THE OCCURRENCE OF SEX CHROMATIN IN EARLY HUMAN AND MACAQUE EMBRYOS

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INTRODUCTION

It is still uncertain at what stage of human development, and in which tissues, sex chromatin first appears. This paper gives the findings of an investigation carried out on the early human and macaque embryos filed at the Carnegie Institution of Washington (Department of Embryology), Baltimore. Many of these unique specimens are of great embryological interest and have already been described along other lines by Drs Hertig, Heuser, Rock and others. Sex chromatin has been found in the 19-day cat embryo (Graham, 1954), but I have been unable to find reports on embryos younger than this, apart from that of Glenister (1956) who found sex chromatin in the syncytiotrophoblastic cells of an implanting blastocyst, as well as in six out of thirteen embryos whose gonad had not yet differentiated to a testis or an ovary.

MATERIALS AND METHODS

Serial sections (H. & E.) were available of human embryos from the 1-cell stage (Horizon I according to the classification of Streeter, 1942) onwards; and macaque embryos from the free blastocyst stage (Horizon III in the human scale) onwards. Wherever possible, 200 nuclei were examined (2 mm. objective), a procedure often requiring prolonged search since 'readable' nuclei might be widely scattered. Particles were not accepted as sex chromatin unless they lay against the nuclear membrane and possessed the characteristic pyknotic density and plano-convex outline of the sex chromatin body, because this material had not been prepared primarily for cytological studies. The figures for incidence of the sex chromatin may thus be rather low.

The frequency of the sex chromatin within trophoblastic nuclei, however, is even more difficult to assess than in nuclei of the embryo proper because of the nuclear size in trophoblast. For example, a histological section 5μ thick would include the whole of a nucleus measuring $20 \times 5\mu$ only if it lay completely 'flat' within the section, an occurrence which is unlikely. Thus, a large part of a nucleus might be clearly seen in a section, yet its sex chromatin be present in the part of the nucleus outside the section; a false negative reading thus results. To compensate for this difficulty, a method of calculation was used whereby the figure for percentage incidence of sex chromatin obtained by counting the number of particles actually seen in 200 nuclei (or parts of nuclei)—the *apparent incidence*—was corrected so as to produce a figure more nearly approaching the true value. This corrected figure is called here the *calculated incidence*. Details of the method of correction have been given elsewhere (Park, 1957); it consists essentially in multiplying the figure for

apparent incidence by a factor which takes into account the diameter of the nucleus (d) and the thickness of the section (t), both measured in microns. The calculated incidence is the apparent incidence multiplied by $\frac{(2d-4)+t.}{t}$ Although young embryonic cells are more uniform and generally smaller than those of trophoblast, this method of correction was applied to them also. All the figures of incidence mentioned in the text and tables refer to *calculated incidence.*

In trophoblastic syncytium the sex chromatin was sometimes difficult to distinguish because of nuclear pyknosis, although usually it could be seen in both forms of trophoblast with approximately equal ease and frequency. The figures of incidence quoted for trophoblast are the means of the figures obtained for both forms of the tissue or for cytotrophoblast alone.

RESULTS

The total findings for human and macaque embryos are shown in Tables 1 and 2. The specimens (Carnegie Collection numbering) are listed by Horizons, in age order as estimated.

DISCUSSION

So far as present knowledge goes, there seems no theoretical reason why sex chromatin should not be present in the zygote, but in the human and macaque at any rate this appears not to be so. The youngest specimen to show sex chromatin amongst the human embryos was aged 10–12 days, the youngest macaque was aged 10 days. In both, the sex chromatin was seen in the trophoblast only, and in neither did the incidence exceed 3 %. These were the only two specimens, out of the eighteen 'readable' specimens aged up to 12 days, that showed any sex chromatin. Since it is unlikely that all eighteen were male the virtual failure to find sex chromatin may be taken as showing it to be normally absent up to this age. In younger specimens it is not always easy to be sure that cells containing sex chromatin at the junctional zone are not maternal cells. As may be gathered from Table 1, no sex chromatin was seen in the large, certainly trophoblastic cells in this zone in specimens of Horizons III and IV; but it was seen in many of the smaller cells lying amongst them. These small, mature nuclei, which, as pointed out by Hertig & Rock (1945), tend to congregate near the maternal tissue, were thought by these authors to be formed by amitotic division of the original nuclear mass of syncytium. The presence of sex chromatin within some of them (and we are almost certainly both referring to the same nuclei) makes it seem likely that these nuclei at any rate belong to maternal stromal cells blended with the developing trophoblast.

The first appearance of the sex chromatin is in the trophoblast and chorionic mesoderm. Although, as the tables show, this is not absolute, there seems to an earlier rise there than in the embryo itself and it is difficult to believe that this could be due to any artifacts of histological technique. This differential first appearance would seem to support the validity of the finding that at an earlier stage there are in fact no sex chromatin particles.

In the human embryo, sex chromatin particles in significant number were seen first at the eighteenth day, in the macaque at the nineteenth. The sixteen specimens,

(eleven human, five macaque) from the eighteenth day onwards can be divided into two main groups: six cases where some part of the embryo or its trophoblast showed an incidence of sex chromatin of 40 % or more, suggesting female tissue, and ten cases with a corresponding incidence of 13 % or less, suggesting male tissue (in four of these latter no sex chromatin was seen in any area). It seems, therefore, that a definite sex difference exists certainly by the eighteenth day in both species and, on

Table 1. *The distribution of sex chromatin in embryonic tissue of the human*

Specimen number and stage of development	Percentage incidence of sex chromatin in: Embryo	Trophoblast	Chorionic mesoderm
Horizons I–IV (up to 9 days)			
8698* (2-cell egg, 36 hr.)	Nil	Nil	Nil
8450* (abnormal 8-cell morula, 48–72 hr.)			
8452* (abnormal 12-cell morula, 72 hr.)			
8155† (8 days)			
8215 (8 days)			
8225 (8 days)			
8171† (9 days)			
Horizon V (10 to 12 days)			
7770	Nil	Nil	Nil
7950			
8558			
8000	Nil	< 1	Nil
Horizon VI (13 to 14 days)			
7801‡	Nil	16	10
8290	Nil	Nil	Nil
8602	< 1	9	24
Horizon VII (about 16 days)			
7762	12 (yolk sac)	Nil	10
7802‡	< 1 (yolk sac)	Nil	Nil
8752	Nil	Nil	Nil
Horizon VIII (about 18 days)			
7666	21 (amnion) 63 (notochord)	37	30
7701	26 (amnion) 45 (yolk sac)	22	25
7949	Nil	Nil	Nil
8727			
Horizon IX (about 20 days)			
7650	31 (body wall) 6 (neural plate)	20	43
Horizon X (about 22 days)			
2795	Nil	< 1	Nil
3710	Nil	Nil	Nil
5074	< 1	6	10
Horizon XI (about 24 days)			
7611	2 (neural tube)	Nil	Nil
7851	77 (neural tube)	40	80
8005	< 1	Nil	< 1

* Hertig *et al.* 1954. † Hertig & Rock, 1949. ‡ Heuser *et al.* 1945.

the findings in the human Horizon VI specimens, possibly by about the fourteenth day, although such sex chromatin as was seen at this stage was present almost exclusively in the trophoblast, not in the embryo itself. Incidentally the sexing of early embryos has depended till recently on recognition of changes within the gonad, for example the sex cords of the male gonad, appearing at about the fortieth day,

and thus this present work confirms the suggestions of Graham (1954) and Glenister (1956) that identification of sex chromatin would prove a way of sexing embryos before the fortieth day.

Table 2. *The distribution of sex chromatin in embryonic tissues of macacus rhesus*

Specimen number and stage of development	Percentage incidence of sex chromatin in		
	Embryo	Trophoblast	Chorionic mesoderm
9 days			
C560	Nil	Nil	—
C610	Nil	Nil	—
10 days			
C548	Nil	Nil	—
C532	Nil	Nil	—
C496	Nil	1 in unattached portion. 3 in 'deep' portion	—
11 days			
C599	Nil	Nil	—
C524	Nil	Nil	—
13 days			
C595	Nil	<1	—
C466	Nil	1	—
C562	Nil	Nil	—
C467	Nil	1 in unattached portion. 4 in 'deep' portion	—
15 days			
C571	Nil	Nil	Nil
17 days			
C457	Nil (germ disk) (2) amnion	12	2
18 days			
C546	Nil	Nil	Nil
19 days			
C508	66	43	66
20 days			
C421	12	13	7
29 days			
C477	6	Nil	Nil
34 days			
C479	54 (neural tube) 80 (paraxial mesoderm)	22	16

It may be concluded that in the human and macaque embryo, the period of absent sex chromatin is followed by a gradual appearing of these bodies from about the fourteenth day, predominantly at first in the trophoblast, and then in the embryo proper. By about the eighteenth day there are enough to indicate that the sex difference has been established. As soon as the incidence of sex chromatin in the embryo proper is enough to allow a reasonable comparison with the trophoblastic zone, the possibility arises of determining whether the sex of the trophoblast and that of the embryo is always the same. The findings in this material have shown that they are always identical.

Finally, the study of embryos during the period when the sex chromatin is in process of appearing has revealed an unusual variation from place to place in the rate of appearance. The present material is inadequate in amount and had not been processed by techniques ideal for study of the sex chromatin, and it is therefore not possible even to discern a pattern of sequence. The interesting possibility remains,

however, that different tissues respond differently and perhaps sequentially to the stimulus that begins to call forth, from about the twelfth day, the sex chromatin.

SUMMARY

1. A series of thirty-three human and eighteen macaque embryos has been examined in an attempt to assess the time of appearance and distribution of sex chromatin at early stages of development. The human embryos were aged from approximately 36 hr. (a two-cell egg) to 24 days, and the macaque embryos from 9 to 34 days.

2. In the human, sex chromatin was first seen at the age of approximately 12 days, in the trophoblast; and in the embryo itself at approximately 16 days. In the macaque, it was seen very occasionally from the tenth day onwards in the trophoblast, and in greater number in the embryo itself at the nineteenth day.

Other specimens of equal age showed no sex chromatin in any area, suggesting that a reliable sex difference is established in both species between about the twelfth and nineteenth days.

3. In the older specimens, sex chromatin was always present in the trophoblast of embryos showing sex chromatin, and absent from the trophoblast of embryos not showing sex chromatin.

4. The present study suggests that sex chromatin appears at different times or with different rates in the different tissues of the embryo.

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