night within the same subject. The discontinuous and arrhythmic nature of gonadotropin release is consistent with the pattern of release of other anterior pituitary hormones in man, particularly ACTH (*20*).

The modest but statistically significant increase in LH during REM sleep is of interest for several reasons. First, norepinephrine as a central neurotransmitter has been implicated both in REM sleep (*6*) and in the secretion of this hormone (*7*). Second, peak secretion occurs during paradoxical (REM) sleep in unanesthetized, unrestrained female rats at all times of the estrous cycle (*21*). Third, REM sleep-associated increases in testosterone, the release of which is stimulated by LH, were reported recently in male subjects (*11*). And fourth, penile erections occur during REM sleep (*22*).

Of major interest is the fact that the fluctuations in both LH and FSH are small in comparison with the changes in cortisol and growth hormone which occur during sleep. Recent studies of reproductive physiology by sensitive radioimmunoassay techniques indicate that major physiologic events may be associated with only small changes in LH and FSH concentrations (*23*). For example, the process of sexual maturation is accompanied by statistically significant increases in average concentrations of LH and FSH, but there is great overlap of values between sexually mature persons and prepubertal children. Also, the FSH increase which initiates follicle growth during the menstrual cycle is small. Thus the modest increase in LH found during REM sleep may have important, but as yet unknown, physiologic significance. However, it is also possible that these changes reflect only the modulation of control systems within the brain and are without importance to gonadal function.

Note added in proof: Similar repetitive, abrupt elevations in LH recently were reported in four normally active men sampled every 15 minutes between 6 a.m. and 6 p.m. (*24*).

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23 September 1971

Activation of Viruses in Human Tumors by 5-Iododeoxyuridine and Dimethyl Sulfoxide

Abstract. *Dimethyl sulfoxide added to cultures first treated with 5-iododeoxyuridine increased C-type virus production approximately tenfold in a human rhabdomyosarcoma cell line. 5-Iododeoxyuridine followed by dimethyl sulfoxide also activated a similar C-type virus in a metastatic tumor from a bronchial node taken from a 52-year-old male.*

Recently we described a method for activating a virus in a nonproducing, established cell line from a human rhabdomyosarcoma. This was accomplished by growing the cells in the presence of 5-iododeoxyuridine (IdU) for 3 to 4 days (*1*). The activated virus resembles the murine C-type oncornaviruses in size and morphology. It differs, however, from the animal oncornaviruses in that it does not bud from the outer cell membrane of the cell, but only from the endoplasmic reticulum. One to ten virus particles were observed in about 1 percent of the cells examined.

We now describe a modification of the former procedure, whereby we can now produce much greater quantities of virus from the same rhabdomyosarcoma cell line. The method has also been applied to an established cell line developed from an adenacarcinoma metastasis to a bronchial node and has again yielded virus from a nonproducer line.

Dimethyl sulfoxide (DMSO), which is known to readily penetrate cell membranes, has been used to attempt enhancing the penetration of virus into

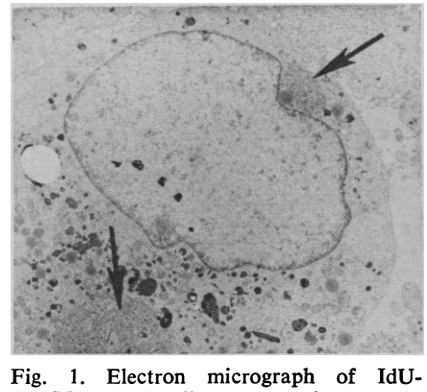
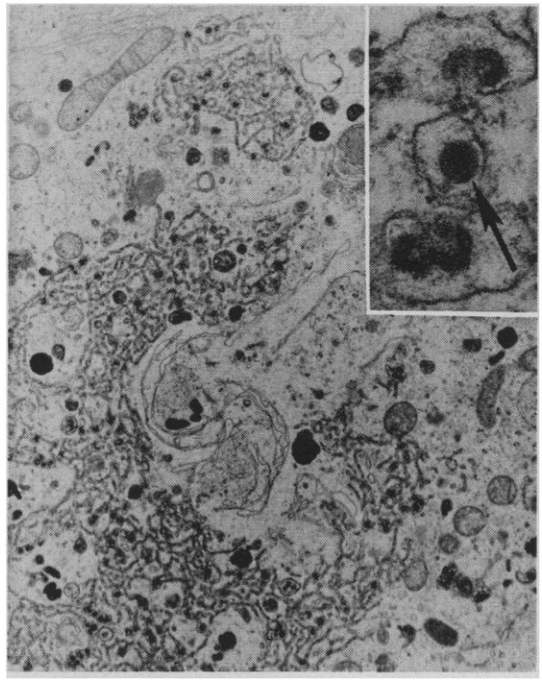


Fig. 1. Electron micrograph of IdU-DMSO treated cell. Arrows point to cytoplasmic inclusions (× 2400).



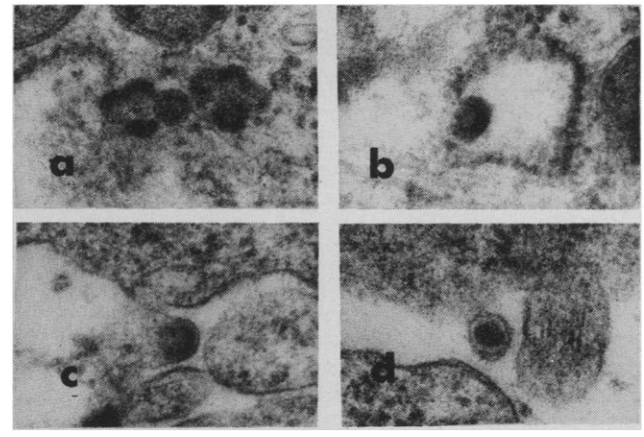


Fig. 2. Cytoplasmic inclusions consist of proliferating agranular endoplasmic reticulum with viruses budding into the cisternae (× 7500). (Inset) Four budding viruses can be seen. Arrow points to complete virus particle within the cisternae of the endoplasmic reticlulum (× 60,000). Fig. 3. (a) Eight virus particles in the cisternae of the endoplasmic reticulum which has been sectioned tangentially to its membrane. (b) Virus budding into cisternae of endoplasmic reticulum. (c) Virus budding from plasma membrane. (d) Mature C-type particle in extracellular space (× 60,000).

cells (*2*). It has also been found to favor cell maturation in tissue culture (*3*). Because viral transformation of normal to malignant cells is often associated with the loss of virus (virus-transformed cells frequently become nonvirus producers), the DMSO was added to transformed tissue culture cells in which virus had been activated by IdU. This was done to determine (i) whether the addition would result in greater virus yield because of a change in the tumor cell to a more differentiated stage or (ii) whether it might affect cell membranes to bring about greater virus replication (budding).

Tumor cells in culture were grown for 3 days in the presence of medium containing IdU (20 μg/ml). The fluid was then removed, and Eagle's basal medium with 15 percent heat-inactivated fetal bovine serum, glutamine 29.2 mg/ml, Kantrex 0.1 ml/100 ml, and 2 percent DMSO was added. The same medium was added to control cultures not exposed to IdU, and all were incubated at 37°C for 4 days. At this time, the cells were examined for virus by electron microscopy.

When the IdU treatment was followed by the DMSO, virus production was greatly increased in the rhabdomyosarcoma cell lines. More than 10 percent of the cells yielded virus; some cells had more than 100 particles per field surveyed. This is a tenfold increase over that observed when the cell line was treated with IdU only. Many cells developed numerous inclusions that consisted of proliferating agranular endoplasmic reticulum with multiple virus particles budding from these membranes (Figs. 1 and 2). Although many of the particles appeared to be incomplete, viruses with dense nucleoids were also observed. The 2 percent DMSO medium added to cells that had not been treated with IdU did not activate virus production.

Using the IdU-DMSO activation procedure, we have also been able to demonstrate a virus in a tissue culture developed in July 1968, from a metastatic adenocarcinoma obtained from a bronchial node. The tumor grew out as fibroblasts that transformed to epitheloid-like cells after 2 years of culture (*4*). The node had been taken from a 52-year-old male who formerly had had polymyositis. The virus resembles the one observed in the rhabdomyosarcoma in that it is a C-type particle (90 to 110 nm) budding from the endoplasmic reticulum. Here, however, it was also found budding from the plasma membrane. Electron microscopic examination was not done on the tumor before culture, and virus was not found in the cultured cells until after the activation procedure. In this culture only small quantities of virus were observed (Fig. 3). Most of the virus particles budded from the endoplasmic reticulum, but budding from the plasma membranes was also found. This culture is contaminated with mycoplasma, which were probably derived from the patient's lungs. It is possible that the mycoplasma has an inhibitory effect on the virus.

These two viruses are both different from the murine and feline oncornaviruses in that they bud from the endoplasmic reticulum; although in the culture made from the adenocarcinoma, viruses budding from the plasma membrane were also observed. It is necessary to carry out further experiments to determine whether these viruses are significant in the etiology of human neoplasms.

However, we wish to caution against the use of this method for activating viruses in human tumors unless the laboratories where such work would take place are equipped with complete virus-containment areas.

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5. This study was conducted under contract 43-65-53 within the Special Virus Cancer Program of NIH.

16 December 1971