therefore, any evaporation may render it toxic to the cysts. These observations and the experiments presented above indicate that it is unnecessary to postulate any "volatile factor" other than water vapor (*5*).

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4. The spermatocytes of the Cecropia silkworm occur as hollow balls of several hundred cells surrounded by follicle cells. Such an aggregation of cells is called a spermatocyst. The cells within one cyst develop synchronously.
5. I am grateful to Prof. C. M. Williams for his very helpful criticism of the manuscript.

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Changes in Incidence of Sex Chromatin in Subcultured Cells

Abstract. Sex chromatin counts of subcultured cells of both female human mammary tumor and female rabbit kidney show a considerable drop from an initial high level. Cultures in which sex chromatin persists also retain the viral insensitivity of their source material.

The presence of sex chromatin in tissue of female origin has been demonstrated in explants and primary trypsinized cell cultures (*1–3*), but generally not in cell lines after prolonged cultivation in vitro (*1, 4*).

The first report of sex chromatin in subcultured female cells was in human mammary tumor tissue cultured for 10 weeks, through nine transfers (*1*). In another report, sex chromatin was not found in human female tissue cultures aged 2½ months to 3½ years, after nine or more transfers, but was present in "younger" cultures aged 2 to 55 days, after three to five transfers (*5*).

More recently, the presence of sex-chromatin-like chromocenters in long-term cultures of both female and male human tissues was reported (*6*). Such chromocenters were found in 5 percent of the HeLa cells studied, and in 27 percent of the D-189 cells, of male origin. Multiple chromocenters, resembling those found in D-189 and, presumably in HeLa, were also found in cell nuclei in a study of 15 successive passages of stock HeLa cells maintained in our laboratory, but we found no typical sex chromatin, as described by Barr *et al.* (*7*).

Since the reported "sex-chromatin-like chromocenters" were found in male as well as in female subcultures, in contrast to other negative findings of sex chromatin in cultures of male origin (*5*), these counts may have included chromatin clumps simulating sex chromatin. The alteration of the distribution of sex chromatin normally found in explants and primary cultures that occurs with prolonged subculturing could be due either to actual loss of sex chromatin or to its masking by increased clumping of the chromatin granules. In either case, these nuclear changes could provide a useful marker of in vitro cell transformation.

The human mammary tumor cell line in which the presence of sex chromatin was first reported (*1*) subsequently was maintained through 24 weeks and 18 transfers, in Lactal (*8*) with human, horse, or rabbit serum. During the subculturing, sex chromatin dropped steadily, from a high of 63 percent to a final count of 9 percent. After the 19th transfer, this culture failed. Therefore it seemed desirable to follow sex chromatin changes in an uninterrupted culture.

Accordingly, counts have been made of the sex chromatin in a line of rabbit kidney cells from their initial cultivation to the present. These cells started as a trypsinized suspension of kidney from a female New Zealand rabbit. The medium was a modified Lactal with cow serum (*9, 10*). At the time of the last count, this culture had surpassed the human mammary tumor line in both duration of maintenance (38th week) and number of transfers (20th). Its continuing vigor is indicated by the fact that at the third day after transfer the number of cells was doubling in 24 hours and an average of 10 percent of the cells were undergoing mitosis.

The following characteristics found in primary explants were used as criteria for sex chromatin in the nuclei of the subcultured cells: (i) one to three well-defined nucleoli in nucleoplasm composed of relatively fine granules which are Feulgen-positive and deeply basophilic with hemotoxylin-eosin staining, and (ii) a wedge-shaped or plano-convex sex chromatin mass, larger than any of the Feulgen-positive granules and more basophilic than the nucleoli. Nuclei with large multiple chromatin clumps were not counted.

Sex chromatin both adjacent to and free of the nuclear membrane was counted in 200 cells from duplicate cultures from each passage, with the exception of the last count (38th week) of the rabbit kidney cells. Because the incidence of sex chromatin was declining, the procedure for that count was modified to reduce the possibility of counting chromatin clumps of any size. Duplicate cultures of two successive days of cultivation were fixed and stained with hemotoxylin and eosin, and in each slide 25 random fields were examined at a magnification of 1000. Only the most typical sex chromatin masses were counted in nuclei with the specified characteristics, and even deeply stained chromatin masses were rejected if they were small, spherical, or not adjacent to the nuclear membrane. Sex chromatin was found in all four of the cultures, and the average did not vary significantly from earlier counts (Fig. 1).

As Fig. 2 shows, there was no significant change, between the primary

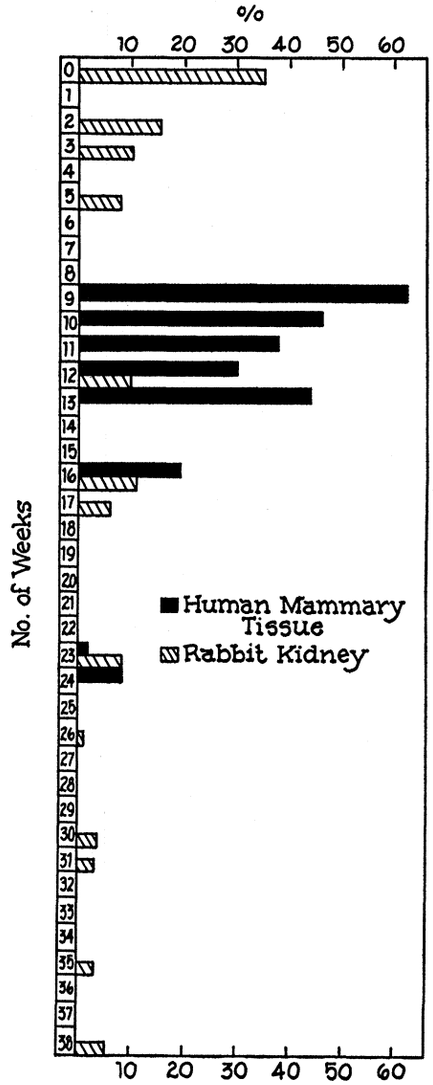

Fig. 1. Percentage of sex chromatin in maintained cultures of female human mammary tumor and of female rabbit kidney cells, by number of weeks. Percentages are average counts of duplicate cultures fixed and stained 3 to 5 days after transfer. Human mammary tumor cultures were not available for counting until the 9th week, representing the 5th transfer. They terminated during the 25th week, after the 19th transfer. Rabbit kidney cultures at the 38th week represent the 20th transfer.

and the last culture, in cell morphology or in the size of nuclei or sex chromatin.

In early transfers of both the human mammary tumor and the rabbit kidney cell lines, counts correspond, respectively, to the incidence of sex chromatin in somatic human tissue (*7, 11*) and in explants and primary cultures of cells of rat (*3*), dog, and cat (*12*). Although sex chromatin incidence in early transfers was higher in the human tumor than in the rabbit cells, in both cell lines it dropped with subculturing to 2 to 9 percent (Fig. 1).

Both cell lines also retained the insensitivity of their source material to certain viruses. In contrast to such established human cell lines as HeLa and H.Ep.No.1 (*13*), the human tumor cultures did not support the virus of infectious bovine rhinotracheitis. In contrast to an established rabbit-kidney cell line obtained from Drew (*14*) and propagated further in this laboratory, preliminary studies with our rabbit kidney cultures indicate that, up to the current passage, they fail to support poliovirus.

Culturing of the rabbit kidney cells continues for the purpose of investigating any relationship between a possible complete loss of sex chromatin and concomitant changes in morphology and viral sensitivity (*15*).

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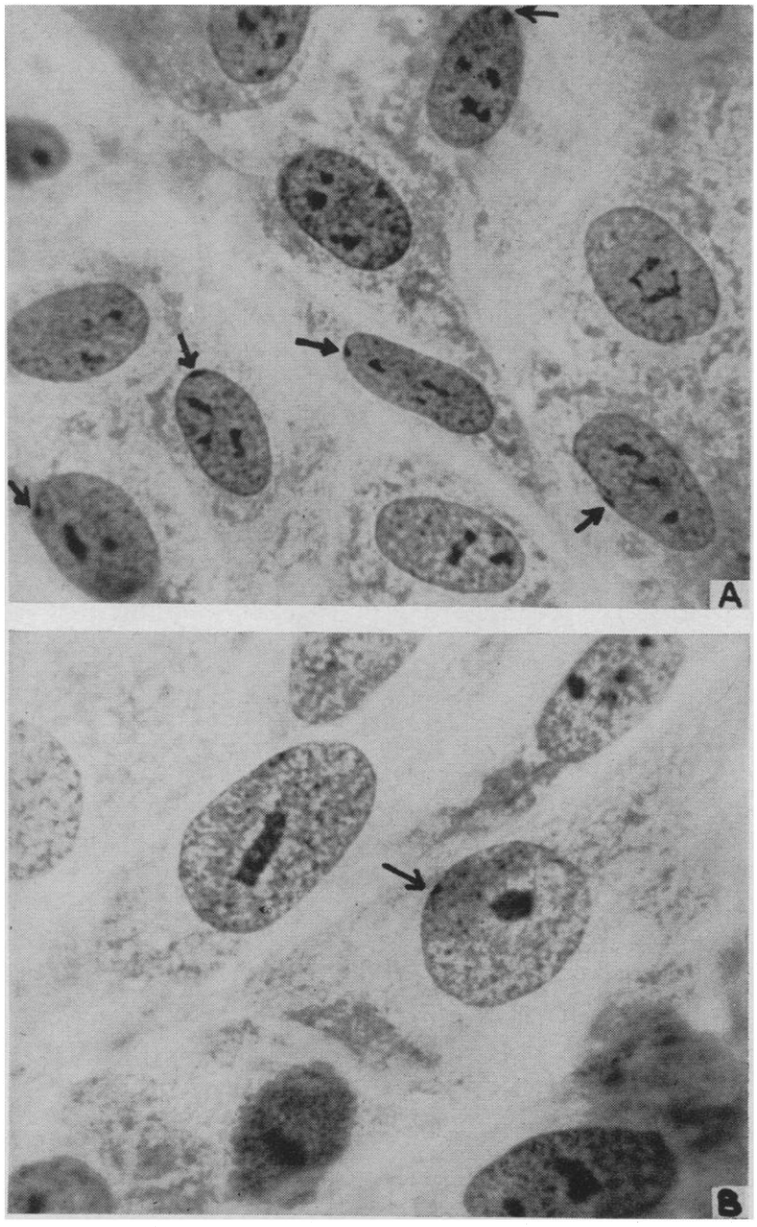

Fig. 2. Female rabbit kidney cultures, about × 1300, Wratten green filter No. 11, hemotoxylin and eosin stained. Arrow indicates sex chromatin. *A*. Primary culture, sex chromatin 35 percent. Width of nuclei average is 14 μ, range 8 to 24 μ. Average size of sex chromatin: width 1.2 μ, length 1.6 μ. *B*. 38th week, 20th transfer, sex chromatin 6 percent. Width of nuclei average is 14 μ, range 8 to 28 μ. Average size of sex chromatin: width 1.1 μ, length 1.8 μ. Culture retains whorl-like arrangement of cells and oval nuclei and elongated cytoplasm of earlier transfers. Note typical wedge-shaped chromatin mass larger than nearby fine chromatin granules.

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Excessive Stimulation of Salivary Gland Growth by Isoproterenol

Abstract. In the rat, chronic treatment with isoproterenol can cause a selective growth of the salivary glands to approximately five times their normal size within 17 days. This enlargement is principally due to mitotic proliferation and hypertrophy of the parenchymatous cells.

It was recently observed that chronic treatment with very large doses of isoproterenol is tolerated by the rat if the compound is administered intraperitoneally. By this procedure, certain otherwise undetectable actions of this catecholamine became evident (for example, production of aortic aneurisms and nephrocalcinosis). In the course of this work it was incidentally