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SEX DETERMINATION : DIAGNOSTIC METHODS*

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In the early years of this century the entomologists McClung (1902) and Stevens (1905) recognized differences between the cells of the male and female of certain insects. During mitoses the female had either an additional or a larger chromosome than the male. This was relatively easy with chromosomes in small numbers, but their work has led the way to investigation of the more complicated chromosomal structure in the higher animals, including man. Quite early it was shown that when arranged in order of size the chromosomes fell into a series of pairs, and Painter (1924) settled what had been a 10-year argument by showing conclusively that in man the female was distinguished by the presence of a pair of like, XX, chromosomes, while the male had an unlike, XY, pair, the Y chromosome being much smaller than the X.

This arrangement is not universal. Although present in the mammals there are species of animals in which a chromosome is missing in the male, and even some, including the birds, where the difference is reversed, so that the males have the two X chromosomes.

Geitler (1937) made a further contribution to the study of sex by showing that in the water-skater, *Gerris lateralis*, the extra chromosomal material in the female sex chromosomes could still be detected after the cells had passed into the resting phase by the more intense staining of the nuclei. The observation that there was extra, deep-staining heterochromatin in the nuclei in the female was confirmed in the fruit-fly, *Drosophila melanogaster*, by Caspersson and Schultz (1940), using an elegant absorption-spectra technique, and in 1952 Hyden was able to apply similar methods to rabbit cells.

Nuclear Sex

The accidental discovery in 1949 by Barr and Bertram that in the neurones of the cat there was a nodule in the nuclei characteristic of the female started a new era in genetics. The later application of this observation to other tissues, including the human skin, made it possible to apply this experimental discovery to clinical medicine.

The nodule, described as being formed of deeply staining chromatin material, has a diameter of near 1 μ, and is most characteristically found applied to the inner surface of the nuclear membrane (Fig. 1).

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The discovery of a sex-specific pedunculated nodule, with a drumstick-like appearance, in the neutrophil, eosinophil, and basophil leucocytes, by Davidson and Smith (1954) and Davidson and Winn (1959), and the

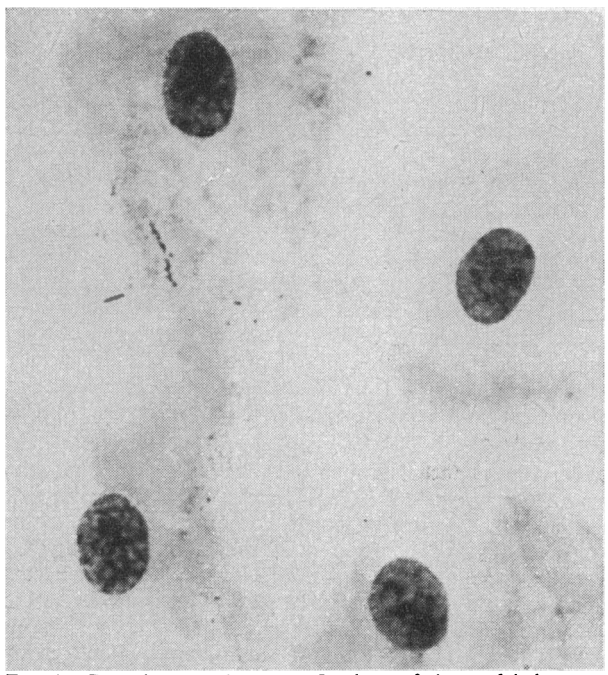

FIG. 1.—Buccal mucosal smear. In three of the nuclei there are the characteristic sex-chromatin nodules applied to the inner surface of the nuclear membrane. Female. (Haematoxylin and eosin. ×1,400.)

application of the original method to buccal mucosal smears by Moore and Barr (1955), provided two mutually confirmatory methods which have since been used widely in the investigation of cases of doubtful anatomical sex.

The neutrophil drumsticks, which have a dense chromatin head 1.5 μ in diameter, are characteristically separated from the rest of the nucleus by a thread-like connecting neck. Nodules with a short thick neck and sessile nodules, although nearly diagnostic of the female neutrophils, are more difficult to distinguish from other nodules found in both sexes (Figs. 2 and 3), but they can be used by an experienced observer in the recognition of the sex from blood films. Although only the drumsticks are sex-specific, all three types have a positive value, while the small sessile nodules and the thread-like

projections, which are commoner in the male, have a negative significance in the nuclear sex-screening test. In this test 100 or possibly 200 neutrophils are examined

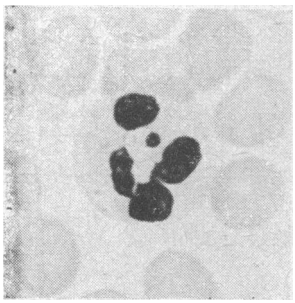

FIG. 2.—Blood film. In the neutrophil there is one of the sex-characteristic drumsticks which mark a proportion of the female granulocytes. (Jenner Giemsa. ×1,400.)

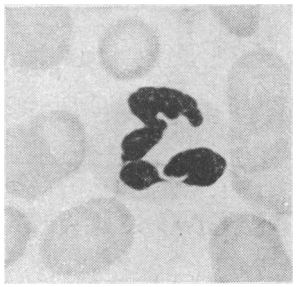

FIG. 3.—Blood film. In the neutrophil leucocytes there is a sessile nodule projecting from the end of the upper lobe of the nucleus. Such nodules are almost sex-specific and should be looked for when sexing blood films. Female. (Jenner Giemsa. ×1,400.)

for these features and the recognition of the sex depends upon the preponderance of one or other group. The result is usually clear-cut.

In cases of difficulty or special importance the classical test of Davidson and Smith should be used. In this, six drumsticks are sought. In normal females and in certain abnormalities, to be discussed later, these are usually found in under 300 neutrophils; on the other hand, in normal males and in such conditions as Turner's syndrome, none are found in 500 or more neutrophils. The results are better recorded as chromatin-positive and chromatin-negative respectively, and not female and male. In exceptional cases the frequency of the drumsticks may be so low that up to 2,000 or even 3,000 neutrophils have to be examined to find six. It is in these cases that the recognition of the nearly sex-specific sessile nodules is of particular value in preventing a premature and erroneous assumption that the film is chromatin-negative.

Davidson and Winn (1961) have shown that the nodule in the nucleus of the tissue cell and the drumstick in the leucocyte are probably homologues, and the size suggests that they represent a major part of the XX chromosome mass (Fig. 4). The absence of such sex-characteristic nodules can be taken to indicate that there are fewer than two X chromosomes in the cells. This raises an intriguing thought that even in the resting nucleus the chromosomes are not merely lost in a fluid state, but may be arranged in an orderly fashion. An interesting development, which if substantiated would cast doubt, not upon the diagnostic value of the sex chromatin, but upon the interpretation of its structure, comes from the work of Kosin and Ishizaki (1959), who showed that, although heterogametic, the females of the duck are still chromatin-positive.

Genetic Sex

In the past few years direct study of the chromosomes has been made possible by improved techniques. Through a combination of the bone-marrow-culture method developed in this country, particularly by Ford and Hamerton (1956) and by Ford *et al.* (1958), the use of colchicine to arrest mitoses in the mid-phase, and the loosening and spreading out of the chromosomes by the judicious use of hypotonic fluids as suggested by Hsu and Pomerat (1953), it has become possible to identify the individual human chromosomes.

The technical methods before this time had been so poor that even the traditional number of chromosomes in man, 48, was found to be wrong, and Tjio and Levan (1956) established the number as 46. Since then Tjio and Puck (1958) and Ford *et al.* (1959a, 1959b) in England, Lejeune *et al.* (1959) in France, and Fraccaro *et al.* (1959) in Sweden have been attempting to recognize the individual chromosomes, and have concluded that in the female the X chromosomes are probably the seventh pair of chromosomes in order of size and are metacentric—that is, when the chromosomes split longitudinally in diakinesis the two chromatids formed remain attached centrally (Fig. 5). The Y chromosome has also been recognized as one of the five small acrocentric

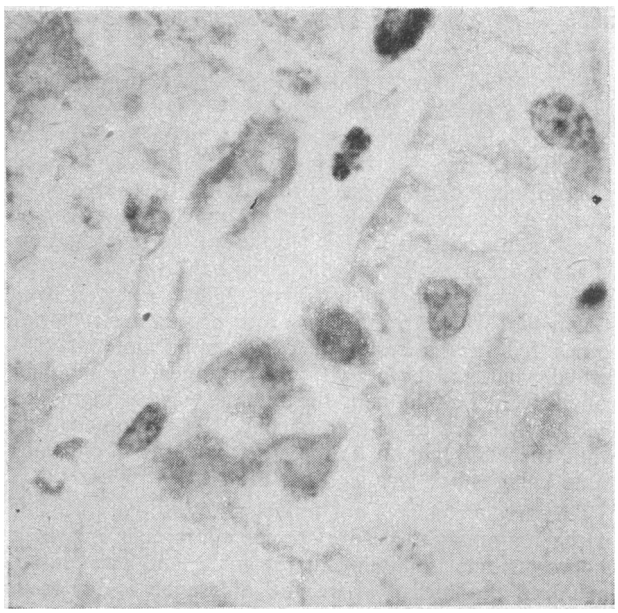

FIG. 4.—Section of liver tissue. In one of the sinusoids there is a neutrophil with a drumstick, and at the other end of the sinusoid there is a liver cell with an intranuclear sex-chromatin mass. Female. (Haematoxylin and eosin. ×1,400.)

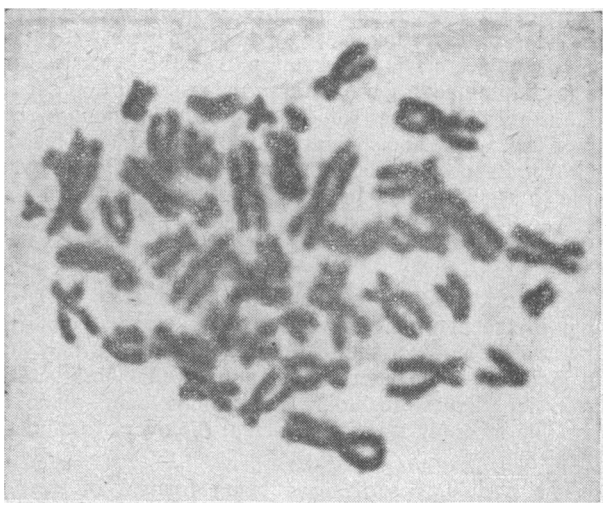

FIG. 5.—Squash preparation from marrow culture. The chromosomes in the process of splitting into chromatids, which are still attached to one another at their centromeres. This mitotic figure is from a female, and the X chromosomes can be recognized by their being about the seventh pair of chromosomes in order of size, and having an almost but not quite centrally placed centromere. (Feulgen stain. ×2,500.)

chromosomes—that is, with the chromatids attached terminally—in the male.

With the introduction of nuclear sexing the brilliant clinical deduction that anatomical females suffering from gonadal dysgenesis (Turner's syndrome) had not the XX chromosome structure, made by Polani *et al.* (1954, 1956) and Bishop *et al.* (1956) from the relatively high frequency of coarctation of the aorta and colour-blindness, was substantiated by finding that these "girls" lacked sex chromatin in their cells. From recent chromosomal studies by Ford and by Fraccaro this has come to have a further meaning: these "girls" are neither male nor female, but have the XO chromosome structure.

In parallel with these morphological developments the chemists have successfully analysed the physico-chemical structure of the chromatin itself. The essential constituent deoxyribonucleic acid has been shown to have in the central part of its double helical molecule a balanced arrangement of the purine and pyrimidine bases, adenine and guanine, thymine and cytosine. The importance of this structure lies in the almost infinite number of variations provided by changes in the order in which the 3,000 or more bases are arranged. This is believed to provide the mechanism for carrying the hereditary pattern—that is to say, a "gene" is really a specific arrangement of the bases at a point on the deoxyribonucleic acid molecules.

This hereditary configuration determines in turn the detail of the structure of the more simple form of nucleic acid, ribonucleic acid, which is intimately connected with the formation of protein, and is present more abundantly in the cytoplasm than in the nuclei of the cells. Thus it seems that the ribonucleic acid passes on the hereditary structural information stored in the deoxyribonucleic acid by acting as the template from which the amino-acid molecules in the specific proteins, including the enzymes, are arranged. Finally, these enzymes which are formed play an all-important part in every cellular function, including the formation of hormones and probably also the reaction of the tissues to the hormones.

In this way the hereditary pattern, the genes, controlling sex is represented in the nuclei of every cell in the body, and can influence the development of the tissues and their reaction to normal and abnormal hormonal stimulation. The inheritance of this sex pattern, the genetic sex, is apparently the result of a balance between the genes having a male influence, which are scattered on the autosomes, and those having a female influence, concentrated on the X chromosomes. Normally the genetic sex agrees with the chromosomal sex—that is, whether in mammals the individual bears the like XX pair or the dissimilar XY pair of chromosomes—and as in theory this determines whether the amount of heterochromatin present is sufficient to form a sex-specific nuclear nodule or not, it has been believed that the nuclear sex must be directly linked to the chromosomal sex. Despite the new work by Ohno *et al.* (1959) suggesting that only one X chromosome forms the chromatin nodule, and the difficulty, already mentioned, which has been aroused by Kosin and Ishizaki (1959) finding sex chromatin in female ducks, it still seems that there must be a basic linkage even if it is more complicated than formerly anticipated.

Normal females are thus chromatin-positive and normal males chromatin-negative, but in abnormalities of sexual development this relationship is not always maintained, and particularly it must be realized that the nuclear sex does not distinguish between XXY, XXX, and XX, nor between XO and XY.

Somatic Sex

Gonadal Sex.—The specific development of the cortex of the primitive gonad to an ovary or of the medulla to a testis, although determined genetically, is mediated through cellular and hormonal factors. The arrival of the germinal cells in the gonadal blastema after their migration from the region of the yolk sac seems to initiate the development, but embryonic hormones, probably produced by the developing pituitary gland, also play an important part (Jost, 1950). This has been confirmed by the sex-reversal experiments on very immature opossums taken from the marsupial sac of the mother (Burns, 1956).

Development of the Sex Ducts and External Genitalia. — The gonads influence the subsequent development of the genital tracts from the sixth to eighth weeks of embryonic life onwards. The developing testis seems to have a positive action, causing the regression of the mesonephric (Müllerian) in favour of the paramesonephric (Wolffian) duct; the male type of development of the urogenital sinus; enlargement of the genital tubercule; and secondary fusion of the genital folds to form the penile urethra. In contrast the ovary is relatively inactive, and Jost (1950) has shown that if all gonadal tissue is removed, in either sex, the subsequent development of the genital tracts follows the female pattern. At this stage abnormal suprarenal

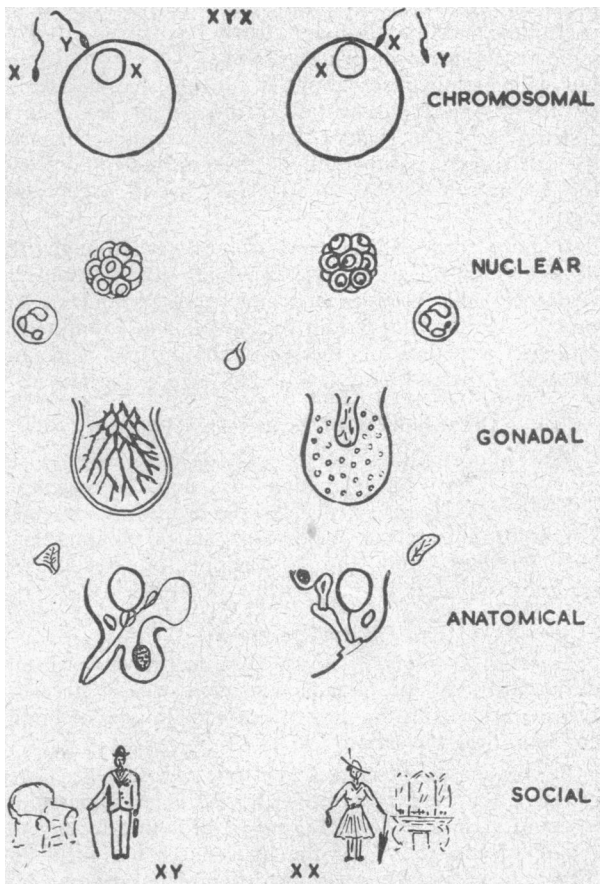

FIG. 6.—Diagrammatic representation of the various levels of sex representation.

hormones can produce sufficient disturbance to cause difficulty in recognizing the anatomical sex (Prader and Siebenmann, 1958).

Development of the Secondary Sex Characteristics.—While there is a fundamental agreement between the genetic, chromosomal, nuclear, and anatomical levels of sex development, the subsequent maturation of the structures and the formation of the secondary sex characteristics, although greatly influenced by the gonadal hormones, also involves other less directly connected factors. These include pituitary and suprarenal hormones.

Social Sex

The anatomical or somatic sex, and particularly the structure of the external genitalia, has a dominating influence upon the development of those physical and mental qualities expressing themselves in dress, friendships, choice of occupation, and hobbies, by which we recognize the social sex. Even in normal individuals there is considerable variation in the degree of expression of social sex, but in some the aberration exceeds the wide limits we have set, and occasionally the outlook is so distorted that the individual cannot fit into any ordinary form of community life (Fig. 6).

Abnormalities of Sex

Although in the vast majority of people there is agreement throughout the sexual structure, every variety of discrepancy may be met in abnormal cases. The determination of the nuclear sex, a relatively easy procedure, is an important tool in investigating these developmental abnormalities if its usefulness and limitation are clearly recognized. It only indicates the presence or absence of two X chromosomes, but with certain reservations it can be used as a guide to the more fundamental chromosomal sex. It is essential to have such a test for screening purposes, for the direct investigation of the chromosomal sex is too complicated and can only be used in exceptional cases.

It must be realized, however, that although the development of these two methods has formed an important link between academic genetics and clinical practice, we are not yet in the position to examine the intimate structure of the deoxyribonucleic acid and determine the ultimate genetic sex of any individual.

Abnormalities of Sexual Development

The abnormalities of sexual development to be discussed can be classified as gonadal agenesis, gonadal dysgenesis, true hermaphroditism, pseudohermaphroditism, the hyposexual states of impotence and frigidity, and the distortions of social sex, transvestism, and homosexuality.

Gonadal Agenesis

Gonadal agenesis, in which there has never been any development of the gonadal blastema, will of necessity be associated with the negative or female type of bodily configuration (Overzier, 1957).

In the cases described the nuclear sex has been negative, but the origin of the disorder is not understood. It could arise as a local failure of growth affecting the region of the gonad, or the blastema could form and then at a very early stage in development be partially or here totally destroyed by a noxious substance passing from the mother to the foetus. This substance could come from a hormonal disturbance or, as Witschi *et al.* (1957) have suggested, a tissue incompatibility, comparable to that in erythroblastosis foetalis.

Gonadal Dysgenesis

Gonadal dysgenesis occurs in two forms, Klinefelter's and Turner's syndromes, two very interesting disturbances of sexual development. In the former the gonad is a maldeveloped testis, in the latter a maldeveloped ovary.

A minority of the cases of both conditions appear to arise as a simple local anomaly affecting the gonad, with no discrepancy between the nuclear, gonadal, and anatomical sex. In the majority, however, there is a striking aberration. In such cases of what is sometimes called true Klinefelter's syndrome, the nuclear sex is chromatin-positive despite the completely male anatomical structure, including the presence of testes. After puberty, however, the testes fail to mature properly and most of the tubules are found to be degenerate. Those embedded in the large masses of degenerate interstitial cells tend to retain their size and lining of Sertoli cells, but the remainder undergo a rather characteristic type of hyalin degeneration (Fig. 7).

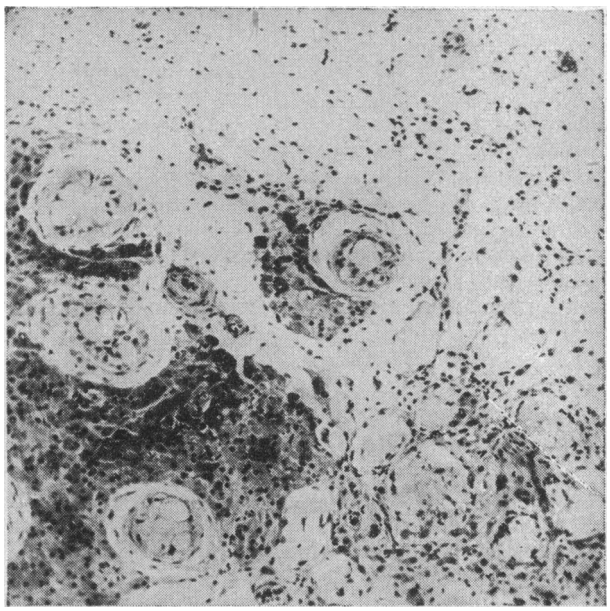

FIG. 7.—Tissue section. The testicular tissue from Klinefelter's syndrome showing the characteristic hyaline degeneration of the tubules and the massing of the Leydig cells. (Haematoxylin and eosin. ×100.)

Occasional areas of spermatogenesis have been found, and even a few cases, including one of my own (Davidson and Smith, 1958), have had spermatozoa in the seminal fluid. So far no instance of a sufferer from Klinefelter's syndrome having fathered a child has been found.

In the cases of what might be termed true Turner's syndrome a similar discrepancy is found, except that it is in the opposite sex. Here the cells are chromatin-negative yet the anatomical structure is feminine, even to the positioning of the gonads. These, however, are poorly developed and usually composed entirely of ovarian stroma, although in one personal case there were some ill-defined cell-cords with germinal cells (Davidson and Winn, 1959) (Fig. 8), and even ova have been described by Atria *et al.* (1948).

Colour-vision studies in these syndromes by Polani *et al.* (1958) have suggested that, despite the gonadal structure, nuclear sexing is still correct in indicating the presence of two X chromosomes in Klinefelter's

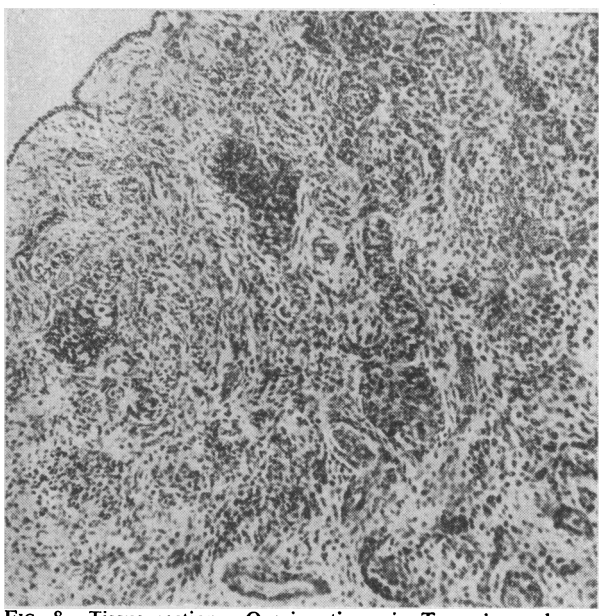

FIG. 8.—Tissue section. Ovarian tissue in Turner's syndrome. A few undeveloped germinal cells are to be seen in a cell cord or tubular remnant buried in the ovarian stroma. (Haematoxylin and eosin. ×100.)

syndrome and, conversely, only one in Turner's syndrome. Recent chromosomal analyses by Ford and Jacobs (Jacobs and Strong, 1959) have confirmed this, but have explained the discrepancy by revealing an XXY structure in Klinefelter's syndrome and an XO structure in Turner's syndrome. Cases of Klinefelter's syndrome could thus be regarded as males with an additional X chromosome, or females with an added Y, and cases of Turner's syndrome as females lacking an X chromosome, or males lacking a Y chromosome.

Such chromosomal patterns are believed to arise through non-disjunction of the sex chromosomes at meiosis—that is, during the formation of the gametes in either the father or the mother. During either the first or the second division, instead of the sex chromosomes separating, both go to one nucleus and none are left for the other. If the maldivision occurs in the father, either a spermatozoa-carrying XY or one lacking any sex chromosome could unite with a normal ovum carrying one X and result in a child with, in the former instance, Klinefelter's, and, in the latter, Turner's syndrome. With non-disjunction in the mother there are two further possibilities—the union could also produce XXX or YO zygotes. If non-disjunction takes place at the first meiotic division it would seem that Klinefelter's and Turner's syndromes should occur with the same order of frequency, and more commonly arise from the father (Fig. 9). This does not appear to be the case, for in practice, according to Moore (1959), Klinefelter's syndrome is the commoner, and Stern (1959) believes that it is more often due to non-disjunction in the mother. So that non-disjunction at the second meiotic division must also be considered (Fig. 9). A sterile female has recently been found to have the third viable product of non-disjunction, the XXX structure. Aberrations during early mitoses in the zygote could produce mosaicism.

Why this mechanism of non-disjunction should cause the gonadal dysgenesis is not clear. It may be that the abnormal chromosomal complement interferes with the migration of the germinal cells or that the enzymes formed from the abnormal deoxyribonucleic acid complement are inimical to the development of the gonad.

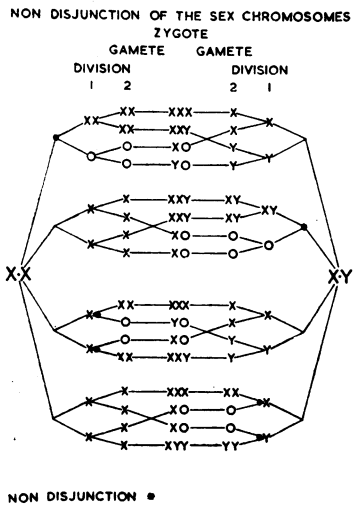

FIG. 9.—A diagram to show the effect of non-disjunction during the first or second reduction divisions in the formation of the gametes. The possible zygotes which result from the union of a product of non-disjunction and a normal gamete are arranged in the centre of the diagram. These are the combinations which give rise to gonadal dysgenesis and certain allied conditions (Klinefelter's syndrome, XXY; Turner's syndrome, XO; superfemales, XXX; and lethal combinations such as YO and XYY).

True Hermaphroditism

In true hermaphroditism there can be no simple agreement between the nuclear and gonadal sex, as both testicular and ovarian tissues are present (Fig. 10). Although both chromatin - positive and chromatin-negative forms occur, most of the cases studied have been chromatin - positive. In these the frequency of the neutrophil drumsticks and of the tissue nodules is within the normal limits, suggesting that the condition is not a mosaic state. Barr (1955) has also confirmed this by finding that the skin from both sides of the body had the same chromatin structure and was not male and female as in gynandromorphism in animals.

A form of mosaic state has been met in man in the blood chimeras. In these binovular twins fusion of the placentae at an early stage of development had allowed an exchange of marrow elements, resulting in a mixing

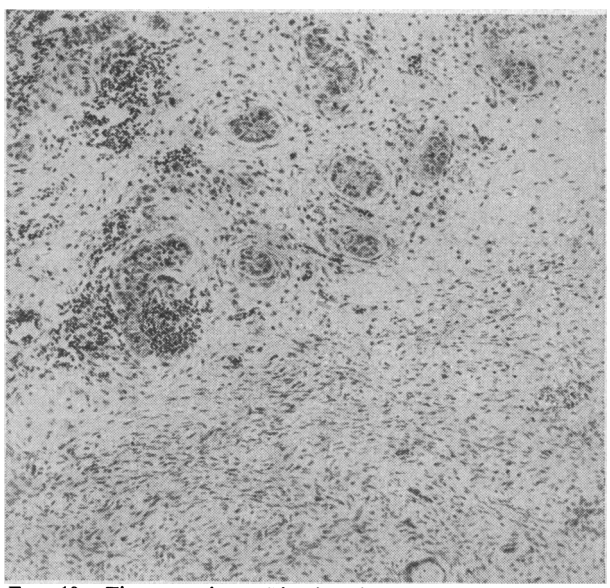

FIG. 10.—Tissue section. The junction between the testicular and ovarian tissue in the gonad from a true hermaphrodite. (Haematoxylin and eosin. ×100.)

of the blood groups and both male and female leucocytes (Davidson *et al.*, 1958). Except for the lack of genital abnormalities, this condition is comparable to the freemartin state in cattle.

Pseudohermaphroditism

Pseudohermaphrodites are classified as male or female according to the gonads present, regardless of other variations. The nuclear sex and the gonadal sex are in agreement in this form of intersex, but there is divergence of the development of the genital ducts, external genitalia, and secondary sex characteristics, ranging from a slight distortion of the genitalia to complete reversal of the anatomical structure, so that it simulates or is even identical with that of the opposite sex.

It is perhaps in the relatively common group of pseudohermaphrodites that nuclear sexing has its greatest use, but the mere fact that by it we are able to determine with reasonable certainty the type of gonad present should not lead us prematurely into the more drastic forms of plastic surgery. Even at birth—and it is very important to make the decision as soon as possible—it may be unwise to attempt to correct the genital defect, and, once the child has been reared for a time in the " wrong " sex, the social sex adopted may have become so deeply rooted that it would be meddlesome to interfere.

Psychological Disturbances

Although the social sex is mainly dependent upon the anatomical sex, rearing has an important effect and there is an intricate psychological background to the errors. Social sex may be so poorly developed or so disturbed as to result in impotence or frigidity, even in the absence of any anatomical error or any discrepancy between the gonadal, nuclear, and genetic sex. A severe aberration of the mental outlook results in those reversals of the social sex known as transvestism and in homosexuality. These disturbances may have some genetic predisposition, but this does not usually show as any morphological alteration in the sex chromosomes and the nuclear sex is in keeping with the gonads and the anatomical structure. Only in the rare instances of an admixture of homosexuality and Klinefelter's syndrome, mentioned by Davidson, Siebenmann, Bishop (see Davidson and Smith, 1958) and Overzier (1958), has any abnormality of the chromosomes been found.

General Conclusion

The discovery of the method of recognizing the sex from nuclei in the resting-phase cells has opened new fields of interest, and the stimulation it has given to the new work on the chromosomal structure has for the first time formed a real bridge linking the theories of genetics with the practical problems of clinical medicine.

This work is only beginning, and is undoubtedly to be extended. Already we recognize a chromosomal abnormality as the cause of mongolism. There must be also other morphological changes, dependent upon chromosomal abnormalities, whose significance has not been recognized. Another attack upon these problems is being made by the physical chemists who are painstakingly analysing the structure of complex organic substances. The abnormal haemoglobins have been elucidated by Ingram (1957), protein structures are becoming understood, and there are hopes of advances in the study of deoxyribonucleic acid, the key to inheritance.

Summary

While the determination of the nuclear sex is of the greatest practical value in the group of pseudohermaphrodites, it must be regarded as a fundamental investigation in anomalies either affecting the genitalia or with a sex preponderance. Although it only marks the presence, or absence, of two X chromosomes, and must be interpreted with this in mind, it is still the test of choice in clinical work while chromosomal studies remain as a research tool.

The buccal-mucosal-smear and blood-film techniques are mutually confirmatory examinations, and in cases of difficulty or special importance both should be made.

Investigations of the chromosomal structure of the dividing marrow cells has yielded results of the greatest importance in the field of abnormalities of the sex organs. The discovery of the abnormal XXY structure in Klinefelter's syndrome and of XO in Turner's syndrome has gone a long way to explain the discrepancy between the gonadal structure and the nuclear sex in these conditions, and has transformed theoretical genetics to a subject with practical application.

References

Atria, A., Sanz, R., and Donoso, S. (1948). *J. clin. Endocr.*, **8**, 397.
Barr, M. L. (1955). In *Modern Trends in Obstetrics and Gynaecology*, edited by K. Bowes, 2nd series, p. 116. Butterworth, London.
—— and Bertram, E. G. (1949). *Nature (Lond.)*, **163**, 676.
Bishop, P. M. F., Polani, P. E., and Lessof, M. H. (1956). *Lancet*, **2**, 843.
Burns, R. K. (1956). *Arch. Anat. micr. Morph. exp.*, **45**, 173.
Caspersson, T., and Schultz, J. (1940). *Proc. Nat. Acad. Sci. (Wash.)*, **26**, 507.
Davidson, W. M., Fowler, J. F., and Smith, D. R. (1958). *Brit. J. Haemat.*, **4**, 231.
—— and Smith, D. R. (1954). *Brit. med. J.*, **2**, 6.
—— —— (1958). In *Symposium on Nuclear Sex*, edited by D. R. Smith and W. M. Davidson, p. 93. Heinemann, London.
—— and Winn, S. (1959). *Postgrad. med. J.*, **35**, 494.
—— —— (1961). *Conference on Human Chromosomal Abnormalities, London, September, 1959*, edited by W. M. Davidson and D. R. Smith. Staples Press, London.
Ford, C. E., and Hamerton, J. L. (1956). *Nature (Lond.)*, **178**, 1020.
—— Jacobs, P. A., and Lajtha, L. G. (1958). Ibid., **181**, 1565.
—— Jones, K. W., Miller, O. J., Mittwoch, U., Penrose, L. S., Ridler, M., and Shapiro, A. (1959a). *Lancet*, **1**, 709.
—— —— Polani, P. E., de Almeida, J. C., and Briggs, J. H. (1959b). Ibid., **1**, 711.
Fraccaro, M., Kaijser, K., and Lindsten, J. (1959). Ibid., **1**, 886.
Geitler, L. (1937). *Z. Zellforsch.*, **26**, 641.
Hsu, T. C., and Pomerat, C. M. (1953). *J. Morph.*, **93**, 301.
Hyden, H. (1952). Coll. Ges. Physiol. Chemie, Mosbech, 1. (Cited by T. Lüers, *Berl. Med.*, 1956, **7**, 120.)
Ingram, V. M. (1957). *Nature (Lond.)*, **180**, 326.
Jacobs, P. A., and Strong, J. A. (1959). Ibid., **183**, 302.
Jost, A. (1950). *Gynéc. et Obstét.*, **49**, 44.
Kosin, I. L., and Ishizaki, H. (1959). *Science*, **130**, 43.
Lejeune, J., Gautier, M., and Turpin, R. (1959). *Lancet*, **1**, 885.
McClung, C. E. (1902). *Biol. Bull. Wood's Hole*, **3**, 43.
Moore, K. L. (1959). *Lancet*, **1**, 217.
—— and Barr, M. L. (1955). Ibid., **2**, 57.
Ohno, S., Kaplan, W. D., and Kinosita, R. (1959). *Exp. Cell Res.*, **18**, 415.
Overzier, C. (1957). *Schweiz. med. Wschr.*, **87**, 285.
—— (1958). *Arch. Psychiat. Nervenkr.*, **198**, 198.
Painter, T. S. (1924). *Amer. Nat.*, **58**, 506.
Polani, P. E., Bishop, P. M. F., Lennox, B., Ferguson-Smith, M. A., Stewart, J. S. S., and Prader, A. (1958). *Nature (Lond.)*, **182**, 1092.
—— Hunter, W. F., and Lennox, B. (1954). *Lancet*, **2**, 120.
—— Lessof, M. H., and Bishop, P. M. F. (1956). Ibid., **2**, 118.
Prader, A., and Siebenmann, R. E. (1958). In *Symposium on Nuclear Sex*, edited by D. R. Smith and W. M. Davidson, p. 62. Heinemann, London.
Stevens, N. M. (1905). *Publ. Carnegie Instn.*, No. 36.
Stern, C. (1959). *Nature (Lond.)*, **183**, 1452.
Tjio, J. H., and Levan, A. (1956). *Hereditas (Lund.)*, **42**, 1.
—— and Puck, T. T. (1958). *Proc. nat. Acad. Sci.*, **44**, 1229.
Witschi, E., Nelson, W. O., and Segal, S. J. (1957). *J. clin. Endocr.*, **17**, 737.