SEX CHROMATIN IN ORAL SMEARS

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[WITH SPECIAL PLATE]

A sex difference in the morphology of intermitotic nuclei was first described as a feature of nerve cells in the female cat by Barr and Bertram (1949). This difference is characterized by a mass of chromatin which, from its intimate relationship to the nucleolar membrane in nerve cells, was first called the nucleolar satellite, but as its position varies with the condition of the cell and the type of tissue examined it was renamed the sex chromatin. Further studies have shown that sex chromatin may be demonstrated, not only in many tissues of the cat, but also in the cells of other species, including man (Moore and Barr, 1954). These findings have been of value in the study and elucidation of cases of abnormal sex development by the examination of nuclei in biopsy specimens of skin.

Moore and Barr (1955) in Canada and Marberger *et al.* (1955) in the United States, in order to simplify still further the diagnosis of chromosomal sex, examined smears of epithelial cells taken from the mucous membrane of the oral cavity, and found that in the nuclei of these cells also the sex chromatin could be identified.

Recently it has been pointed out (Barr, 1956) that, as the precise relationship of the female mass of sex chromatin to the XX chromosome complex has not yet been established, the use of such terms as male or female chromosomal sex should be replaced, in clinical use, by "chromatin negative" (male) or "chromatin positive" (female).

The purpose of the present investigation is twofold: first, to examine smears from a large number of individuals with a view to testing the validity of the oral-smear method, as it has not yet been extensively used in this country; and, secondly, to establish a number of criteria by which the method may be standardized and the diagnosis of chromosomal sex made more or even quite certain.

Materials and Methods

Oral smears were taken from 260 individuals, of whom 98 were female and 162 male. Ages ranged from 16 months to 60 years and there was some racial variation. The smears were obtained by gently scraping the inner surface of the cheek with a wood orange-stick and transferring the material thus removed to a slide previously coated with a thin layer of albumen. Fixation in equal parts of 95% ethyl alcohol and ether was begun immediately, and continued for 24 hours. Following careful hydration, staining was carried out in 1% cresyl violet at 57° C. for 20 minutes. Excess stain was removed by brief rinsing in 95% alcohol (two changes), dehydration was completed in absolute alcohol, and the specimens were then cleared in xylol and mounted in Canada balsam. Other staining techniques, such as the Feulgen reaction, which demonstrates the sex chromatin because of its high content of deoxyribose nucleic acid, were tried, but preference was given to the cresyl-violet method as it was found to give adequate differential staining of the nucleus and has the advantage of greater simplicity.

The slides were numbered serially and examined independently by each investigator, without reference to the records

of the sex of the subjects. A 1/12-inch oil-immersion objective was used, as lower magnifications were not satisfactory. Twenty-five suitable cells were counted in each smear, and the incidence of sex chromatin, the first cell in which it was seen, and the degree of bacterial contamination were noted. Only cells which fulfilled the following criteria were included in the counts: (1) they must have large, circular, lightly stained nuclei; (2) there must be absence of crenation of the nuclear membrane and no undue folding of the cell body; (3) there must be no obscuring of the nucleus due to overlapping by adjacent cells; (4) freedom from bacterial contamination in the vicinity of the nucleus is essential; (5) only plano-convex, darkly stained bodies in actual continuity with the nuclear membrane were considered to be sex-chromatin masses. Figs. 1 and 2 on the Special Plate show male and female nuclei typical of those included in the counts, while Figs. 3 and 4 show nuclei of the type which were excluded because of bacterial contamination and folding of the cell body respectively.

Observations

Three of the 260 smears were unsuitable for examination. In the 162 male smears examined no sex chromatin was seen, whereas, by contrast, in the majority of the 95 female smears between 30% and 50% of the cells counted contained a sex-chromatin mass. The average incidence is shown in the

Incidence of Sex Chromatin in 95 Females

Percentage	20	24	28	32	36	40	44	48	52	56	60	64
No. of subjects	3	4	5	11	12	21	14	10	6	6	2	1

Table. The incidence range, which is unaffected by variations in age or race, is more clearly shown in the Graph. Some errors in diagnosis were made, but these were less than 5% of the total and occurred in the initial stages of

Graph showing incidence range in females.

the investigation. As experience in the examination of the smears was gained the accuracy of our estimations improved, in great part owing to the application of the criteria for suitable cells which have been enumerated above. The smears in which errors had been made were re-examined at the end of the investigation, and no difficulty was then experienced in providing a correct diagnosis.

Discussion

It has already been established that there is a well-recognized difference between the nuclei of male and female epithelial cells, and our results fully confirm this finding. Other investigators have given different values for the percentage

of female cells possessing sex chromatin, but this variation in values would appear to be due to differences in the type of cells counted and also to the fact that some estimations have included cells in which the mass of chromatin was not in an absolutely peripheral position in the nucleus. Unless it is in such a position it is partially obscured by the substance of the nucleus and cannot be positively identified. This probably explains why, in a proportion of cells in any smear from females, the sex chromatin is not visible. The limiting criteria we have used tend to give a low percentage incidence, but in our opinion greater accuracy of forecast.

In female smears the sex chromatin was usually first seen in one of the first five cells counted. As this finding occurred with such regularity we decided that a count of 25 cells was quite adequate for sex determination. This fact at first sight may not seem significant, but in the case of children, and more particularly of young infants, we have found great difficulty in obtaining smears in which more than 25 cells fulfilling the above essential criteria can be seen.

Summary

The examination of oral smears from 260 individuals, representative of a wide age and racial variation, has enabled their chromosomal sex to be determined with considerable accuracy. With increasing experience in assessment the diagnosis becomes progressively more accurate. The procedure is simple in its application and if necessary may be readily repeated.

The findings of previous workers have been confirmed, and a number of criteria established the use of which may help to standardize the method and ensure correct diagnosis.

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EFFECT OF CORTISONE ON THE DEFENCE MECHANISMS OF THE BODY

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[WITH SPECIAL PLATE]

Many records have been published showing that cortisone depresses the reaction of the body to all common bacterial infections in both animals and man (Thomas, 1953). In the mouse it was found that cortisone increased the severity of a tuberculous infection (Hart and Rees, 1950), and in the human subject there is evidence that it may cause a quiescent tuberculous lesion to become active. Furthermore, cases of sudden death from acute fulminating pneumonia in patients with rheumatoid arthritis undergoing cortisone therapy have been reported. In a number of animal experiments the doses, weight for weight, exceeded those given

to patients, and it was thought that overdosage might account for some of the unfavourable results. Further clinical trials largely substantiated this view, and it is now accepted that severe intercurrent infection tends to occur only when very large doses are given. Wayne (1955) is of the opinion that the critical dose of cortisone in the human subject is in the region of 75 mg. per day, since nearly all patients receiving more than 100 mg. of the hormone daily develop some adverse side-effect.

Present Investigations

The research now described was designed to investigate the mechanism by which cortisone depresses the defences of the body, and also that by which it brings about improvement in certain diseases. Studies were therefore made of the effect of cortisone on (1) the phagocytic activity of the reticulo-endothelial system; (2) the total and differential leucocyte counts of the blood; and (3) the gamma-globulin level in the serum, which may be taken as the measure of the antibody level present, since most antibodies are found in association with the gamma-globulin fraction of the serum protein. All three are known to be important in defence against infection.

To study the effect of cortisone on the phagocytic activity of the reticulo-endothelial system 79 mature male guinea-pigs were used. Each received one daily subcutaneous injection of trypan blue for the last six days before being killed by chloroform, the dosage of the dye being calculated on the basis of 0.8 ml. of a 1% solution in distilled water for each 100 g. body weight. Seven of the animals were given dye only and used as controls. The remaining 72 were divided into three groups of 24 and given cortisone acetate (Roussel) once daily intramuscularly in addition to the dye. The animals in the first group each received 5 mg. of the hormone daily, those in the second 10 mg. daily, and those in the third 25 mg. daily, and all were killed in batches of six at the end of the first, second, third, and fourth weeks. These doses were planned to be proportionately much higher than those given therapeutically to the human subject. Specimens were taken from the spleen, liver, and lymph nodes and fixed in Heidenhain's "Susa." Sections were cut 10 μ thick and stained with weak eosin, dilute carbol fuchsin, or alum carmine. The degree of phagocytic activity was assessed by studying the intensity of the vital staining as judged by the number of dye granules seen within the cells. Only cells containing dye granules were regarded as belonging to the reticulo-endothelial system.

Twelve male guinea-pigs were used to investigate the effect of cortisone on the total and differential leucocyte counts and the gamma-globulin level in the serum. Each received 10 mg. of cortisone intramuscularly once daily for a period of five weeks. Blood samples were taken from each animal by heart puncture before treatment started and thereafter at weekly intervals, the blood films being stained with Giemsa's stain. The gamma-globulin level in the serum was estimated by first separating the gamma-globulin fraction by electrophoresis, then treating the electrophoretic paper with dye and estimating the optical density of the protein dye complex by means of a photoelectric cell. In this manner the percentage of the gamma globulin in relation to the total protein in the serum could be assessed.

Results

The phagocytic activity of the reticulo-endothelial system was greatly depressed in the animals which received cortisone treatment for one or two weeks, as indicated by reduced concentration of the dye in the macrophages of the spleen, liver, and lymph nodes compared with the appearances in the controls (Special Plate, Figs. 1-5). Moreover, the lymph nodes were atrophied and the mortality rate was high. Post-mortem examination of animals which died showed gross reduction in size of the lymph nodes and a slight reduction

J. D. ARNEAUD *ET AL.*: TRUE HERMAPHRODITISM

FIG. 1.—Internal genital organs at operation.

FIG. 2.—Ovarian component of left gonad. ($\times 480$.)

FIG. 3.—Testicular component of left gonad. ($\times 480$.)

FIG. 4.—Epidermis showing sex-chromatin body on nuclear membrane. ($\times 5,350$.)

FIG. 5.—Interstitial tissue of testis showing sex chromatin. ($\times 5,350$.)

A. D. DIXON AND J. B. D. TORR: SEX CHROMATIN IN ORAL SMEARS

FIG. 1.—Male nucleus typical of those included. ($\times 1,125$.)

FIG. 2.—Female nucleus typical of those included. ($\times 1,125$.)

FIG. 3.—Nuclei excluded because of bacterial contamination. ($\times 1,125$.)

FIG. 4.—Nuclei excluded because of folding of cell body. ($\times 1,125$.)