in relaxed muscle and muscle in rigor, given above.

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Sex Chromatin in Newborns: Presumptive Evidence for External Factors in Human Nondisjunction

Abstract. *An incidence of 0.6 percent of sex-chromosome aberrations in newborns was found during a 5-month period, while no aberrations occurred in similar populations before and after this period. Down's syndrome also exhibited an elevated frequency during this same critical interval. A severe rubella epidemic may have influenced this pattern.*

With the development of means for determining chromosomal constitution in any human subject (*1*), a new departure in human genetics was instituted which led to the discovery that chromosomal nondisjunction in man is a common cause of disease. It has been estimated that at least 0.5 percent of all human live births are attended by diseases arising from nondisjunction of the chromosomes, which produces serious metabolic disturbance, often including mental deficiency. In addition, it has been calculated that 10 to 15 percent of all human conceptions may result in spontaneous abortion because of chromosomal abnormalities (*2*), and supporting experimental evidence has been presented (*3*).

The discovery by Barr (*4*) of the existence of nuclear chromatin in somatic cells in a number equal to one less than the total number of X chromosomes made possible rapid and convenient study of the presence of abnormalities in the sex chromosomes, in a fashion applicable to large human populations. Studies carried out in different parts of the world (*5–7*) have revealed frequencies of nondisjunction in the sex chromosomes which, when averaged over both sexes, demonstrated values for different human populations, varying between a minimum of zero (*5*) and a maximum of 0.18 percent (*6*). The seriousness of the diseases resulting from such aberrations, as well as the need to understand the basis for this genetic variability in the human species, makes it necessary to answer the following questions: (i) Are the differences in the frequency of the sex chromosomal anomalies reported by different investigators real—or do they simply reflect fluctuations occasioned by the limited size of the samples utilized, or differences in efficiency of the techniques employed? (ii) If the differences are real, do they imply intrinsic, hereditable differences associated with some or all human genetic constitutions, or do they reflect an action of extraneous factors such as diet, temperature, altitude, or exposure to toxic or infectious agents? Since several human autosome pairs such as 13–15, 21, and 18 also participate appreciably in nondisjunctional processes which result in severe developmental anomalies, a definite possibility exists that these chromosomes may be equally susceptible to whatever factors cause nondisjunction in the sex chromosomes, so that the seriousness of the health problem may be large indeed.

A study was undertaken to determine the status of the sex chromatin in somatic cells of the newborn population in the Colorado General Hospital and the General Rose Memorial Hospital in Denver. Details of the mounting, fixation, and staining procedures are described elsewhere (*8, 9*). The sampling in each hospital included all of the babies born on that day. The number of samplings gradually increased to include virtually every baby born on weekdays in both hospitals. The samples were taken by one person and mounted on serially numbered microscope slides, and the frequency of single (and double, if any) chromatin-positive cells was scored by another person who was unacquainted with the identities or phenotypic sex distribution of the babies. Only clear, unwrinkled, well-stained cells were scored, and at least 200 cells were read on each slide. The resulting determination of sex chromatin condition was completed and compared with the phenotypic sex of the baby within 48 hours after birth, so that whenever an unreadable slide or a discrepancy between the sex chromatin status and the phenotypic sex was obtained, additional studies, including full chromosomal analysis and physical examination of the infant, were performed.

During the first 12 months of this study, sex chromatin determinations were carried out by means of buccal smears alone. Approximately 15 percent of the slides so prepared were classified as unreadable because of gross bacterial contamination, poor staining, or an insufficient number of undamaged cells. However, when such samples were repeated on the same babies, clear results were always obtained. In contrast to reports from other laboratories (*10*), the frequency of chromatin-positive cells found in buccal smears of newborn females treated by our procedure was found to be 26 ± 5 percent, a value not significantly different from that found in older females (*8*). Males of any age in no case demonstrated a frequency greater than 3 percent.

The practice was adopted whereby no attempt was made to assess the sex chromatin condition of any doubtful sample, but rather to repeat the sampling procedure immediately, so as to obtain an unequivocal slide. In 1.8 percent of the cases such resampling was impossible because the baby had already left the hospital. Such slides were discarded, unless there was even a suggestion on the original slide of an anomaly. In the latter cases the baby in question was followed up and a clear determination was obtained. By all indications, unreadable samples were due only to technical reasons. A test of the reliability of the method showed the frequency of error in a first reading of 700 slides to be 0.28 percent.

After 1475 cases had been studied, the technique was modified to utilize a sex chromatin determination on the amniotic membrane (*11*). Amniotic preparations differ from buccal smears in displaying a fraction of chromatin-positive cells in normal females of 91 ± 4 percent, instead of only 26 percent, while the frequency in cells from males is still zero (*12*). Consequently, scoring is faster when amniotic membranes are utilized, and marginal mosaics of certain kinds (such as XX/XO) can more readily be detected. The amniotic procedure has the important advantage of making it possible to obtain placentas routinely from the hospital delivery room without disturbing the newly born infant. Enough amnion is available to

Table 1. Sex chromatin determinations on somatic cells of 3367 newborns in two Denver hospitals during the period December 1962 to February 1965, inclusive.

Dates encompassed	No. of months	Tissue sampled	No. of patients	No. of aberrations scored
Dec. 1962 to May 1963	6	Buccal mucosa	486	0
June 1963 to Nov. 1963	6	Buccal mucosa	293	0
Dec. 1963 to May 1964	6	Buccal mucosa and amnion	762	0
		Subtotal	*1541*	
June 1964	1	Amnion	264	1
July 1964	1	Amnion	234	1
August 1964	1	Amnion	224	1 (mosaic)
September 1964	1	Amnion	150	1
October 1964	1	Amnion	137	2
		Subtotal	*1009*	
Nov. 1964 to Feb. 1965	4	Amnion	817	0

prepare several slides, if the first results are questionable. The regimen was modified so as to carry out only amnion determinations initially and to obtain buccal smears and full chromosome analyses in addition on any baby whose amniotic preparation revealed aberrant or doubtful results. The buccal smear is highly reliable in the detection of uniform nondisjunctional anomalies of the sex chromosomes. While it could miss some mosaic individuals possessing only a small percentage of aberrant cells, the effect on the present study would appear small, since only one mosaic was encountered in all of the data collected.

The results obtained during the 27 months of the study to date are shown in Table 1. All of the aberrant babies yielded similar findings on buccal smears and amniotic preparations, when both samples were available. The results are summarized, together with the chromosomal analysis and physical findings, where these were obtainable, in Table 2. These data reveal that during the first 18 months of the study 1541 patients were sampled without the appearance of a single discrepancy. In the following 5 months, however, a total of 1009 newborns studied resulted in five babies with uniformly abnormal chromosomes and one with a mosaic condition. Finally, in the next 4 months 817 newborns were examined and again no anomalies occurred. Thus the total population of 3367 newborns forms two groups: one with a high incidence of sex-chromosome anomalies, which is centrally located in time; and one with no anomalies which precedes and follows the other group.

These data may be examined on the basis of three principal hypotheses: (i) The actual probability of occurrence of chromosomal anomalies in both populations is constant and close to 6/3367, or 0.18 percent, but chance dictated that all of the cases were confined to the central 1009 individuals studied. (ii) The true frequency is close to that found in the central portion of the study, which is equal to 6/1009 or 0.595 percent, but failure to discover any anomalies in the two sub-sets of the second group was due to experimental error. (iii) The two populations under consideration possessed different intrinsic frequencies of chromosomal aberration, that of one being close to zero, and that of the other, 0.595 percent.

The first of these alternatives is unlikely, since the probability of the given distribution of cases in two parts of a uniform population possessing the same intrinsic probability of occurrence of anomalies is less than one part per thousand. Therefore, a systematic difference between the two series is strongly suggested. The second hypothesis can be excluded with an even greater probability, by virtue of the error rate on first reading in the buccal smear technique being only 0.28 percent, so that the probability of missing any single nonmosaic anomaly in the first 1541 cases is only 3 parts per thousand, and the probability of missing two or more becomes negligibly small. In the last set of 817 cases in which no aberrations were found, the amnion technique, which has an even smaller possibility of error, was used throughout. Hence, there remains the third hypothesis as the most probable explanation of the data.

The frequency of all aberrations found, computed as an average over the entire population studied, is 0.18 percent, which is quite comparable to some of the higher values previously reported (*5–7*). However, the frequency of anomalies in the central 1009 subjects is 0.595 percent, which is more than three times that of the highest previous rate reported from a presumably normal population. The present data suggest that the unprecedented high frequency of sex-chromosomal anomalies in the middle 1009 cases studied is due to one or more nonrandom factors which operated differently during this part of the test. The complete absence of anomalies in the other cases studied becomes highly important, since it implies that the intrinsic frequency of sex-chromosome nondisjunctional processes in

Table 2. Details of the sex-chromosomal defects recorded in Table 1.

Phenotypic sex	Frequency of cells with chromatin-positive bodies in: Buccal smear (%)	Frequency of cells with chromatin-positive bodies in: Amnion (%)	Chromosomal constitution of leukocytes	Gross physical findings
M	Single 29 Double 0	Single 92 Double 0	100% 47/XXY	Glossoptosis, micrognathia. Died several hours after birth
F	Single 45 Double 30	Single 21 Double 74	100% 47/XXX	Normal
			Mosaic	
F	Single 45 Double 7	Single 40 Double 46	4% 47/XXX 96% 46/XX	Normal
M	Single 42 Double 0	Single 82 Double 0	100% 47/XXY	Normal
F	Single 0 Double 0	Single 0 Double 0	100% 45/XO	Webbing of neck, cubitus valgus, lymphedema, shield chest, and other typical Turner's syndrome findings
M	Not possible	Single 85 Double 0	Not possible	Stillbirth (no gross abnormalities)

human populations may indeed be close to zero in the absence of extraneous factors. The present data make it appear likely that the large differences in frequency of sex chromosomal anomalies reported in earlier studies are real and also reflect differences in action of extrinsic factors.

In order to determine whether a similar relationship might exist in the incidence of autosomal chromosomal anomalies during this same period, the frequency of Down's syndrome (due in the overwhelming majority of such cases to trisomy of chromosome 21) was studied. Tabulation from the hospital records was made of the number of cases of Down's syndrome which had occurred in the newborn population of these two hospitals during the same time intervals listed in Table 1. During the first 18 months, December 1962 to May 1964, four cases of Down's syndrome occurred in a total of 5311 births. In the succeeding 5 months, June to October 1964, during which all of the sex chromatin anomalies occurred, five of a total of 2155 births were similarly affected. Finally, in the last 4 months, November 1964 to February 1965, a total of 1012 births occurred with no cases of Down's syndrome. Thus during the critical 5-month period in which all of the sex-chromosome aberrations occurred, the frequency of Down's syndrome was 0.23 percent, while in the intervals before and after, during which no sex-chromosome abnormalities were found, the average frequency of Down's syndrome was only 0.063 percent. Hence the data suggest that the autosomes display a similar sensitivity.

Search is in progress for factors which might account for the difference in behavior displayed by these two populations. Maternal age was not the determining factor, since only one of the six mothers in the sex chromatin aberrational group was as old as 39, the others ranging from 19 to 27 years of age. Similarly, only three of the nine mothers of babies with Down's syndrome were older than 33 years, and these three cases were distributed among both periods of the study. The only correlative factor so far uncovered is an epidemic of rubella in this geographic area during the last quarter of 1963 and the first half of 1964 (*13*) in which the incidence of this disease reached exceedingly high levels.

The production of developmental anomalies by rubella infection of the mother during the first trimester of pregnancy is well known, as is the induction of chromosomal anomalies in mammalian cells by several viruses (*14*). However, while aberrations involving chromosomal breakage and reconstitution through virus action have been frequently described, nondisjunction has not been demonstrated as a major viral action. In the present study, while one case of mosaicism occurred, meiotic or very early mitotic chromosomal nondisjunction was the predominant lesion. Thus, the principal biological insult would have had to occur before or very shortly after conception, and so may involve a different kind of biological action from those underlying other viral developmental defects. The dynamics of such a process would appear of great importance.

Several suggestions have appeared in the literature to the effect that autosomal chromosomal abnormalities may be distributed in a nonrandom fashion. Collmann and Stoller (*15*) have demonstrated a "clustering" of cases of Down's syndrome in no fewer than 40 percent of such anomalies encountered in an extensive survey in Australia. They suggested on theoretical grounds that an infective agent, most probably a virus, might be the cause. During the course of our study, Heinrichs *et al.* (*16*) reported a clustering of patients with Trisomy 18 and Trisomy 21 in South Dakota. Most recently, Hecht *et al.* (*17*) have summarized several lines of evidence for nonrandom distribution of autosomal chromosomal errors in human populations, with particular reference to an association between Down's syndrome and Trisomies 18 and 13. They have considered a variety of intrinsic and extrinsic mechanisms that could produce an increase in incidence of such anomalies, and they have pointed out that on theoretical grounds viruses would appear to be excellent candidates for the role of the presumed causal agency.

The present data add further support to the nonrandom character of human chromosomal nondisjunction; afford presumptive, though preliminary, evidence that the cause resides in extrinsic factors; demonstrate that the sex chromosomes also share this tendency and, thus, a large number of additional and easily investigated diseases becomes available for such studies; and reveal that the present episode of a burst of chromosomal anomalies occurred at a time suggestive of a relationship to an extremely severe epidemic of a specific virus disease which occurred in this region.

As long as the number of cases involved is so small, great caution must be exercised in the interpretation of these results, since there is always the possibility of operation of unrecognized factors or of chance alone. Nevertheless these data are suggestive to the point of demanding further investigation.

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