period of 60 to 90 minutes was compared with that during a period of 15 to 45 minutes. A dose of α-toxin (16,000 HU) that produced maximal suppression of SCC and PD was used. The addition of toxin prior to incubation produced an increase in mean oxygen consumption in paired tissue from 4.5 to 6.5 μl per minute ($P < .001$) (Table 1). When toxin was added to bladder suspensions which had been allowed to respire for 45 minutes, oxygen consumption observed at 60 to 90 minutes was increased by 15.4 μl over that observed at 15 to 45 minutes. Oxygen consumption by untreated tissue declined by 4.6 μl during the same interval ($P < .001$).

The inhibition of both the short-circuit current and potential difference of the isolated toad bladder by staphylococcal α-toxin suggests that an alteration in epithelial ion movement has occurred. This does not appear to be secondary to inhibition of oxygen consumption since this was stimulated by the toxin. Active sodium transport has been shown to be directly related to SCC across the isolated bladder in a variety of circumstances (*6*). Whether the inhibition of SCC and PD by this toxin reflects a specific effect on active transport mechanisms, a destructive action on cell membranes, or is mediated by other metabolic or structural changes remains to be determined.

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Mammalian X-Chromosomes: Change in Patterns of DNA Replication during Embryogenesis

Abstract. *One arm of both X-chromosomes in female eight-cell embryos of the golden hamster replicates late in the period of DNA synthesis. Mid-gestation embryos and adult fibroblasts show an increase in late-replicating DNA. Here, one X-chromosome is labeled in one arm; the other is labeled throughout.*

In somatic cells of female mammals, one of the two X-chromosomes has the following characteristics: it is relatively inactive, both genetically and metabolically (*1*); it shows positive heteropycnosis (*2*); and its DNA is synthesized later in the DNA-synthetic or S period than is most of the other chromosomal DNA (*3*). According to the Lyon hypothesis (*4*), this differentiation is a random event involving either X-chromosome during early embryogenesis. However, once a given chromosome has been inactivated it will remain so in all subsequent cell generations.

Interphase and prophase heteropycnosis of the X-chromosome does not appear until the time of implantation (*5*). Likewise, estimates of the time of inactivation from genetic studies do not implicate the first few cell divisions (*6*). Therefore, if the previously mentioned relationships hold, the patterns of DNA replication in the X-chromosomes during the early stages of cleavage should differ from those found after implantation.

In this report, comparisons are made of autoradiographs of metaphase cells from 3-day-old, eight-cell embryos before implantation, and 7½- to 9½-day-old embryos after implantation, and of fibroblasts from adult skin. All cells have been continuously exposed to tritiated thymidine during the latter part of the period of DNA-synthesis, a time when few chromosome segments become labeled. The golden or Syrian hamster (*Mesocricetus auratus*) was chosen for this study because it can be easily hand-bred, it produces litters of 9 to 13 young, and its early developmental patterns are known (*7*). Even with these advantages, over 300 females have been sacrificed in the course of this study.

The eight-cell embryos were flushed from the uterine horns of pregnant females and placed in a medium, used by Brinster (*8*), containing 8 μc of tritiated thymidine per milliliter, (New England Nuclear Corporation; specific activity > 10.6 c/mmole) for 7 to 8½ hours. Colchicine (0.2 mg/ml) was added for the last hour. Mid-gestation embryos and adult fibroblasts were cultured in enriched Eagle's medium containing 15 percent human and 5 percent calf serum and 2 μc of tritiated thymidine per milliliter for 5½ to 6¼ and 6 hours, respectively. Colchicine was present during the last ½ hour in the embryo cultures and during the last 4 hours in the fibroblast cultures. All cells were treated with hypotonic citrate, fixed, and squashed in 1.5 percent aceto-orcein either by direct pressure or by heating the slide; autoradiographs were then made (*9*).

The X-chromosome is the largest of the golden-hamster complement: it is nearly metacentric and is morphologically distinguishable (*10*). The patterns of DNA replication of the sex chromosomes have been described for cultures of 15-day fetal cells and for adult cells (*11*). These published reports are confirmed by our findings in 35 cells from adult skin fibroblasts from females, which have been labeled during the latter part of the S period. Here, one of the X-chromosomes is labeled along its whole length, the other being labeled in only one arm. Credence is given to this by the finding of one and a half heteropycnotic X-chromosomes in prophase cells (*12*) and one and one-half X-chromosomes in metaphase cells showing an alteration in morphology after special fixation and spreading (*13*). Our examination of 29 cells from 7½-day-old and of 29 cells from 9½-day-old female embryos showed that the replication pattern just described is clearly established by these times in development.

The patterns of replication in X-chromosomes reported for the Syrian hamster differ in some respects from those found in most mammalian species (*3, 14*). Usually, pronounced asynchrony is found in the times at which the two X-chromosomes replicate; one chromosome is labeled at the end of the S period, the other at an earlier time. As in other mammals, asynchronous replication of X-chromosome DNA is present in the Syrian hamster, but only in one arm. The other arm of both X-chromosomes is late replicating. The presence of this late-replicating DNA in both chromosomes may be related to their larger size (*12*), since other mammals with large X-chromosomes show replication pat-

terns with this characteristic (*15*).

We have found that the patterns of DNA replication in the X-chromosomes of female eight-cell embryos are different from those of embryos after implantation and from adult fibroblasts. In the cleavage embryos both X-chromosomes are labeled in only one arm (Fig. 1). This is thought to represent a true difference in the time of replication for several reasons: (i) Of 34 cells labeled relatively late in the period of DNA synthesis, no case was found in which one and a half X-chromosomes were labeled. The X-chromosomes were never labeled in more than one arm each. (ii) We tried to compare cells which contained similar amounts of label on the autosomes. In the implanted embryos and fibroblasts, cells with relatively little label showed the autosomes to have the grains mainly concentrated in their short arms (*11*). Autosomes from eight-cell embryos tended to be slightly more diffusely labeled but retained many of the similarities just noted. Even though the autosomes were more diffusely labeled in the cleavage embryos, the X-chromosomes contained less label. (iii) An additional ten cells were examined in which many autosomes were diffusely labeled. In these, both X-chromosomes were labeled throughout their length. This increase in labeling of the X-chromosomes does not occur until the autosomes have become diffusely labeled, a period which is not representative of the end of the S period. Since we have observed cells from a stage when there is little labeling to one where many chromosomes are completely labeled without finding the intermediate labeling pattern of one and a half X-chromosomes, it is unlikely that our results are spurious.

It is reasonable to assume, then, that the X-chromosomes in the embryos before implantation differ from those after implantation. Our findings indicate the basic pattern of replication in eight-cell female embryos to be the late replication of one arm in both X-chromosomes. Between cleavage and midgestation one of the other arms of an X-chromosome shifts from an early to a later time of replication, resulting in the late replication of one and a half X-chromosomes. Presumably this change in DNA replication is a manifestation of the genetic inactivation of that chromosome segment.

In the male, the single X-chromosome is labeled in not more than one arm at the end of the S period (*11*). We have made similar observations in 12, 26, and 15 cells from cleavage embryos, implanted embryos, and adult fibroblasts, respectively. The Y-chromosome is not morphologically distinct (*10*). Nevertheless, in adult fibroblasts, as well as in cells from midgestation embryos, a single completely labeled chromosome is found when all others, except the X-chromosome, are relatively unlabeled. This unpaired chromosome is inferred to be the Y-chromosome. In observations of eight-cell embryos, the typically labeled Y-chromosome has not been detected.

We conclude that there is X-chromosome differentiation during mammalian embryogenesis, as predicted by the Lyon hypothesis. This involves an increase in the amount of late-replicating DNA in one of the two X-chromosomes of females. Once established, these patterns of replication are apparently maintained in the later stages of embryogenesis and in the various adult somatic tissues (*11*, *16*).

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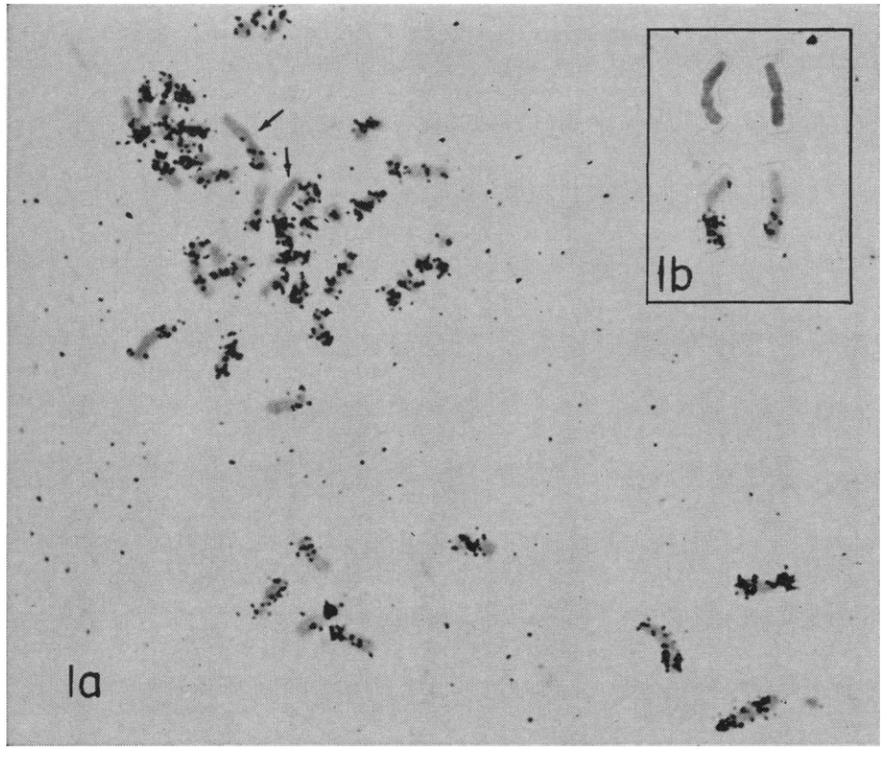

Fig. 1. Metaphase chromosomes from a cell of a female Syrian hamster, eight-cell embryo labeled at the end of the S period. (a) Diploid metaphase spread ($2n = 44$). Arrows mark the two X-chromosomes. (b) X-chromosomes from this cell photographed before and after autoradiography.

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