Note on Nucleolonemata in Human Cultured Cells.* By A. R. T. Denues,†
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In the course of treatments of cultured cells with a hypotonic salt solution to
spread the chromosomes (after Hsu, 1952; Hsu and Pomerat, 1953), certain filament-
ous structures were seen by phase contrast microscopy within the nucleoli of liv-
ing normal and neoplastic human somatic cells. Such indications of filaments
in nucleoli seem of some interest in relation to earlier reports of similar observa-
tions following the use of other materials and of widely different techniques. Thus,
filamentous structures have been described within the nucleoli of a variety of
plant and animal cells processed by special methods (Estable, 1930; Sosa, 1945;
and Estable and Sotelo, 1951), and similar observations have also been reported
for certain germinal cells examined in vito. Detailed descriptions of such coiled
structure in stained nucleoli of several Conjugales have also appeared (God-
ward, 1950). Independent electron microscopic observations of such structures in
the nucleoli of various rodent and human cells have also been reported (Borysko
and Bang, 1951; Bernhard et al., 1951 and 1952). These filaments seen by the special
techniques of electron microscopy are of
about the same size as those described by
the other techniques (0.1 to 0.2 μ diam-
eter). The present observations with liv-
ing and mildly treated human cells (cf.
Hughes, 1952) thus support the reality of
the nucleolonema and call for studies of
its nature and function in normal and
neoplastic human cells.

For these studies, pieces (1 to 3 mm.)
of tissues from flourishing cultures of
minced human embryo and of two hu-
man epidermoid carcinomas (HeLa and
Helleis),† or from heterologous propaga-
tion of a human epidermoid carcinoma
(HEp 3) and of a human sarcoma (H.S. 1)
in rats§ were planted on coverslips in clots
composed of equal parts of chicken
plasma and culture fluid. These covers-
lip cultures were then placed in roller
tubes and fed with 1 ml. of culture fluid
initially and every 3rd day thereafter.
Incubation was at 37°C. After 2 to 6 days’
growth, the cultures were pretreated for
various times by immersion at 37°C. in a
hypotonic salt solution comprising 20
parts of Gey’s standard salt solution and
80 parts of a solution with the same in-
gredients except for NaCl which was
omitted. Following this pretreatment the
coverslips were mounted in a drop of the
hypotonic solution, sealed with paraffin,
and examined immediately and at fre-
quent intervals on a warm-stage phase

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* The culture fluid comprised 5 parts of a balanced salt solution (X-6) with antibiotics,
3 parts of human placental serum, and 2 parts of chick embryo extract.
contrast microscope with dark M oil immersion objective (NA 1.25).

Observations and photomicrographs of many cells indicated internal structures of a generally filamentous character in the nucleoli of all the various types of tissues examined. Examples are shown in Figs. 1 to 7. The filaments were found to be about 0.2 μ in diameter, in good agreement with the diameter deduced from electron micrographs of cells of a human melanoma. These filaments were not generally apparent in non-pretreated cells (see Fig. 8) but were clearly evident after even the briefest pretreatments. They were not seen to change strikingly during observations lasting up to one-half hour. Dispersion of the nucleoli did appear to increase with time.

These filamentous structures in nucleoli of both normal and neoplastic human cells in living tissue cultures thus appear comparable with the nucleolomata first reported for animal cells by Estable et al. in silver-stained preparations and by Bernhard, Borysko et al. in thin sections by electron microscopy, as cited above. The absence of marked change of the filaments and presence of continued dispersion of the nucleoli with prolonged exposure to hypotonic medium would agree with the bipartite structure described by Estable and Sotelo. The marked accentuation of the filaments by hypotonic treatment would suggest that the nucleolomata may represent a structure often highly dispersed in the normal interphase nucleus.

Note: Lettré and Siebs in a recent short note have suggested from stainings of nucleoli of cultured chick fibroblasts that the filamentous structures are of chromosomal nature (Naturwissenschaften, 1954, 41, 458).

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EXPLANATION OF PLATE 49
All figures are phase contrast photomicrographs at a magnification of 2330 diameters. The cultures, except that shown in Fig. 8, were pretreated with hypotonic salt solution.

Fig. 1. Minced human embryo, 6 day culture, 15 minute pretreatment.
Fig. 2. Human epidermoid carcinoma HeLa, 5 day culture, 5 minute pretreatment, after 20 minutes’ observation.
Fig. 3. Human epidermoid carcinoma Helleis, 4 day culture, 5 minute pretreatment.
Fig. 4. As Fig. 3, different cell, 20 minute pretreatment.
Fig. 5. Human epidermoid carcinoma Helleis, 3 day culture, 5 minute pretreatment, after 5 minutes’ observation.
Fig. 6. As Fig. 5, same cell at different focal level after 30 minutes’ observation.
Fig. 7. Human epidermoid carcinoma HEp No. 3, 3 day culture, 15 minute pretreatment.
Fig. 8. Human epidermoid carcinoma HeLa, 4 day culture, in isotonic medium (Gey’s solution), after 3 minutes’ examination.