that a blood film from a normal female should contain at least 6 drumsticks per 500 neutrophils, and though this rule is obeyed in the majority of instances, there is a tail end of the distribution that falls outside this range. Whether high or low, the drumstick counts of individual women show considerable constancy on repeated occasions (Briggs and Kupperman, 1956; Briggs, 1958; Mittwoch, 1961).

One factor that influences the incidence of drumsticks is the degree of lobing of the polymorphonuclear nucleus, the more highly lobed cells having a higher incidence of drumsticks (Davidson and Smith, 1954; Briggs, 1958). Drumsticks are rare in the Pelger anomaly (Davidson and Smith, 1954; Lüers and Petzel, 1958; Siebner, Klaus, and Heni 1963). Thus, many but not all women with low drumstick counts also have low nuclear lobe counts (Briggs and Kupperman, 1956). Mittwoch (1964a) found that in 12 apparently normal women, in whom a detailed analysis of drumsticks was undertaken, the incidence varied between 1.6 and 7.2%, and the correlation coefficient between the incidence of drumsticks and the mean lobe count was 0.56.

TABLE

DRUMSTICKS PER 100 NEUTROPHILS, AND MEAN LOBE COUNTS IN PATIENTS WITH CHROMOSOMAL ABNORMALITIES AND IN CONTROLS (from *Nature* (1964), 201, p. 317)

Patients	No. of Patients	Groups					% Drumsticks For All Lobe Numbers	Mean Lobe Counts
		I	II	III	IV	V		
Non-mongol mentally retarded girls	28	0.48	1.59	3.19	5.68	6.83	2.74	2.61
Mongol girls	28	0.22	0.79	1.53	2.39	3.22	0.97	2.19
Klinefelters	12	0.77	0.83	1.87	2.36	(3.45)	1.27	2.26
Triple X females	3	0.77	2.26	2.20	3.92		2.07	2.26
Triple X Klinefelters	3	0.45	1.23	2.07	(1.56)		1.45	2.22

Groups I-III. 500 cells were investigated from each patient.
Groups IV and V. 1,000 cells were investigated from each patient.
Numbers in brackets are based on less than 100 cells.

When cells with constant lobe numbers were considered, it could be shown that in all women the drumstick count rose as the lobe number increased from 1 to 4 or 5, but for each given lobe number the drumstick count of the woman with the highest number was about 3 or 4 times that of the woman with the lowest number.

There is, as yet, no explanation for the observed variation in drumstick counts. However, there is evidence that the frequency of drumsticks is somewhat lower during the menstrual cycle and that this is due to hormonal influences (Caratzali, Phleps, and Turpin, 1957; Caratzali, 1963). Hormonal influences might also account for the increased incidence of drumsticks in newborn infants, and of their mothers immediately before and after parturition, which has been reported (von Harnack and Strietzel, 1956; Peiper and Oehme, 1956). An increase in the incidence of drumsticks as a result of adrenocorticotrophin (ACTH) and insulin has also been noted (Caratzali and Phleps, 1958). In this connexion, it is of interest that in the case of Barr bodies a decrease has been observed both in the newborn (Smith *et al.*, 1962; Taylor, 1963b) and as a result of cortisone (A. I. Taylor, 1964, personal communication). A slight decrease in the incidence of drumsticks with increasing age of the individual has been described by Wiedemann (1958) and Tonomura, Toyofuko, and Matsunaga (1962). de Castro (1963) reported a low incidence of drumsticks in cachectic women, while fasting rabbits showed first a rise, and then a drop, in the drumstick count.

Since the degree of lobulation of the neutrophil nucleus is an important factor in determining drumstick formation, lobe counts must be carried out whenever quantitative findings on drumsticks are of importance, or where the absence of drumsticks requires to be evaluated. Unfortunately, the criteria employed in enumerating lobes are very

variable, so that different investigators counting lobes on the same blood film may arrive at widely differing counts. It is clearly essential that whenever the results of lobe counts are reported these must be compared with the values obtained from a sample of apparently normal individuals counted by the same investigators.

Drumsticks in Patients with Chromosomal Abnormalities

In mongolism (Down's syndrome) it was shown (Turpin and Bernyer, 1947) that the nuclei of the polymorphonuclear leucocytes have, on an average, fewer lobes than those of unaffected subjects, and Mittwoch (1957) reported that the over-all incidence of drumsticks in 18 girls with mongolism was only a fraction of that of 18 non-mongol mentally defective girls.

In Klinefelter's syndrome, an incidence of drumsticks considerably below that of normal females was noted by Davidson and Smith (1958). Mittwoch (1961) found the low incidence of drumsticks in these patients to be associated with decreased polymorphonuclear lobing. A low drumstick count coupled with a low lobe count has been noted in a few individual patients with XXXXY Klinefelter's syndrome (Miller, Breg, Schmickel, and Tretter, 1961; Fraccaro *et al.*, 1962; Barr, Carr, Pozsonyi, Wilson, Dunn, Jacobson, and Miller, 1962).

The Table contains drumstick frequencies and mean lobe counts of patients with various chromosomal abnormalities and of mentally defective children, the majority of whom may be assumed to have normal chromosomes. The incidence of drumsticks in the mentally retarded controls is very close to that found in normal women (Davidson and Smith, 1954), but all groups of patients with chromosomal abnormalities have a lower incidence

of drumsticks, coupled with a lower mean lobe count, than the controls. Thus the mean frequency of drumsticks for mongol girls actually falls below the value of 6 drumsticks per 500 neutrophils, often regarded as the lower limit for normal female blood films.

One rather remarkable fact, which is borne out by the data in the Table, is that the patients with chromosomal abnormalities not only have lower drumstick and lobe counts than the controls when the average for all cells is considered, but that the incidence of drumsticks is lower in cells of constant lobe numbers. This suggests that in at least some conditions associated with chromosomal abnormalities, the formation of drumsticks is inhibited to an even greater extent than that of lobes (Mittwoch, 1964a). It is conceivable that hormonal influences, as described by Caratzali *et al.* (1957) and Caratzali (1963), may be responsible for the additional shortage of drumsticks.

The drumsticks in patients with 3 or more X chromosomes present a special problem. As was mentioned on p. 55, the Barr bodies in such patients present a very characteristic picture, since the maximum number of Barr bodies per cell is one less than the number of X chromosomes. In addition, the proportion of cells containing Barr bodies is also higher than in normal females (e.g. Jacobs *et al.*, 1959; Barr *et al.*, 1959; Ferguson-Smith *et al.*, 1960). By contrast, the incidence of drumsticks in such patients appears to be below normal. This is particularly pronounced if a Y chromosome is also present (e.g. Barr *et al.*, 1959; Ferguson-Smith *et al.*, 1960; Maclean 1962). Regarding female patients with XXX sex chromosomes, although Johnston *et al.* (1961) reported a

high incidence of drumsticks in 3 patients, the incidence in 5 further patients quoted by them is below normal. In 8 adult patients with XXX sex chromosomes studied by Maclean (1962) the average incidence of drumsticks was only 1.5%; though four newborn infants had an average incidence of 5%, this is below the average incidence of drumsticks reported for newborn and premature infants (von Harnack and Strietzel, 1956; Peiper and Oehme, 1956). The data so far suggest that, although there may be considerable variation in individual cases, the average incidence of drumsticks in patients with three X chromosomes, both with and without a Y chromosome, is below normal; while the admittedly limited data shown in the Table indicate that in this condition also there may be an inhibition of nuclear lobing.

Jacobs *et al.* (1959) reported the presence of a single cell with 2 drumsticks in a patient with XXX sex chromosomes. Both the existence and the rarity of double drumsticks has been confirmed by Maclean (1962) who, surveying a large number of cells in 12 patients with XXX sex chromosomes, found an incidence of such cells of less than 1 in 2,000 (Fig. 19). Two sessile nodules (Fig. 20) or 1 drumstick and 1 sessile nodule occur somewhat more frequently than 2 fully filamented drumsticks in the same cell. In an infant with 5 X chromosomes, described by Kesaree and Woolley (1963), there were a number of cells with 2 drumsticks and some with 3, though no cells were found with 4 drumsticks. The conclusion seems justified that the theoretical maximum number of drumsticks, like that of Barr bodies, is one less than the number of X chromosomes, but that owing to the low frequencies of drumsticks, particularly in chromo-

FIG. 19. Polymorphonuclear leucocyte with two drumsticks from a triple X female. (May-Grünwald-Giemsa, $\times 2,200$.) (By courtesy of the Editor of *Cytogenetics*.)

FIG. 20. Polymorphonuclear leucocyte with two sessile nodules from a triple X patient. (May-Grünwald-Giemsa, $\times 2,200$.)

FIG. 21, a, b. Polymorphonuclear leucocytes with large drumsticks from (a) an XXX female, (b) an XXXY male. Large drumsticks occur rarely in patients with multiple X chromosomes, but are frequent in patients with iso-chromosomes of the large arm of the X.

somal abnormalities, the maximum number may be rarely, if ever, reached.

Maclean (1962) also compared the size of drumsticks from patients with structurally abnormally X chromosomes with that of drumsticks from normal females. The 'drumstick/nucleus' ratio was estimated as

$$\frac{D \times 100}{N - D},$$

where D = area of drumstick and N = area of nucleus, in 3 patients with iso-chromosomes of the long arm of the X, 1 patient with a deletion of the short arm of the X, and 2 normal female subjects. These studies indicated that the drumsticks of iso-X chromosomes were larger, and those from deleted X chromosomes were smaller, than those of normal females. The results of Bamford, Cassin, and Mitchell (1963) are in agreement with this.

Although large drumsticks are primarily associated with iso-chromosomes of the long arm of the X, they may also be found, though much more rarely, in patients with multiple X chromosomes (Fig. 21). In general, the detection of large drumsticks will present little difficulty to an experienced observer, since their intense staining and regular outline distinguishes them from other parts of the nucleus. On the other hand, it is sometimes not possible to distinguish small drumsticks from other non-specific nuclear appendages.

The Relation of Drumsticks to Barr Bodies

The significance of drumsticks and their relation to Barr bodies remained in doubt for some time for a number of reasons. To begin with the morphological appearances of the 2 structures in stained preparations are different. The drumstick is larger,

a head of normal size having a diameter of 1.5μ while the diameter of the Barr body is about 1μ . Davidson and Winn (1961), who measured the ratios of the areas of drumsticks and Barr bodies to their respective nuclei from enlarged prints, found that the drumstick occupied a larger relative area than the Barr body in relation to the rest of the nucleus. Although the authors concluded that this difference might be explained by difference in the shape and thickness of the nuclei in polymorphonuclear and epithelial cells, the alternative, i.e. that drumsticks and Barr bodies are intrinsically different structures, remained for some time a possibility (Klinger, 1957; Ashley, 1962). Ashley (1957) thought that he could detect Barr bodies and drumsticks in the same cells. It should be remembered, however, that peripherally placed chromatin masses occur not infrequently in the leucocytes of both sexes.

In the majority of instances the results of tests on Barr bodies and drumsticks in the same individuals are in agreement, but a number of apparent discrepancies have been described. For instance, Kosenow (1957) found that among 15 patients with gonadal dysgenesis, 11 had neither Barr bodies nor drumsticks, 3 had both Barr bodies and drumsticks, while 1 had Barr bodies in the buccal mucosa but no drumsticks in the blood. The opposite situation, i.e. patients who lacked Barr bodies but in whom drumsticks were found, was reported by Ashley and Jones (1958). Other apparent discrepancies between findings in the buccal mucosa and in the blood were described by Overzier (1958), Izakovič (1960), Greenblatt, Manautou, Tigerman, Rogers, and Sheffield (1957), Greenblatt, Aydar, and Gibson (1962), and Turner and Zanartu (1962).

While a few of such results may be due to faulty techniques, the results of chromosome analysis have shown that a considerable proportion of patients with abnormalities of the sex chromosomes are, in fact, mosaic, with different sex chromosome complements in different cells (see Ford, 1963). In the extensive data by Lindsten (1963), over one-third of the patients with Turner's syndrome were mosaics with XO cells and others containing a second X chromosome. In some cases the different karyotypes are intermingled while in others different tissues are characterized by different chromosome constitutions. Jacobs *et al.* (1960) described a patient with Turner's syndrome, whose bone marrow cells appeared to be exclusively XO, while the skin was predominantly XXX, but contained also XO cells. The buccal mucosa contained cells with single and double Barr bodies, but no drumsticks were present in the leucocytes. Thus the sex chromatin findings are exactly as in the patient described by Overzier (1958) before the advent of chromosomal analysis. The results of chromosome studies have shown that many of the so-called discrepancies between Barr body and drumstick findings provide, in fact, confirmation that both structures represent the sex chromatin in the cells in which they occur.

Other apparent differences between drumsticks and Barr bodies have also been largely explained. As pointed out by Sohval (1963), some of the so-called discrepancies between the 2 techniques are merely reflections of differences in the frequencies with which Barr bodies and drumsticks occur. While Barr bodies may be seen in more than 90% of suitable cells (Klinger, 1957), drumsticks occur only rarely in as much as 10% of neutrophils, and their average incidence in normal females is about 3%. Moreover, in patients with Klinefelter's syndrome the incidence of Barr bodies appears to be no different from that of normal females, but the frequency of drumsticks is abnormally low (see p. 64). It should be pointed out that, if only a few drumsticks are to be expected within a given number of cells, none at all may be found in certain samples merely by chance. As was described above (p. 64), evidence has now been adduced that both the incidence of drumsticks and the degree of lobing is likely to be below normal in a variety of chromosomal abnormalities, and that, of the two, the reduction in the frequency of drumsticks is the more severe.

It is becoming clear that the drumstick is an appendage of a morphologically highly specialized cell, and that the factors that cause the nucleus to divide up into lobes also affect the extrusion of

drumsticks. It is considered by most haematologists that the lobulation of the nucleus gives a fairly good indication of the age of the cell, the youngest cells having a nucleus shaped like a letter C, while old cells have 4 or 5 lobes (Whitby and Britton, 1963). As it appears that chromosomal abnormalities may interfere with the normal development of these nuclei, the term 'maturity' may be preferable to 'age'.

Many problems of drumstick formation remain to be solved, together with related problems of the physiology and pathology of the leucocytes. Similarly, problems remain regarding details of the formation of Barr bodies, and further studies on the effect of hormones and other external agents on the formation of drumsticks and Barr bodies are required.

Nevertheless, work of recent years has made it clear that the original suggestion by Davidson and Smith (1954) that the drumstick is equivalent to the Barr body was valid. In present-day terms, it may be concluded that both the Barr body and the drumstick of mammals are true sex chromatin, representing heteropycnotic X chromosomes that are present in addition to the first one.

Sex Chromatin in Mammals Other than Man

A sex difference is found in the interphase nuclei of many mammals, and the domestic cat was the first species in which this was described.

Moore and Barr (1953) undertook a systematic investigation of the nerve cell nuclei of eutherian mammals, with the following results. A sex difference was found in the dog (*Canis familiaris*), mink (*Mustela vison* Schreber), marten (*Martes americana* Turton), ferret (*Mustela furo* L.) raccoon (*Procyon lotor* L.), and skunk (*Mephitis mephitis* Schreber), all of which belong to the order Carnivora; and in the goat (*Capra hircus* L.) and the deer (*Odocoileus virginianus* Boddaert), both of which are members of the Artiodactyla. Cells from five regions of the nervous system were examined (pyramidal cells, Purkinje cells spinal cord, dorsal root ganglion cells, and stellate ganglion cells). More than 80% of such cells from female animals contained a sex chromatin mass, most of them adjacent to the nucleolus; similar masses were observed in up to 13% of male cells, but usually the frequency was less than 10%.

In the following animals a sex difference could not be observed: rat (*Rattus norvegicus* Exleben), mouse (*Mus musculus* L.), hamster (*Cricetus auratus* Waterhouse), and groundhog (*Marmota monax* L.), belonging to the order Rodentia; and the rabbit (*Oryctolagus cuniculus* L.), a member of the Lago-

morpha. In these animals the cells of both sexes tended to contain coarse chromatin clumps, usually adjacent to the nucleolus, which obscured any sex chromatin that might have been present. The fact that sex chromatin is present in members of the carnivores and absent, or at least difficult to detect, in rodents and rabbits, has been largely confirmed by 1 ter workers (see Barr, 1963, for review). In the rat (rodent), Klinger (1957) was able to demonstrate sex chromatin only in spinal cord nerve cells; Shantaveerappa and Bourne (1962) found sex chromatin in pia-arachnoid membrane and perineural epithelium of female rats. Klinger was also unable to show sex chromatin in the rabbit, but Hulliger, Klinger, and Allgöwer (1963) found sex chromatin to be clearly visible in rabbit cells cultured *in vitro*. Melander (1962) found sex chromatin in the rabbit from the fifth day of embryonic life onwards. Hay (1960) was unable to demonstrate sex chromatin in the armadillo (*Edentata*) and the prairie dog and the mouse, both of them rodents. However, in another rodent, the porcupine, sex chromatin was present. Prince, Graham, and Barr (1955) found a well-marked sex difference in the rhesus monkey, *Macacus rhesus*. Hamerton, Fraccaro, de Carli, Nuzzo, Klinger, Hulliger, Taylor, and Lang (1961) and Hamerton, Klinger, Mutton, and Lang (1963) found sex chromatin in the buccal mucosa of the female gorilla and other apes belonging to the Hominioidea.

In the hoofed animals (*Artiodactyla*) sex chromatin can be distinguished in neurones, but in other cells the chromatin tends to be too coarse for the distinction to be made (see Barr, 1963). A particularly interesting application of the nuclear sexing method is the demonstration of sex chromatin in the neurones of the bovine freemartin by Moore, Graham, and Barr (1957). A freemartin is a sterile heifer, which is always born as a twin to a normal male. The external genitalia are predominantly female, while the gonads resemble testes. The findings of sex chromatin confirm the interpretation (Tandler and Keller, 1911; Lillie, 1916) that the freemartin is a genetical female that has become masculinized as a result of male hormones, following the fusion of chorions and vascular anastomosis in early embryonic life.

Drumsticks too are present in certain animals but not in others. The subject has been recently reviewed by Davidson and Smith (1963). It is interesting that, in the various groups of animals, there appears to be a close correlation between the presence or absence of drumsticks with that of Barr bodies. Thus drumsticks have been demonstrated in primates, carnivores, and hoofed animals, but

none have been found in rodents in which both sexes have non-specific appendages. Drumsticks are present in the rabbit.

The reason why sex chromatin is clearly visible in some animals and not in others, or only in certain cells of some animals, is not yet known. The question is of fundamental importance, however, for it touches on the significance of the sex chromatin body itself. The problem that must be answered is whether the difficulty of demonstrating sex chromatin in certain animals, e.g. rodents, is merely a technical one due to the 'coarseness' or clumping of the chromatin, or whether the presence or absence of such clumps reflects a fundamental difference in the organization of the DNA in different cells. This question will be further discussed later (p. 71).

Sex Chromatin in Animals with Female Heterogamety

Following the discovery of a heteropycnotic body in cells of female larvae of the spruce budworm, *Choristoneura (Archips) fumiferana* by Smith (1944, 1945a, b), Frizzi (1948) found a similar sex difference in the silk worm (*Bombyx mori* L.). Cells from the storage cells of the silk glands of larvae, which were known to be female from the histology of the gonads, contained a heteropycnotic body that was never found in the cells from males. The position of the heteropycnotic body was variable, occurring either in the centre of the cells or at the poles of elongated cells. Occasionally 2 bodies were seen in the same cell. This body provided a simple means for sexing larvae from the second instar onwards, when other means of sexing are very laborious.

The silkworm is an animal of economic importance and, though in common with other Lepidoptera its chromosomes are not favourable for cytological studies, a good deal is known about its genetics (Tanaka, 1953). At least 5 genes are known that show sex-linked inheritance (Tanaka, 1953), and there can be no doubt that the female is the heterogametic sex. Frizzi (1948) postulates that the heteropycnotic body represents the condensed Y chromosome.

Another class of animals, in which the female is the heterogametic sex, are the birds. Kosin and Ishizaki (1959) found sex chromatin in interphase cells of female chickens (*Gallus domesticus*). Cells of smooth muscle of the duodenum as well as dermal and epidermal cells at the base of growing feathers were particularly favourable for the demonstration of the sex chromatin body. This occurred with an incidence varying between 15 and 56% in cells from female birds, while a similar body was

seen in 0.4–5.4% of male cells. The sex chromatin body was situated at the periphery of the nucleus and its appearance resembled that of the Barr body in mammals.

Kosin and Ishizaki conclude from their findings that the sex chromatin is an incompletely sex-limited trait which is not necessarily related to the X chromosome.

The finding of sex chromatin in the fowl was confirmed by Ohno, Kaplan, and Kinoshita (1960) and by Moore and Hay (1961), while the presence of sex chromatin in female chick embryos was also reported by Ishizaki and Kosin (1960).

On the other hand, neither Ashley and Theiss (1959) nor Miles and Storey (1962) were able to demonstrate sex chromatin in the fowl. Ashley and Theiss examined sections of various tissues from the fowl, duck, parrot, and parakeet. They were unable to establish a sex difference, since cells from both males and females contained many chromatin crosses. Miles and Storey who examined cells from the fowl, both grown *in vitro* as well as sections from various tissues, also found chromocentres present in both sexes.

In the fowl at least 11 sex-linked genes are known proving beyond doubt that the female is the heterogametic sex (Hutt, 1949). Chromosome analysis in birds has always proved difficult owing to the presence of large numbers of so-called microchromosomes, some of which are at the limit of the resolving power of the light microscope in size. In addition, there are a number of pairs of chromosomes of ordinary size, the macro-chromosome. According to Ohno (1961), the domestic fowl has 8 pairs of macrochromosomes, of which the fifth largest is unpaired in the female and paired in the male and therefore represents the X chromosome. Many authors, including Ohno, use the terminology ZZ and WZ to represent the X and Y chromosomes in animals with female heterogamety. However, the XY terminology in female heterogamety is simpler and is in accordance with the usage of Winge (1932); it has been adopted here. Regarding the presence of a Y chromosome in female chickens, Ohno (1961) did not arrive at a definite conclusion, but Frederic (1961) provided evidence that a Y chromosome is present in the form of one of the largest microchromosomes. The total number of micro-chromosomes in the fowl is thought to be about 60 (Ohno, 1961; Frederic, 1961; Stenius, Christian, and Ohno, 1963).

The problem of sex chromatin in the fowl was further tackled by Schmid (1962), who treated chicken cells grown in tissue culture with radioactive thymidine. He found that the labelling

pattern of both X chromosomes in the male, as well as the single X chromosome in the female, was not different from that of the autosomes. However, in the female, one of the larger micro-chromosomes, which appeared to be identical with the Y chromosome described by Frederic, exhibited a very heavy and comparative late uptake of thymidine.

Although the data on sex chromatin in animals with female heterogamety available so far are scanty compared with those obtained in mammals, the problems that are raised are of the greatest importance for a general understanding of the subject. Schmid's (1962) finding that the Y chromosome of the hen shows late DNA replication is in agreement with the observation (Schmid, 1963) that the Y chromosome in man is similarly late labelling. On the other hand, the evidence available so far suggests that the situation with regard to the X chromosome is very different from that found in mammals, as there appears to be no sex chromatin in the interphase nuclei of cocks, while the DNA-labelling pattern of both X chromosomes in the male and the single X chromosome is like that of autosomes. Since any comprehensive theory on the significance of sex chromatin and heteropycnosis in mammals will have to take account of the findings in animals with female heterogamety, further studies of these phenomena in birds and Lepidoptera will clearly be of the greatest importance.

Some Practical Applications of Sex Chromatin Determinations

The results of sex chromatin studies provided the first indications of discrepancies between the genetical and phenotypic sex in some errors of human sexual development. Similarly, in present-day medical genetics, the presence or absence of Barr bodies and drumsticks will usually give the first information regarding the numbers of X chromosomes present in patients in whom abnormalities of the X chromosome may be suspected.

In general, the sex chromatin test will be most useful in detecting the classical XO type of Turner's syndrome and the XXY type of Klinefelter's syndrome, as well as karyotypes with multiple X chromosomes, but the test will not usually detect chromosomal mosaics, unless the X chromosome constitution is strikingly different in different tissues, as in the patient of Jacobs *et al.* (1960). For instance, the detection of a single Barr body in a proportion of buccal mucosa cells in a male patient will suggest the presence of an XXY karyotype, but it will not give any information regarding further cell lines that might have XO, XY, or XX sex

chromosome constitutions. Also, in rare instances, the karyotype might be XYYY (Muldal and Ockey, 1960).

In patients with Turner's syndrome, who are XO/XX mosaics, sex chromatin is usually present (de la Chapelle, 1962), and such patients will not generally be detected as a result of sex chromatin testing. A low incidence of sex chromatin bodies may raise the suspicion of a mosaicism of this type, but, particularly in buccal mucosa, a low incidence of Barr bodies may occur for other reasons.

Patients with iso-chromosomes of the long arm of the X chromosome (X_1) tend to have a high proportion of the large Barr bodies (Jacobs *et al.*, 1960; de la Chapelle, 1962; Lindsten, 1963). The drumsticks are also larger and much more frequent than normal (Maclean, 1962; Bamford, Cassin, and Mitchell, 1963; de la Chapelle, 1962); since they occur with a frequency of 10 per 100 neutrophils, large drumsticks occurring with lesser frequencies may suggest the presence of mosaicism of the type XX_1/XX_0 with a fair degree of accuracy. Smaller than normal Barr bodies are associated with deleted X chromosomes (Jacobs *et al.*, 1960, 1961; Lindsten, 1963).

Although the drumsticks in such cases may also be smaller than normal, small drumsticks are difficult to distinguish from similar looking appendages of the neutrophils that are not sex specific.

From a genetical point of view, the most important application of sex chromatin testing has been in establishing the incidence of abnormalities of the sex chromosomes as a result of screening large populations. Preparations made from the oral mucosa are the most suitable for this purpose and have been used almost exclusively. The survey by Shapiro and Ridler (1960) is exceptional in being based on leucocyte morphology, while Ridler, Shapiro, and McKibben (1963) have utilized both buccal mucosa and leucocytes.

Sex chromatin surveys have been carried out on series of newborn infants as well as on populations of the mentally retarded. The possibility of making sex chromatin surveys as the basis of post-mortem material has recently been suggested by Edwards and Cameron (1964).

The largest survey on the sex chromatin of newborns was carried out by Maclean, Harnden, Court Brown, Bond, and Mantle (1964) who examined 10,725 liveborn males, born consecutively, and 10,000 females. Among the male infants, 21 were found to contain single Barr bodies, and among the females, 12 had double Barr bodies, and in 4 no Barr bodies could be found. On the basis of this and 2 earlier surveys by Moore (1959) and by

Bergemann (1961), comprising 3,800 males and 3,600 females, the incidence of Klinefelter's syndrome at birth may be estimated at about 2 per 1,000, that of the triple X condition at about 1 per 1,000 and that of Turner's syndrome without sex chromatin about 4 per 10,000. As was pointed out above, this approach is likely to miss those cases of Turner's syndrome that are mosaics or have iso-chromosomes. In the data by Lindsten (1963), sex chromatin was present in the buccal mucosa of 20 out of 57 patients with Turner's syndrome, and in the data by de la Chapelle (1962) there were 10 such patients out of 23. As a first approximation it may be assumed that the incidence of Turner's syndrome is about a third to a quarter higher than would be estimated from sex chromatin studies. While the results of these three surveys are in good mutual agreement a sex chromatin survey on newborn babies in India (2,058 males and 1,832 females) failed to show any abnormalities (Subray and Prabhaker, 1962).

Sex chromatin surveys on male patients in mental deficiency hospitals have been carried out by Mosier, Scott, and Cotter (1960), Barr, Shaver, Carr, and Plunkett (1960), Ferguson-Smith *et al.* (1960), Sanderson and Stewart (1961), Maclean, Mitchell, Harnden, Williams, Jacobs, Buckton, Baikie, Court Brown, McBride, Strong, Close, and Jones (1962), Hamerton, Jagiello, and Kirman (1962) and Davies (1963). Out of a total of 7,305 patients examined, 67 were found to have sex chromatin, giving an incidence of just under 1%. This incidence is several times as high as that found at birth. The incidence of Klinefelter's syndrome is also increased among boys attending schools for the educationally subnormal (see Israelsohn and Taylor, 1961).

Surveys on female patients were carried out by Fraser, Campbell, MacGillivray, Boyd, and Lennox (1960), Sanderson and Stewart (1961), Johnston *et al.* (1961), Maclean *et al.* (1962), Hamerton *et al.* (1962), Davies (1963), and Ridler *et al.* (1963). The total number of patients' surveys was 4,575, and among these 9 patients with duplicated Barr bodies were found, giving an incidence of about 4 per 1,000. In these patients an XXX sex chromosome constitution was either verified or may be assumed, and it is clear that this condition also has a higher incidence among the mentally retarded than is found at birth. Among this group of patients only one had no Barr bodies (Maclean *et al.*, 1962), and another patient with an iso-chromosome of the long arm of the X was identified by Hamerton *et al.* (1962). Judging from these data, the incidence of Turner's syndrome among the mentally retarded is about 4

per 10,000, and there is, therefore, no evidence that this is any higher than among the newborn.

Thus, in spite of certain limitations of the buccal smear technique for sex chromatin investigations, it has provided results of the greatest interest to human cytogenetics. It is clear that without this technique estimates of the frequencies of the various abnormalities of the X-chromosome could not have approached the accuracy of those that are now available.

Some Theoretical Considerations Concerning Sex Chromatin

The possibility that the sex chromatin body might represent the second X chromosome which, being genetically superfluous, is present in a condensed and inactivated form, was suggested by Stewart (1960). Russell (1961) on the basis of data relating to the effect of rearrangements of the X chromosome in the mouse formulated the hypothesis that in mammals any X chromosome in addition to the first assumes the properties of heterochromatin. However, the inactive X hypothesis in its wider form was advanced and developed by Lyon (1961, 1962, 1963).

The Lyon hypothesis consists of 3 parts. First, it postulates that the genes on the sex chromatin forming X chromosome are inactive; secondly, that the decision as to which of a pair of X chromosomes is to be inactivated is made early in embryonic life and that, once the decision is made, the descendants of each X chromosome will be like the parent chromosome; and thirdly, that the original inactivation in each embryonic cell occurs at random, so that in some cells a paternal and in others a maternal X chromosome will be inactivated. Therefore, as the embryo grows, patches of cells with inactive X chromosomes of paternal origin will alternate with inactive X chromosomes of maternal origin. The theory was originally developed to explain the fact that female mice that are heterozygous for sex-linked genes are piebald (Lyon, 1961). Since the effect of the theory would be that animals of both sexes have only a single dose of sex-linked genes, it is thought to provide the basis for dosage compensation in mammals (Lyon, 1963).

The term 'dosage compensation' was introduced by Muller (1932) to describe the fact that in *Drosophila* the effect of genes borne on the X chromosome is about the same in males where they occur in single dose, and in females where they are present in double dose. Muller's explanation was that the genes on the X chromosome were balanced in such a way that the effects of dosage were cancelled.

There can be no doubt that dosage compensation occurs also in mammals. For instance in man, the gene controlling the production of glucose-6-phosphate dehydrogenase in erythrocytes is located on the X chromosome, yet the enzyme is present in equal amounts in males and females (Marks, 1958) as well as in patients with abnormal numbers of X chromosomes (Grumbach, Marks, and Morishima, 1962; Harris, Hopkinson, Spencer, Court Brown, and Mantle, 1963). In the mouse, animals with an XO karyotype are viable and fertile females (Welshons and Russell, 1959).

In the mouse, Lyon (1963) has presented evidence that in females heterozygous for 2 pairs of genes, with one gene borne on the X chromosome and the other on a piece of autosome translocated to the other X chromosome, only 1 of the genes is acting in any patch of cells. It is postulated that the X chromosome bearing the translocation acts an integral whole. Ohno and Cattanaach (1962) have combined genetical studies of this type with cytological observations. They used female mice having one normal X chromosome, X^a , and one bearing a translocation X^t , which made it 18% longer than normal. The translocated chromosome contained the wild type allele of the gene *albinism* which, in this combination, produces patches of wild-type colour alternating with patches of white. The authors found that in the prophase of mitosis in skin cells, one chromosome was more condensed than the rest. Moreover, it appeared that the condensed chromosome in cells from the white patches was larger than the condensed chromosome from the wild type patches, suggesting that the genes on the condensed chromosome do not affect the phenotype.

Further work along these lines would be most desirable. At present, it is one of the ironies of this subject, that although the theory of X-inactivation was originally formulated on the basis of genetic data on the mouse, this species has proved so recalcitrant to the cytological demonstration of sex chromatin. It may well be that the difficulties in this respect are merely technical, due to the presence of non-specific chromocentres. Nevertheless, the significance of these chromocentres requires elucidation.

In man, work on 2 genes borne on the X chromosome is of particular relevance to the Lyon hypothesis. Women who are heterozygous for the gene causing glucose-6-phosphate dehydrogenase deficiency tend to have an enzyme level that is intermediate between that of normal and deficient subjects, and Beutler, Yeh, and Fairbanks (1962) and Beutler and Baluda (1964), have presented evidence that the erythrocytes of such

women are composed of 2 classes, with normal and very low enzyme levels respectively. This finding was confirmed by Davidson, Nitowsky, and Childs (1963) on cells grown in tissue culture. These authors succeeded in growing clones from single cells and found that such clones from heterozygous women either had a normal enzyme level or were deficient.

However, in women who are heterozygous for the sex-linked blood groups antigen Xg (Mann, Cahan, Gelb, Fisher, Hamper, Tippet, Sanger, and Race, 1962), Gorman, di Re, Treacy, and Cahan (1963) were unable to separate 2 classes of cells and Reed, Simpson, and Chown (1963), using a fluorescent antibody technique, found that all the red cells from such a woman contained the antigen. In a recent editorial (*Lancet*, 1963) the following reasons for this discordant finding were suggested: the X chromosome may not be inactivated over its entire length (evidence that inactivating is not total has been obtained by Russell (1963) for the mouse); the antigen may be diffusible; or (unlikely in this case, but very likely in some other instances) one race of cells may have a selective advantage over the other. It is clear that far more work is required on the behaviour of sex-linked genes in man and, indeed, on the behaviour of autosomal genes also, to enable one to make a full evaluation of the Lyon hypothesis in terms of gene action on the human X chromosome.

On a more strictly cytological level, there are also many nuclear aspects that need to be resolved. For instance, the maximum number of Barr bodies is not present in every nucleus (p. 54). The reason for this is not yet known, and until it is we cannot say what effect such nuclei have with respect to the action of their X chromosomes.

A finding that is not strictly in accordance with the Lyon hypothesis of random inactivation of the paternal or maternal X chromosome is that in patients with iso-chromosomes of the long arm of the X, this chromosome appears to be invariably the late labelling one (Muldal *et al.*, 1963; Giannelli 1963). However, in the present state it seems hardly fair to test the hypothesis in such an extreme situation as that provided by the presence of an iso-chromosome. On the other hand, there is as yet no cytological evidence that in the case of normal X chromosomes a Barr body forming X chromosome invariably gives rise to another Barr body following mitotic division.

At present, we do not know the reason for the observed abnormalities in patients with abnormal numbers of X chromosomes. It is conceivable that such abnormalities are laid down before the sex

chromatin is formed in the embryo (p. 54) and therefore all X chromosomes might be presumed to be active. The possibility has also been raised that the X chromosome may have other effects on embryonic development in addition to the monogenic ones with which the Lyon hypothesis is specifically concerned (McKusick, 1962). This, in turn, is connected with the bigger problem of the role of the X chromosome in sexual differentiation (Stern, 1963).

The past 10 years or so have seen a tremendous increase in our knowledge of what we might call formal human cytogenetics. Chromosomal abnormalities have been shown to be the cause of a variety of human malformations; and the discovery of the sex chromatin, and the development of suitable techniques to demonstrate it, have added a valuable test to routine pathology. At the same time, we are still far removed from any real understanding as to the physical part played by the chromosomes in the development of the cells and the organism which we know they control. We do not know the function of the chromosomes in normal development or in the pathogenesis of abnormalities. The Lyon hypothesis has focused attention on some of the problems involving the X chromosome. To solve these problems we shall require much more knowledge of the relation between cytogenetics and the emergence of the phenotype in man and other animals.

Stern (1960) wrote: 'Dosage compensation forms 1 chapter in the history of genetic systems. Its analysis is not yet a closed chapter.' Indeed, it has only just begun and current work on sex chromatin will undoubtedly advance its development.

REFERENCES

- Ashley, D. J. B. (1957). Occurrence of sex chromatin in the cells of the blood and bone marrow of man. *Nature (Lond.)*, **179**, 969.
- (1962). *Human Intersex*. Livingstone, Edinburgh and London.
- , and Jones, C. H. (1958). Discrepancies in the diagnosis of genetic sex by leucocyte morphology. *Lancet*, **i**, 240.
- , and Theiss, E. A. (1959). Nuclear sex in species showing male homogamety. *Anat. Rec.*, **135**, 115.
- Atkin, N. B. (1960). Sex chromatin and chromosomal variation in human tumours. *Acta Un. int. Cancr.*, **16**, 41.
- , and Klinger, H. P. (1962). The superfemale mole. *Lancet*, **2**, 727.
- Atkins, L., Böök, J. A., Gustavson, K. H., Hansson, O., and Hjelm, M. (1963). A case of XXXXY sex chromosome anomaly with autoradiographic studies. *Cytogenetics*, **2**, 208.
- Austin, C. R., and Amoroso, E. C. (1957). Sex chromatin in early cat embryos. *Exp. Cell Res.*, **13**, 419.
- Bamford, S. B., Cassin, C. M., and Mitchell, B. S. (1963). Sex chromatin determinations in selected cases of developmental sex abnormalities with an assessment of results. *Acta cytol. (Philad.)*, **7**, 151.
- Barr, M. L. (1951). The morphology of neuroglial nuclei in the cat, according to sex. *Exp. Cell Res.*, **2**, 288.
- (1955). The sex chromatin and its application to errors in sex development. In *Modern Trends in Obstetrics and Gynaecology*, 2nd series, ed. K. Bowes, p. 117. Butterworth, London.

- (1963). The sex chromatin. In *Intersexuality*, ed. Claus Overzier, p. 48. Academic Press, London and New York.
- , and Bertram, E. G. (1949). A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature (Lond.)*, **163**, 676.
- , Bertram, L. F., and Lindsay, H. A. (1950). The morphology of the nerve cell nucleus, according to sex. *Anat. Rec.*, **107**, 283.
- , and Carr, D. H. (1960). Sex chromatin, sex chromosomes and sex anomalies. *Canad. med. Ass. J.*, **83**, 979.
- , —, Pozsonyi, J., Wilson, R. A., Dunn, H. G., Jacobson, T. S., and Miller, J. R. (1962). The XXXXY sex chromosome abnormality. *ibid.*, **87**, 891.
- , Shaver, E. L., Carr, D. H., and Plunkett, E. R. (1959). An unusual sex chromatin pattern in three mentally deficient subjects. *J. ment. Defic. Res.*, **3**, 78.
- , —, and — (1960). The chromatin-positive Klinefelter syndrome among patients in mental deficiency hospitals. *ibid.*, **4**, 89.
- Barton, D. E., David, F. N., and Merrington, M. (1963). Numerical analysis of chromosome patterns. *Ann. hum. Genet.*, **26**, 349.
- Bergemann, E. (1961). Geschlechtschromatinbestimmungen am Neugeborenen. *Schweiz. med. Wschr.*, **91**, 292.
- Bertram, E. G., Bertram, L. F., and Lindsey, H. A. (1950). The nucleolus-associated chromatin in nerve cells of cat and man. *Anat. Rec.*, **106**, 299.
- Beutler, E., and Baluda, M. C. (1964). The separation of glucose-6-phosphate-dehydrogenase-deficient erythrocytes from the blood of heterozygotes for glucose-6-phosphate-dehydrogenase deficiency. *Lancet*, **1**, 189.
- , Yeh, M., and Fairbanks, V. F. (1962). The normal human female as a mosaic of X-chromosome activity. Studies using the gene for G-6-Pd deficiency as a marker. *Proc. nat. Acad. Sci. (Wash.)*, **48**, 9.
- Bishop, A., and Bishop, O. N. (1963). Analysis of tritium-labelled human chromosomes and sex chromatin. *Nature (Lond.)*, **199**, 930.
- Böök, J. A., and Santesson, B. (1961). Nuclear sex in triploid XXY human cells. *Lancet*, **2**, 318.
- Bradbury, J. T., Bunge, R. G., and Boccabella, R. A. (1956). Chromatin test in Klinefelter's syndrome. *J. clin. Endocr.*, **16**, 689.
- Briggs, D. K. (1958). The individuality of nuclear chromatin with particular reference to polymorphonuclear neutrophil leukocytes. *Blood*, **13**, 986.
- , and Kupperman, H. S. (1956). Sex differentiation by leukocyte morphology. *J. clin. Endocr.*, **16**, 1163.
- Caratzali, A. (1963). Sex appendages, ARNETH formula, and the menstrual cycle. *Cytogenetics*, **2**, 15.
- , and Phleps, A. (1958). Considérations sur le déterminisme des corpuscules sexuels des leucocytes. *C.R. Soc. Biol. (Paris)*, **152**, 1114.
- , —, and Turpin, R. (1957). Variations quantitatives des corpuscules sexuels des granulocytes neutrophiles au cours du cycle menstruel. *Bull. Acad. Méd. (Paris)*, **141**, 496.
- Carpentier, P. J., Stolte, L. A. M., and Visschers, G. P. (1956). Determination of genetic sex by the vaginal smear. *J. clin. Endocr.*, **16**, 155.
- Carr, D. H. (1963). Chromosome studies in abortuses and stillborn infants. *Lancet*, **2**, 603.
- , Barr, M. L., and Plunkett, E. R. (1961). An XXXX sex chromosome complex in two mentally defective females. *Canad. med. Ass. J.*, **84**, 131.
- de Castro, N. M. (1963). Frequency variations of "drumsticks" of peripheral blood neutrophils in the rabbit in different alimentary conditions. *Acta anat. (Basel)*, **52**, 341.
- de la Chapelle, A. (1962). Cytogenetical and clinical observations in female gonadal dysgenesis. *Acta endocr. (Kbh.)*, Suppl. 65.
- Davidson, R. G., Nitowsky, H. M., and Childs, B. (1963). Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. *Proc. nat. Acad. Sci. (Wash.)*, **50**, 481.
- , and Smith, D. R. (1954). A morphological sex difference in the polymorphonuclear neutrophil leukocytes. *Brit. med. J.*, **2**, 6.
- , and — (1958). The neutrophil sex nodules in Klinefelter's syndrome. In *Symposium on Nuclear Sex*, ed. D. R. Smith, and W. M. Davidson, p. 93. Heinemann, London.
- , and — (1963). The nuclear sex of leucocytes. In *Intersexuality*, ed. C. Overzier, p. 72. Academic Press, London and New York.
- , and Winn, S. (1961). The relationship between the sex nodule and the sex chromosomes. In *Proceedings of the Conference on Human Chromosomal Abnormalities, London 1952*, ed. W. M. Davidson, and D. R. Smith, p. 28. Staples Press, London.
- Davies, T. S. (1963). Buccal smear surveys for sex chromatin. *Brit. med. J.*, **1**, 1541.
- DeMars, R. (1962). Sex chromatin mass in living, cultivated human cells. *Science*, **138**, 980.
- Edwards, J. H., and Cameron, A. H. (1964). Postmortem nuclear sexing. *Lancet*, **1**, 328.
- Ferguson-Smith, M. A., Johnston, A. W., and Handmaker, S. D. (1960). Primary amentia and micro-orchidism associated with an XXXY sex-chromosome constitution. *ibid.*, **2**, 184.
- Ferrier, P., Ferrier, S., Stalder, G., Bühler, E., Bamatter, F., and Klein, D. (1964). Congenital asymmetry associated with diploid-triploid mosaicism and large satellites. *ibid.*, **1**, 80.
- Ford, C. E. (1963). The cytogenetics of human intersexuality. In *Intersexuality*, ed. C. Overzier, p. 86. Academic Press, London and New York.
- Fraccaro, M., Ikkos, D., Lindsten, J., Luft, R., and Kaijser, K. (1960a). A new type of chromosomal abnormality in gonadal dysgenesis. *Lancet*, **2**, 1144.
- , Kaijser, K., and Lindsten, J. (1960b). A child with 49 chromosomes. *ibid.*, **2**, 899.
- , Klinger, H. P., and Schutt, W. (1962). A male with XXXXY sex chromosomes. *Cytogenetics*, **1**, 52.
- , and Lindsten, J. (1959). Observations on the so-called 'sex chromatin' in human somatic cells cultivated *in vitro*. *Exp. Cell Res.*, **17**, 536.
- , and — (1960). A child with 49 chromosomes. *Lancet*, **2**, 1303.
- Fraser, J. H., Campbell, J., MacGillivray, R. C., Boyd, E., and Lennox, B. (1960). The XXX syndrome; frequency among mental defectives and fertility. *ibid.*, **2**, 626.
- Frederic, J. (1961). Contribution à l'étude du caryotype chez le poulet. *Arch. Biol. (Liège)*, **72**, 185.
- Frizzi, G. (1948). L'eteropinciosi come indice di riconoscimento dei sessi in 'Bombyx mori L.'. *Ric. sci.*, **18**, 119.
- Geitler, L. (1937). Die Analyse des Kernbaus und der Kernsteilung der Wasserläufer *Gerris lateralis* und *Gerris lacustris* (Hemiptera, Heteroptera) und die Somadifferenzierung. *Z. Zellforsch.*, **26**, 641.
- (1938). Über den Bau des Ruhekerens mit besonderer Berücksichtigung der Heteropteren und Dipteren. *Biol. Zbl.*, **58**, 152.
- (1939). Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. *Chromosoma (Berl.)*, **1**, 1.
- German, J. L., III (1962a). Synthesis of deoxyribonucleic acid during interphase. *Lancet*, **1**, 744.
- (1962b). DNA synthesis in human chromosomes. *Trans. N.Y. Acad. Sci.*, **24**, 395.
- Giannelli, F. (1963). The pattern of X-chromosome deoxyribonucleic acid synthesis in two women with abnormal sex-chromosome complements. *Lancet*, **1**, 863.
- Gilbert, C. W., Muldal, S., Lajtha, L. G., and Rowley, J. (1962). Time-sequence of human chromosome duplication. *Nature (Lond.)*, **195**, 869.
- Glenister, T. W. (1956). Determination of sex in early human embryos. *ibid.*, **177**, 1135.
- Gorman, J. G., di Re, J., Treacy, A. M., and Cahan, A. (1963). The application of -Xg^a antiserum to the question of red cell mosaicism in female heterozygotes. *J. Lab. clin. Med.*, **61**, 642.
- Graham, M. A. (1954a). Detection of the sex of cat embryos from nuclear morphology in the embryonic membrane. *Nature (Lond.)*, **173**, 310.
- (1954b). Sex chromatin in cell nuclei of the cat from the early embryo to maturity. *Anat. Rec.*, **119**, 469.
- , and Barr, M. L. (1952). A sex difference in the morphology of metabolic nuclei in somatic cells of the cat. *ibid.*, **112**, 709.
- Greenblatt, R. B., Aydar, C. K., and Gibson, H. H. (1962). Discrepancies in the evaluation of sex chromatin in oral smears and peripheral blood specimens. *Acta cytol. (Philad.)*, **6**, 103.
- , Manautou, J. M., Tigerman, N. R., Rogers, R. L., Jr., and Sheffield, F. H. (1957). A simplified staining technique for the study of chromosomal sex in oral mucosal and peripheral blood smears. *Amer. J. Obstet. Gynec.*, **74**, 629.

- Grumbach, M. M., Marks, P. A., and Morishima, A. (1962). Erythrocyte glucose-6-phosphate dehydrogenase activity and X-chromosome polysomy. *Lancet*, **1**, 1330.
- , and Morishima, A. (1962). Sex chromatin and the sex chromosomes: on the origin of sex chromatin from a single X chromosome. *Acta cytol. (Philad.)*, **6**, 46.
- , —, and Taylor, J. H. (1963). Human sex chromosome abnormalities in relation to DNA replication and heterochromatinization. *Proc. nat. Acad. Sci. (Wash.)*, **49**, 581.
- Guard, H. R. (1959). A new technic for differential staining of the sex chromatin, and the determination of its incidence in exfoliated vaginal epithelial cells. *Amer. j. clin. Path.*, **32**, 145.
- Guthertz, S. (1907). Zur Kenntnis der Heterochromosomen. *Arch. mikr. Anat.*, **69**, 491.
- Hamerton, J. L., Fraccaro, M., de Carli, L., Nuzzo, F., Klinger, H. P., Hulliger, L., Taylor, A., and Lang, E. M. (1961). Somatic chromosomes of the gorilla. *Nature (Lond.)*, **192**, 225.
- , Jagiello, G. M., and Kirman, B. H. (1962). Sex-chromosome abnormalities in a population of mentally defective children. *Brit. med. j.*, **1**, 220.
- , Klinger, H. P., Mutton, D. E., and Lang, E. M. (1963). The somatic chromosomes of the Hominoidea. *Cytogenetics*, **2**, 240.
- von Harnack, G. A., and Strietzel, H. N. (1956). Die Altersabhängigkeit der geschlechtsbedingten Leukocytenmerkmale. *Klin. Wschr.*, **34**, 401.
- Harnden, D. G. (1961). Nuclear sex in triploid XXY human cells. *Lancet*, **2**, 488.
- Harris, H., Hopkinson, D. A., Spencer, N., Court Brown, W. M., and Mantle, D. (1963). Red cell glucose-6-phosphate dehydrogenase activity in individuals with abnormal numbers of X-chromosomes. *Ann. hum. Genet.*, **27**, 59.
- Hay, J. C. (1960). Further observations on the sex chromatin of mammalian cells. *Anat. Rec.*, **136**, 315.
- Heitz, E. (1928). Das Heterochromatin der Moose. I. *Jb. wiss. Bot.*, **69**, 762.
- Hulliger, L., Klinger, H. P., and Allgöwer, M. (1963). Sex chromatin as a marker in some rabbit cells. *Experientia (Basel)*, **19**, 240.
- Hunter, W. F., and Lennox, B. (1954). The sex of teratomata. *Lancet*, **2**, 633.
- Hutt, F. B. (1949). *Genetics of the Fowl*. McGraw-Hill, New York, Toronto, London.
- Ishizaki, H., and Kosin, I. L. (1960). Sex chromatin in early chick embryos. *Exp. Cell Res.*, **21**, 197.
- Israelsohn, W. J., and Taylor, A. I. (1961). Chromatin positive presumed Klinefelter's syndrome. Survey of boys in London schools for educationally subnormal children. *Brit. med. j.*, **1**, 633.
- Izaković, V. (1960). Gonadal dysgenesis in two sisters with male nuclear sex pattern and female characteristics in polymorphonuclear leukocytes. *J. clin. Endocr.*, **20**, 1301.
- Jackson, W. P. U., Shapiro, B. G., Uys, C. J., and Hoffenberg, R. (1956). Primary male hypogonadism with female nuclear sex. *Lancet*, **2**, 857.
- Jacobs, P. A., Baikie, A. G., Court Brown, W. M., MacGregor, T. N., Maclean, N., and Harnden, D. G. (1959). Evidence for the existence of the human 'super female'. *ibid.*, **2**, 423.
- , Harnden, D. G., Buckton, K. E., Court Brown, W. M., King, M. J., McBride, J. A., MacGregor, T. N., and Maclean, N. (1961). Cytogenic studies in primary amenorrhoea. *ibid.*, **1**, 1183.
- , —, Court Brown, W. M., Goldstein, J., Close, H. G., MacGregor, T. N., Maclean, N., and Strong, J. A. (1960). Abnormalities involving the X chromosome in women. *ibid.*, **1**, 1213.
- James, J. (1960). Observations on the so-called sex chromatin. *Z. Zellforsch.*, **51**, 597.
- Johnston, A. W., Ferguson-Smith, M. A., Handmaker, S. D., Jones, H. W., and Jones, G. S. (1961). The triple-X syndrome; Clinical, pathological, and chromosomal studies in three mentally retarded cases. *Brit. med. j.*, **2**, 1046.
- Kesaree, N., and Woolley, P. V. (1963). A phenotypic female with 49 chromosomes, presumably XXXXX. *J. Pediatr.*, **63**, 1099.
- Klinger, H. P. (1957). The sex chromatin in fetal and maternal portions of the human placenta. *Acta anat. (Basel)*, **30**, 371.
- (1958). The fine structure of the sex chromatin body. *Exp. Cell Res.*, **14**, 207.
- , and Ludwig, K. S. (1957). A universal stain for the sex chromatin body. *Stain Technol.*, **32**, 235.
- , and Schwarzscher, H. G. (1960). The sex chromatin and heterochromatic bodies in human diploid and polyploid nuclei. *J. biophys. biochem. Cytol.*, **8**, 345.
- , and — (1962). XY/XXY and sex chromatin positive cell distribution in a 60 mm human fetus. *Cytogenetics*, **1**, 266.
- Kosenow, W. (1957). Abweichende Ergebnisse bei der Geschlechtsbestimmung an Leukocyten- und Mundepithel-Kernen. *Klin. Wschr.*, **35**, 75.
- , and Scupin, R. (1956). Die Bestimmung des Geschlechts mit Hilfe einer Kernanhangsformel der Leukocyten. *Acta haemat. (Basel)*, **15**, 349.
- Kosin, I. L., and Ishizaki, H. (1959). Incidence of sex chromatin in *Gallus domesticus*. *Science*, **130**, 43.
- Lancet (1963). [Editorial.] Lyonisation of the X chromosome. **2**, 769.
- Lennox, B. (1963). Sex chromatin in tumours. In *Intersexuality*, ed. C. Overzier, p. 462. Academic Press, London and New York.
- Lillie, F. R. (1916). The theory of the free-martin. *Science*, **43**, 611.
- Lindsay, H. A., and Barr, M. L. (1955). Further observations on the behaviour of nuclear structures during depletion and restoration of Nissl material. *J. Anat. (Lond.)*, **89**, 47.
- Lindsten, J. (1963). *The Nature and Origin of X Chromosome Aberrations in Turner's Syndrome*. Almqvist and Wiksell, Stockholm.
- , Fraccaro, M., Ikkos, D., Kaijser, K., Klinger, H. P., and Luft, R. (1963). Presumptive iso-chromosomes for the long arm of the X in man. Analysis of five families. *Ann. hum. Genet.*, **26**, 383.
- London, D. R., Kemp, N. H., Ellis, J. R., and Mittwoch, U. (1964). Turner's syndrome with secondary amenorrhoea and sex chromosome mosaicism. *Acta endocr. (Kbh.)*, **46**, 341.
- Lüers, T. (1956). Vergleichende Untersuchungen über morphologische Geschlechtsunterschiede der neutrophilen Leukozytenkerne bei Mensch und Kaninchen. *Blut*, **2**, 81.
- , and Petzel, C. (1958). Zellkernmorphologische Geschlechtsdiagnose bei Pelger-Anomalie der Blutkörperchen. *ibid.*, **4**, 185.
- Lyon, M. F. (1961). Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature (Lond.)*, **190**, 372.
- (1962). Sex chromatin and gene action in the mammalian X-chromosome. *Amer. j. hum. Genet.*, **14**, 135.
- (1963). Attempts to test the inactive-X theory of dosage compensation in mammals. *Genet. Res.*, **4**, 93.
- McKusick, V. A. (1962). On the X chromosome of man. *Quart. Rev. Biol.*, **37**, 69.
- Maclean, N. (1962). The drumsticks of polymorphonuclear leukocytes in sex-chromosome abnormalities. *Lancet*, **1**, 1154.
- , Harnden, D. G., Court Brown, W. M., Bond, J., and Mantle, D. J. (1964). Abnormalities of sex chromosome constitution in newborn babies. *ibid.*, **1**, 286.
- , Mitchell, J. M., Harnden, D. G., Williams, J., Jacobs, P. A., Buckton, K. A., Baikie, A. G., Court Brown, W. M., McBride, J. A., Strong, J. A., Close, H. G., and Jones, D. C. (1962). A survey of sex-chromosome abnormalities among 4514 mental defectives. *ibid.*, **1**, 293.
- Mann, J. D., Cahan, A., Gelb, A. G., Fisher, N., Hamper, J., Tippet, P., Sanger, R., and Race, R. R. (1962). A sex-linked blood group. *ibid.*, **1**, 8.
- Marberger, E., Boccabella, R. A., and Nelson, W. O. (1955). Oral smear as a method of chromosomal sex detection. *Proc. Soc. exp. Biol. (N.Y.)*, **89**, 488.
- Marks, P. A. (1958). Red cell glucose-6-phosphate and 6-phosphogluconic dehydrogenases and nucleoside phosphorylase. *Science*, **127**, 1338.
- Melander, Y. (1962). Chromosomal behaviour during the origin of sex chromatin in the rabbit. *Hereditas (Lund)*, **48**, 645.
- Merrington, M., and Penrose, L. S. (1964). Distances which involve satellited chromosomes in metaphase preparations. *Ann. hum. Genet.*, **27**, 257.
- Miles, C. P. (1959). Sex chromatin in cultured human tissues. *Nature (Lond.)*, **184**, 477.
- (1960). Morphology and functional relations of sex chromatin in cultured amnion cells. *Exp. Cell Res.*, **20**, 324.
- (1961). Peripheral position of sex chromatin. *Nature (Lond.)*, **191**, 626.
- , and Storey, S. D. (1962). Nuclear chromocenters of cultured chicken cells. *Exp. Cell Res.*, **27**, 377.
- Miller, O. J., Breg, W. R., Mukherjee, B. B., Gamble, A. Van N., and Christakos, A. C. (1963a). Non-random distribution of chromosomes in metaphase figures from cultured human leuco-

- cytes. II. The peripheral location of chromosomes 13, 17-18 and 21. *Cytogenetics*, **2**, 152.
- , —, Schmickel, R. D., and Tretter, W. (1961). A family with an XXXXY male, a leukaemic male, and two 21-trisomic mongoloid females. *Lancet*, **2**, 78.
- , Mukherjee, B. B., Breg, W. R., and Gamble, A. Van N. (1963b). Non-random distribution of chromosomes in metaphase figures from cultured human leucocytes. I. The peripheral location of the Y-chromosome. *Cytogenetics*, **2**, 1.
- Minouchi, O. (1928). Spermatogenesis of the albino rat (*Mus norvegicus albus*). *Jap. J. Zool.*, **1**, 235.
- Mittwoch, U. (1957). Some observations on the leucocytes in mongolism. *J. ment. Defic. Res.*, **1**, 26.
- (1961). Nuclear sex and chromosome structure in mongolism and the Klinefelter syndrome. In *Proceedings of the Conference on Human Chromosomal Abnormalities, London 1959*, ed. W. M. Davidson, and D. R. Smith, p. 97. Staples Press, London.
- (1963). Barr bodies and nuclear size in cultured human fibroblasts. *Nature (Lond.)*, **198**, 975.
- (1964a). Frequency of drumsticks in normal women and in patients with chromosomal abnormalities. *ibid.*, **201**, 317.
- (1964b). Barr bodies and their relation to nuclear size. *Cytogenetics*, **3**, 62.
- , Atkin, N. B., and Ellis, J. R. (1963). Barr bodies in triploid cells. *ibid.*, **2**, 323.
- , and Delhanty, J. D. A. (1961). Nuclear sex in triploid XXY human cells. *Lancet*, **2**, 552.
- Moore, K. L. (1959). Sex reversal in newborn babies. *ibid.*, **1**, 217.
- , and Barr, M. L. (1953). Morphology of the nerve cell nucleus in mammals, with special reference to the sex chromatin. *J. comp. Neurol.*, **98**, 213.
- , and — (1954). Nuclear morphology, according to sex, in human tissues. *Acta anat. (Basel)*, **21**, 197.
- , and — (1955). Smears from the oral mucosa in the detection of chromosomal sex. *Lancet*, **2**, 57.
- , Graham, M. A., and Barr, M. L. (1953). The detection of chromosomal sex in hermaphrodites from a skin biopsy. *Surg. Gynec. Obstet.*, **96**, 641.
- , —, and — (1957). The sex chromatin of the bovine freemartin. *J. exp. Zool.*, **135**, 101.
- and Hay, J. C. (1961). Sexual dimorphism in intermitotic nuclei of birds. *Anat. Rec.*, **139**, 315.
- Moorhead, P. S., and Defendi, V. (1963). Asynchrony of DNA synthesis in chromosomes of human diploid cells. *J. Cell. Biol.*, **16**, 202.
- Morishima, A., Grumbach, M. M., and Taylor, J. H. (1962). Asynchronous duplication of human chromosomes and the origin of sex chromatin. *Proc. nat. Acad. Sci. (Wash.)*, **48**, 756.
- Mosier, H. D., Scott, L. W., and Cotter, L. H. (1960). The frequency of the positive sex-chromatin pattern in males with mental deficiency. *Pediatrics*, **25**, 291.
- Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Rowley, J., and Fraccaro, M. (1963). Tritiated thymidine incorporation in an isochromosome for the long arm of the X chromosome in man. *Lancet*, **1**, 861.
- , and Ockey, C. H. (1960). The 'double male': a new chromosome constitution in Klinefelter's syndrome. *ibid.*, **2**, 492.
- Muller, H. J. (1932). Further studies on the nature and causes of gene mutation. *Proc. 6th Int. Cong. Genet.*, Ithaca, 1931, **1**, 213.
- Nelson, W. O. (1956). Sex differences in human nuclei with particular reference to the 'Klinefelter-syndrome', a gonadal agenesis and other types of hermaphroditism. *Acta endocr. (Kbh.)*, **23**, 227.
- Ohno, S. (1961). Sex chromosomes and microchromosomes of *Gallus domesticus*. *Chromosoma (Berl.)*, **11**, 484.
- , and Cattanaach, B. M. (1962). Cytological study of an X-autosome translocation in *Mus musculus*. *Cytogenetics*, **1**, 129.
- , Kaplan, W. D., and Kinoshita, R. (1959). Formation of the sex chromatin by a single X-chromosome in liver cells of *Rattus norvegicus*. *Exp. Cell Res.*, **18**, 415.
- , —, and — (1960). On the sex chromatin of *Gallus domesticus*. *ibid.*, **19**, 180.
- , and Makino, S. (1961). The single-X nature of sex chromatin in man. *Lancet*, **1**, 78.
- , —, Kaplan, W. D., and Kinoshita, R. (1961). Female germ cells of man. *Exp. Cell Res.*, **24**, 106.
- Orsi, E. V., and Ritter, H. B. (1958). A report of sex chromatin in human tumor tissue culture. *Exp. Cell Res.*, **15**, 244.
- Overzier, C. (1958). Diskrepanz der Geschlechtsdiagnose. *Klin. Wschr.*, **36**, 845.
- (1963). *Intersexuality*. Academic Press, London and New York.
- Park, W. W. (1957). The occurrence of sex chromatin in early human and macaque embryos. *J. Anat. (Lond.)*, **91**, 369.
- Peiper, U., and Oehme, J. (1956). Die Abhängigkeit geschlechtsgebundener Leukocytenmerkmale bei Feten und Frühgeboreren vor der Reife. *Klin. Wschr.*, **34**, 1067.
- Plunkett, E. R., and Barr, M. L. (1956). Testicular dysgenesis; affecting the seminiferous tubules principally with chromatin-positive nuclei. *Lancet*, **2**, 853.
- Polani, P. E., Hunter, W. F., and Lennox, B. (1954). Chromosomal sex in Turner's syndrome with coarctation of the aorta. *ibid.*, **2**, 120.
- Prince, R. H., Graham, M. A., and Barr, M. L. (1955). Nuclear morphology, according to sex, in *Macacus rhesus*. *Anat. Rec.*, **122**, 153.
- Reed, T. E., Simpson, N. E., and Chown, B. (1963). The Lyon hypothesis. *Lancet*, **2**, 467.
- Reitalu, J. (1957). Observations on the so-called sex chromatin in man. *Acta Genet. med. (Roma)*, **6**, 393.
- Ridler, M. A. C., Shapiro, A., and McKibben, W. R. (1963). Sex chromatin abnormalities in female subnormal patients. *Brit. J. Psychiat.*, **109**, 390.
- Riis, P., Johnsen, S. G., and Mosbech, J. (1956). Nuclear sex in Klinefelter's syndrome. *Lancet*, **1**, 962.
- Ross, A. (1962). The demonstration of sex chromatin in cultured cells. *J. med. Lab. Technol.*, **19**, 112.
- Rowley, J., Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Fraccaro, M., and Kaijser, K. (1963). Synthesis of deoxyribonucleic acid on X-chromosomes of an XXXXY male. *Nature (Lond.)*, **197**, 251.
- Russell, L. B. (1961). Genetics of mammalian sex chromosomes. *Science*, **133**, 1795.
- (1963). Mammalian X-chromosome action: inactivation limited in spread and in region of origin. *ibid.*, **140**, 976.
- Sanderson, A. R., and Stewart, J. S. S. (1961). Nuclear sexing with aceto-orcein. *Brit. med. J.*, **2**, 1065.
- Shantaveerappa, T. R., and Bourne, G. H. (1962). A perineural epithelium. *J. cell. Biol.*, **14**, 343.
- Schmid, W. (1962). DNA replication patterns of the heterochromosomes in *Gallus domesticus*. *Cytogenetics*, **1**, 344.
- (1963). DNA replication patterns of human chromosomes. *ibid.*, **2**, 175.
- Schneiderman, L. J., and Smith, C. A. B. (1962). Non-random distribution of certain homologous pairs of normal human chromosomes in metaphase. *Nature (Lond.)*, **195**, 1229.
- Schrader, F. (1928). *Die Geschlechtschromosomen*. Borntraeger, Berlin.
- Schwarzacher, H. G. (1963). Sex chromatin in living human cells *in vitro*. *Cytogenetics*, **2**, 117.
- Serr, D. M., Ferguson-Smith, M. A., Lennox, B., and Paul, J. (1958). Representation of the X-chromosome in intermitotic nuclei in man. *Nature (Lond.)*, **182**, 124.
- Shapiro, A., and Ridler, M. A. C. (1960). The incidence of Klinefelter's syndrome in a mentally deficiency hospital. *J. ment. Defic. Res.*, **4**, 48.
- Siebnner, H., Klaus, D., and Heni, F. (1963). Ein Extrachromosom bei der Pelger-Huëtschen Kernanomalie. *Med. Welt. (Stuttg.)*, **877**.
- Smith, D. W., Marden, P. M., McDonald, M. J., and Speckhard, M. (1962). Lower incidence of sex chromatin in buccal smears of newborn females. *Pediatrics*, **30**, 707.
- Smith, S. G. (1944). The diagnosis of sex by means of heteropycnosis. *McGill. med. J.*, **13**, 451.
- (1945a). Heteropycnosis as a means of diagnosing sex. *J. Hered.*, **36**, 195.
- (1945b). The diagnosis of sex by means of heteropycnosis. *Sci. Agric.*, **25**, 566.
- Sohval, A. R. (1963). Chromosomes and sex chromatin in normal, and anomalous sexual development. *Physiol. Rev.*, **43**, 306.
- , and Casselman, W. G. B. (1961). Alteration in size of nuclear sex-chromatin mass (Barr body) induced by antibiotics. *Lancet*, **2**, 1386.

- , Gabrilove, J. L., Gaines, J. A., and Soffer, L. J. (1956). Observations on the sex chromatin in testicular disorder. *J. Mt Sinai Hosp.*, **23**, 647.
- Stenius, C., Christian, L. C., and Ohno, S. (1963). Comparative cytological study of *Phasianus colchicus*, *Meleagris gallopavo*, and *Gallus domesticus*. *Chromosoma (Berl.)*, **13**, 515.
- Stern, C. (1960). Dosage compensation—development of a concept and new facts. *Canad. J. Genet. Cytol.*, **2**, 105.
- (1963). The genetics of sex determination in man. *Amer. J. Med.*, **34**, 715.
- Stewart, J. S. S. (1960). Genetic mechanisms in human intersexes. *Lancet*, **1**, 825.
- Subray, N., and Prabhaker, N. (1962). Sex chromatin anomalies in newborn babies in India. *Science*, **136**, 1116.
- Tanaka, Y. (1953). Genetics of the silkworm, *Bombax mori*. *Advanc Genet.*, **5**, 239.
- Tandler, J., and Keller, K. (1911). Ueber das Verhalten des Chorions bei verschiedengeschlechtlicher Zwillingsgravidität des Rindes und über die Morphologie des Genitales der weiblichen Tiere, welche einer solchen Gravidität entstammen. *Dtsch. tierärztl. Wschr.*, **19**, 148.
- Taylor, A. I. (1963a). Nuclear sex of embryonic tumours. *Brit. med. J.*, **1**, 377.
- (1963b). Sex chromatin in the newborn. *Lancet*, **1**, 912.
- Taylor, J. H. (1960). Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. biophys. biochem. Cytol.*, **7**, 455.
- , Woods, P. S., and Hughes, W. L. (1957). The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. nat. Acad. Sci. (Wash.)*, **43**, 122.
- Therkelsen, A. J., and Petersen, G. B. (1962). Frequency of sex-chromatin-positive cells in the logarithmic and postlogarithmic growth phases of human cells in tissue culture. *Exp. Cell Res.*, **28**, 588.
- Tonomura, A., Toyofuko, Y., and Matsunaga, E. (1962). The frequency of so-called drumsticks in the polymorphonuclear neutrophil leucocytes of Japanese females. *Jap. J. hum. Genet.*, **7**, 60.
- Turner, H. H., and Zanartu, J. (1962). Ovarian dysgenesis in identical twins: discrepancy between nuclear chromatin pattern in somatic cells and in blood cells. *J. clin. Endocr.*, **22**, 660.
- Turpin, R., and Bernyer, C. (1947). De l'influence de l'hérédité sur la formule d'Arneth (cas particulier du mongolisme). *Rev. Hémat.*, **2**, 189.
- Welshons, W. J., and Russell, L. B. (1959). The Y-chromosome as the bearer of male determining factors in the mouse. *Proc. nat. Acad. Sci. (Wash.)*, **45**, 560.
- Whitby, L. E. H., and Britton, C. J. C. (1963). *Disorders of the Blood*, 9th ed. Churchill, London.
- Wiedemann, H. R. (1958). The result of haematological determination of the genetic sex in disturbances of sexual development. In *Symposium on Nuclear Sex*, ed. W. M. Davidson, and D. R. Smith, p. 102. Heinemann, London.
- , Romatowski, H., and Tolksdorf, M. (1956). Geschlechtsbestimmung aus dem Blutbilde. *Münch. med. Wschr.*, **98**, 1090, 1108.
- Wilkins, L., Grumbach, M. M., and van Wyk, J. J. (1954). Chromosomal sex in 'ovarian agenesis'. *J. clin. Endocr.*, **14**, 1270.
- Wilson, E. B. (1925). *The Cell in Development and Heredity*, 3rd ed. Macmillan, London and New York.
- Winge, O. (1932). The nature of sex chromosomes. *Proc. 6th Int. Congress Genetics*, Ithaca, 1931, **1**, 343.
- Wintrobe, M. M. (1961). *Clinical Haematology*, 5th ed. Kimpton, London.