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Identification of *Y* and *X* Chromosomes in Amniotic Fluid Cells

A FLUORESCENT staining technique has been described[1–3] for identification of the *Y* chromosome in cells from various sources. The use of this technique to detect *Y* chromosomes in the amniotic fluid cells, in conjunction with a search for sex chromatin bodies in these cells, might provide a rapid and accurate means of prenatal sex determination.

Fresh amniotic fluid from twelve women whose pregnancies were being terminated was centrifuged at 800–1,000 r.p.m. for 20 min, the supernatant discarded and a smear of the cell pellet was prepared. Smears were fixed in methanol for at least 15 min, stained with a 0.5% aqueous solution of quinaquine dihydrochloride for 5 min, washed in running tap water for 3 min and rinsed in 0.2 M acetate buffer (sodium acetate; glacial acetic acid), *p*H 5.5. The preparations were mounted in 1 : 1 glycerol and buffer. A minimum of 100 cells was examined from each specimen. In four specimens a fluorescent body (*Y* chromosome) was detected in 3–9% of the cells.

Sex chromatin was determined in eight specimens. One drop of the cell pellet of the amniotic fluid was mixed and stained immediately with one drop of 2% aceto-orcein and then compressed with a cover slip. Immediately after preparation the slides were examined for sex chromatin bodies. Two were sex chromatin positive, two were negative, four were unsatisfactory because there were too many pyknotic cells and too few cells suitable for analysis according to the selection criteria suggested by Hsu *et al.*[4]. All studies were done before expulsion of the foetuses. The results of *Y* fluorescence, sex chromatin and phenotypic sex determination for each abortus are shown in Table 1.

Pathological examination of the abortuses revealed five males and seven females. Although the four smears which developed fluorescent bodies were from males, one other male could not be detected by the method used. The reasons for our failure to detect a fluorescent body in the amniotic fluid cells are now being investigated. One possibility is that many of the cells were damaged or not viable, and this certainly seemed to account for the unsatisfactory smears for sex chromatin determination. The other possibility is that a small *Y* chromosome may fail to fluoresce. Borgaonkar and Hollander[5] found a male with a small *Y* chromosome which did not fluoresce. We have recently had two cases where no sex chromatin body was found in 200 amniotic fluid cells and karyotypes of the fibroblasts cultured from one amniotic fluid revealed a 46,*XX* chromosomal constitution. No karyotypes were done on the other fluid, but both foetuses were found to be phenotypic females (our unpublished results).

Based on these observations, we feel that extreme caution should be used in prenatal sex determination by examination for fluorescent *Y* and sex chromatin body. While the finding of a fluorescent body does indicate the presence of a *Y* chromosome and the presence of a sex chromatin body indicates the presence of an inactivated *X* chromosome, the failure to detect these bodies does not necessarily indicate the absence of the corresponding chromosome. It seems that until further experience with these techniques is obtained, karyotype analysis of cultured fibroblasts from amniotic fluid cells is the only accurate means of prenatal sex chromosome determination.

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Persistent Alteration of Turnover of Brain Noradrenaline in the Offspring of Rats subjected to Stress during Pregnancy

VARIOUS hypotheses have suggested that environmental stress during development is an aetiological factor in mental illnesses. Such hypotheses have included both biological and psychoanalytical concepts, but they have not explained convincingly any possible mechanism of action of stress. The developing organism has often been shown to be sensitive to various hormonal influences, and steroid and thyroid hormones or stress, given at a critical time during the neonatal period, seem to affect permanently the behaviour and metabolism of growing animals[1–3].

There have been several reports that stress or injections of adrenaline given to pregnant rats can have a persistent, although variable, effect on the behaviour of their progeny[4–10]. Although these changes in the offspring are probably mediated by some biological mechanism, they cannot be explained by any known persistent neurobiological changes. We have therefore made a neurochemical examination of the progeny of stressed pregnant rats, which could be a preliminary step in the construction of an animal model for human mental illnesses. We found that when stress was applied during pregnancy there

Table 1 *Y* Chromosomes, Sex Chromatin and Phenotypic Sex of Twelve Abortuses

Case No.	1	2	3	4	5	6	7	8	9	10	11	12
Y fluorescence	+	−	−	−	−	+	+	−	−	−	−	+
Sex chromatin	ND	ND	ND	ND	NS	NS	−	NS	+	NS	+	−
Phenotypic sex	M	F	F	F	M	M	M	F	F	F	F	M

ND, Not done; NS, not satisfactory; M, male; F, female; +, positive; −, negative.