

A MORPHOLOGICAL SEX DIFFERENCE IN THE POLYMORPHONUCLEAR NEUTROPHIL LEUCOCYTES

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[WITH SPECIAL PLATE]

Since 1949 Barr and his associates (Barr and Bertram, 1949; Barr *et al.*, 1950; Moore and Barr, 1953) have been demonstrating a morphological sex difference in the nuclei of the cells of the nervous system of mammals. In a recent paper (Moore, Graham, and Barr, 1953) they extended their observation to human skin and applied the method to the determination of the genetic sex in hermaphrodites.

For some time we have been considering the possibility of sex differences in the human blood, and at the same time we have been particularly interested in the nuclear morphology of the polymorphonuclear neutrophil leucocytes. Recently we have come to realize that there is a structural difference between the nuclei of the neutrophils in the two sexes, and we present evidence of this in the present paper.

Methods.—The routine blood films going through the department were used for the investigation. These were prepared on coverslips and stained on an automatic staining machine by the Jenner-Giemsa method. Only a very small number of films which were technically unsuitable were rejected. A few were stained by the Feulgen method. Many hundreds of films were examined in the preliminary studies. For the present investigation the neutrophils were examined in each of a series of 500 preparations.

Findings

While there is no doubt that chromatin condensations in the neutrophil nuclei are more striking in the female than in the male, this is very difficult to demonstrate in many films. It is easier to recognize the female by a solitary chromatin nodule, which becomes separated off from the main nuclear lobes in a proportion of the neutrophils (Plate, Fig. 1). This characteristic "drumstick," which is just visible at $\times 90$ magnification, has to be distinguished from other appendages which occur in both sexes.

The drumstick has a well-defined, solid, round head, some 1.5μ in diameter, joined by a single fine chromatin strand to one lobe of the nucleus. Undoubtedly some of these nodules are hidden or partly hidden by one of the lobes of the nucleus, but we have reckoned as characteristic only those which are discrete.

For the present investigation six neutrophils showing such discrete solitary nodules were sought in each of 125 female blood films. These were found on the average in 227 neutrophils; representing approximately 1 to 38 cells, the lowest being 1 to every 6 and the highest 1 to every 98 cells (Fig. A). The correctness of these average figures was confirmed in a further series of 250 female films, in which on the average the first cell showing the typical drumstick was the thirty-sixth counted (Fig. B). Several counts went above 98 cells.

Five hundred neutrophils were examined in each of 125 male blood films. In the majority nothing resembling a characteristic drumstick could be discovered. In an occa-

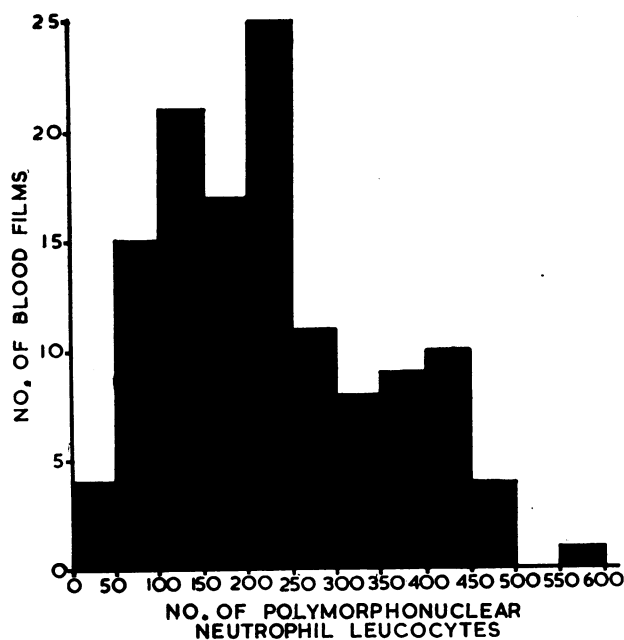


FIG. A.—The frequency distribution of the six drumsticks in the neutrophils in 125 female blood films.

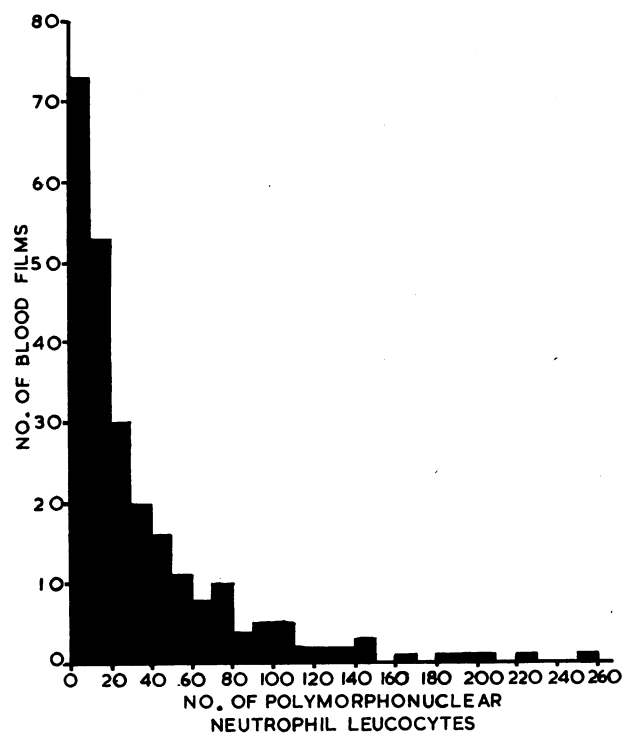


FIG. B.—The frequency distribution of the first drumstick found in the neutrophils in 250 female blood films.

sional preparation appendages were seen which might have been mistaken for the characteristic structure. Never were six of these discovered in 500 cells, and almost without exception they were rejected on closer scrutiny as minor lobes (Fig. 2), "small clubs" (Fig. 3), sessile nodules and tags (Fig. 4), and "racket" formations (Fig. 5). The minor lobes are too large, have two connecting strands, an irregular outline, or a deficiency of chromatin. The small clubs have a head less than 1μ in diameter and are not visible at $\times 90$ magnification; they are often multiple, and are perhaps more common in the male than in the female. Chromatin-rich sessile nodules and tags are more frequent in the female.

The curious racket structures, which differ from the drumsticks only in having a pale centre, although rare, occur more frequently in the male.

The characteristic drumsticks are a feature of the lobulated nucleus of the mature neutrophil, are rarely found in the unsegmented forms, and have not been demonstrated in the precursory cells. They are therefore scarce where there is a shift to the left, particularly when it is of the toxic type, and they have not been demonstrated in the inherited shift to the left of the Pelger-Huët anomaly (Davidson *et al.*, 1954). Conversely, they tend to be more frequent where there is a shift to the right, but even in severe shifts do not appear in the male.

Age has no influence, drumsticks having been demonstrated from birth to 84 years.

Discussion

For many years entomologists have recognized the richness of the chromatin connected with the X chromosome, and have used this to demonstrate a difference between certain male and female cells, particularly when in mitosis (Geitler, 1937). Barr and his associates have extended these observations to the resting phase of mammalian tissue cells. We have found that a similar aggregation of chromatin can be demonstrated near the nuclear membrane of the polymorphonuclear neutrophil leucocytes, but with their irregular form it is difficult by this means to distinguish the female from the male. Taking advantage of the tendency of the neutrophils to form lobes and projections, we have found it easier to demonstrate a difference between the sexes when the female chromatin aggregation separates off as a drumstick-like projection.

It is tempting to think that the chromatin aggregation in the solitary drumstick represents the XX chromosome combination, as Barr and his associates have suggested for the cells of the nervous system and skin, and that the lesser aggregation of chromatin in the XY combination of the male either forms an insignificant projection or remains within one of the lobes of the neutrophil.

In over 50 of the films studied (over 25 from each sex) the sex was not known to the examiner. In no case was there an error in determining the sex. Only two proved difficult. They were both female films and undoubted drumsticks were found, but in one we had to count 585 cells before finding the sixth drumstick. In retrospect we realize that, in the main, the skew distribution of the drumstick counts is due to shifts to the left (Fig. A). This is undoubtedly a difficulty in a random sample of blood films, but we feel that a similar difficulty would arise with Barr's method with certain morbid changes in the skin. If films showing a marked shift to the left are excluded, six typical drumsticks should be found easily in the female within 500 neutrophils and none in the male.

This method of differentiating the sexes may be of medico-legal interest, and it has already proved of value to us in checking the origin of certain blood films.

Summary

A morphological difference between the polymorphonuclear neutrophil leucocytes in the male and those in the female is described.

A solitary nuclear appendage with a drumstick form is found only in the female.

Six such structures occur on the average in 227 neutrophils in the female and none in 500 neutrophils in the male.

The typical drumstick structures have to be distinguished from certain other appendages found in both sexes. These have been described.

We acknowledge our indebtedness to Professor H. A. Magnus for his interest and advice and to Mr. George Harwood, of his department, for the photomicrographs.

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THE TREATMENT OF INTERSTITIAL KERATITIS

BY

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[WITH SPECIAL PLATE]

Since the introduction of cortisone in hospital practice in this country, 20 cases of interstitial keratitis occurring as congenital syphilis have been treated at this hospital and closely observed for at least one year. Of these 20 cases, some had attacks of keratitis prior to the use of cortisone and suffered some visual impairment; in others keratitis was well advanced before such treatment. Eight of the 20 cases had had no previous attacks of keratitis or any visual impairment prior to the illness for which they were under treatment. It is these eight cases which are here reported.

Antisymphilitic treatment seems to have had little or no effect on the course and outcome of interstitial keratitis itself, but when congenital syphilis is first discovered by the appearance of this complication it is important that the patient and his family should be fully investigated and treated as necessary. Treatment with cortisone brings about rapid relief of symptoms and disappearance of signs of inflammation, and by the cutting short of the inflammatory period the likelihood of permanent scarring is greatly reduced. It is not certain that cortisone actually shortens the course of the disease. One must be prepared to continue with cortisone treatment for many weeks or months, and it may be necessary to restart treatment for the typical relapses and recurrences which arise.

Of the eight cases, three (Nos. 6, 7, and 8) had had full syphilitic treatment for at least a year, including not less than 8,000,000 units of penicillin before interstitial keratitis appeared. Case 6 had had attacks of keratitis several years previously and had had the usual treatment with mydriatics and systemic therapy. Of the other seven, who were in their first attacks, Cases 7 and 8 had been under routine general and local treatment for many months and interstitial keratitis was still obviously active before cortisone was given. The remaining five started cortisone and antisymphilitic treatment at the same time. All cases received at least 8,000,000 units of penicillin; most of them much more.

Treatment to the Eye

Cortisone.—In severe cases cortisone is given by subconjunctival injections of 10 mg. every third day, after the conjunctiva has been anaesthetized with 4% cocaine drops. This is continued until the eye is obviously settling. Relief usually appears in two to three days, and the third injection is not often required; subsequently, and for moderate cases, cortisone eye-drops (5 mg. per ml.) are instilled every hour by day. This is continued until the eye is white, when the frequency of instillation is reduced to one drop every four hours by day for two weeks, then three times

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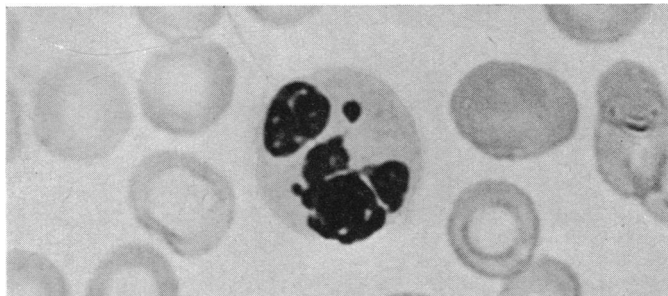


FIG. 1.—The characteristic drumstick found in the female neutrophil leucocyte. The thin strand of chromatin joining the head to a nuclear lobe can be seen clearly. (Jenner-Giemsa. $\times 1,750$.)

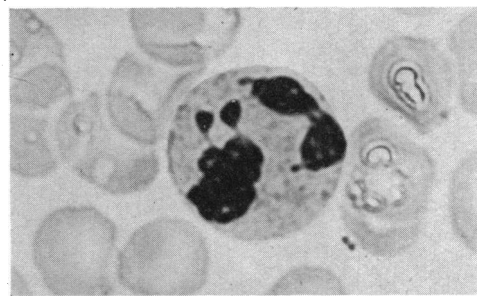


FIG. 2.—A female neutrophil showing a bilobed nucleus. Projecting from the left lobe is a characteristic drumstick, and immediately to its right, connecting the two lobes, is a minor lobe. Two trivial projections from the right main lobe are "small clubs." (Jenner-Giemsa. $\times 1,750$.)

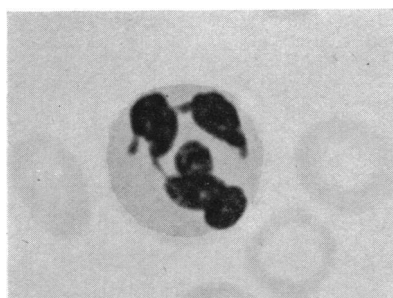


FIG. 3.—A male neutrophil. From the middle lobe a "small club" projects downwards. (Jenner-Giemsa. $\times 1,750$.)

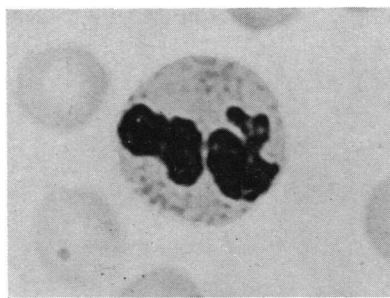


FIG. 4.—A bilobed female neutrophil. The right lobe is constricted, and from the smaller mass a chromatin-rich sessile nodule projects to the left. (Jenner-Giemsa. $\times 1,750$.)

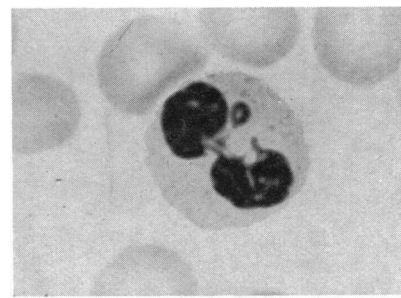
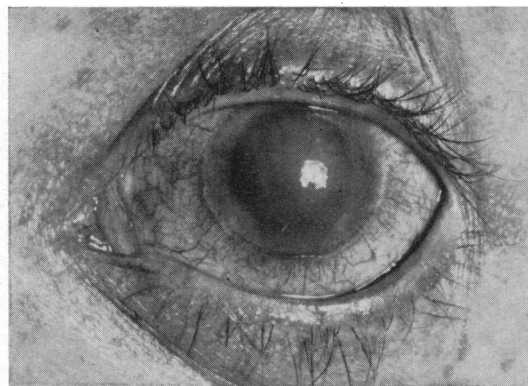
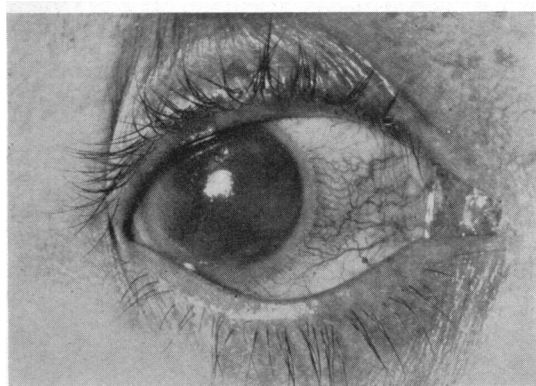
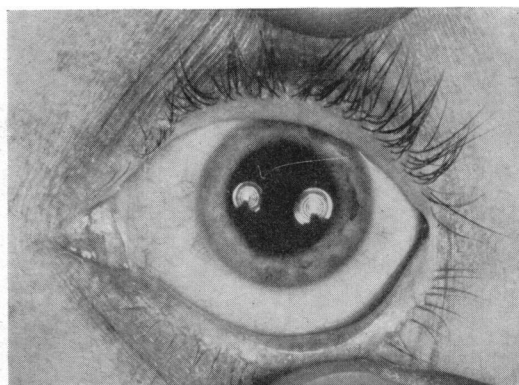
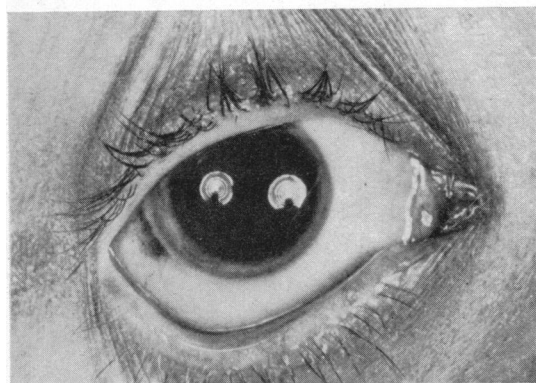


FIG. 5.—A bilobed male neutrophil with one of the rare "racket" structures between the lobes. The head of the racket has a rim of chromatin around a pale centre. (Jenner-Giemsa. $\times 1,750$.)

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FIGS. 1 and 2.—Eyes of Case 1 before treatment with cortisone.



FIGS. 3 and 4.—Eyes of Case 1 on conclusion of therapy.