the fact that the subjects did not know exactly when and where the satellite was to appear.

For watch intervals beyond 15 minutes the decrement increased, reaching a maximum mean difference of 1.1 magnitude at 60 minutes. Observations at 90 and 120 minutes showed reduced mean decrements compared with the value at 60 minutes. The individual subjects, however, varied greatly in this respect, the greatest decrement occurring with S_4, and reaching 1.8 magnitudes at 60 minutes. Subjects S_5 and S_8, at the other extreme, showed very little decrement.

The group of points lying within the box labeled "observers warned that the satellite would appear" are data obtained after the observers had watched for 60 minutes as in other watch periods, but were then suddenly warned that the satellite would appear. These measurements were made to try to separate the decrement due to true fatigue from that caused by other factors. The small decrement was found not to be statistically reliable.

The conclusion reached from these results is that sometime between 15 and 30 minutes watch an additional decremental process sets in. This is distinct from the decrement due to space and time uncertainty and is caused by other factors which are associated with decreasing vigilance. The results obtained at 60 minutes with the observers warned show that this decrement was not caused by a true fatigue of the visual system.

A plausible explanation for the improvement in observer efficiency for watch periods longer than 60 minutes is that the observers knew that the longer they observed, the more probable it became that the satellite would appear.

It was found that training of the observers prior to the experiment resulted in an improvement in detection threshold, in some cases by as much as a magnitude. Training also reduced the number of false reports and the variability of individual thresholds.

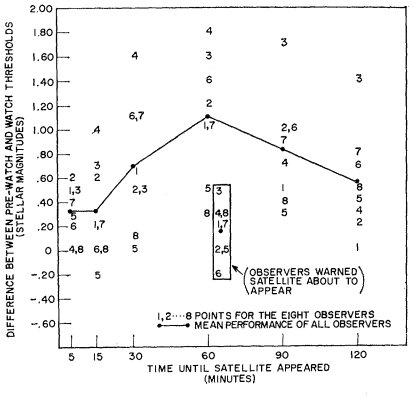

Fig. 1. Decrement in detection threshold as a function of the observing times before the satellite appeared.

The practical conclusion is that satellite observers should be rotated every 30 minutes when possible. However, if no relief is available, it is worth while for an observer to watch continuously for 1 to 2 hours, because the satellite will often be bright enough to be seen in spite of the increase of 1 to 2 magnitudes in his threshold.

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References and Notes

1. Individuals who may be interested in taking part in this program should write to S. A. O., 60 Garden St., Cambridge 28, Mass.
2. This report has been published in greater detail as NRL Report No. 5094 (Feb. 1958).
3. I. S. Gulledge *et al.*, *Science*, this issue.

25 February 1958

Inhibition of HeLa Growth by Intranuclear Tritium

Tritium-labeled thymidine (H^3TDR) of high specific activity is proving to be a useful label for deoxyribonucleic acid (DNA). In conjunction with autoradiography, it has revealed the mechanism of duplication of genetic material (*1, 2*) and the dynamics of cell renewal (*3*). As Robertson and Hughes have pointed out (*4*), such localization of tritium within the cell nucleus should result in almost exclusive irradiation of this radio-sensitive volume because of the very short range (average 1 μ, maximum 6 μ) of the resulting β-radiation. However, to date no evidence of radiation effects has been reported. This report demonstrates that high levels of incorporation of H^3TDR do cause radiation damage as predicted.

HeLa cells were grown in Eagle's basal medium (hereafter referred to as "standard medium") prepared in Hank's balanced salt solution, 20 percent horse serum and 15,000 units of penicillin, 1000 μg of Terramycin, and 5000 units of Mycostatin per 100 ml. The cells were inoculated into 1 ml of standard medium in Leighton tubes. Duplicate cell counts were made with hemacytometer chambers, observed at low (×100) power.

Tritium-labeled thymidine was prepared by catalytic exchange with CH_3COOH^3 and purified by column chromatography. It was homogeneous as judged by recrystallization with carrier thymidine and had a specific activity of approximately 500 mc/mole or about 2 mc/mg.

Incubation of HeLa cells for 24 hours with 2.5 μc/ml (about 1.25 μg/ml) of H^3TDR results in excellent labeling of the nucleus, as shown by autoradiography. Under these conditions, more than 100 silver grains appear over each nucleus after exposure of the film for 24 hours.

In early experiments testing the efficiency of uptake of H^3TDR, an apparent inhibition of growth of cells containing the labeled material was noted. To test the validity of this observation, several subsequent experiments were performed. Although alterations in design of the experiment were made in order to test other parameters, each included a test of the effect of H^3TDR, in various concentrations, in the medium on the growth of the HeLa cells. In all experiments the cells were allowed to attach to the glass for 3 days in the standard medium, which was then removed and replaced by the same medium containing the H^3TDR. After 24 hours at 37°C, this medium was removed and replaced again by standard medium, and growth was allowed to continue for 48 more hours. The cells were then removed from the glass by incubating with trypsin, and total cell counts were made on triplicate samples (with the exception of experiment 2, in which six replications per treatment were used). Finally, an experiment was also performed to test the effect of chronic irradiation from tritium oxide (H^3_2O) in the medium. The results are summarized in Table 1.

The most important point to be noted is that, in all cases, the inclusion of H^3TDR in the medium resulted in inhibition of growth, except where carrier thymidine was added. The fact that the latter material reversed the action of H^3TDR demonstrates that the inhibition was a result of the intracellular incorporation of tritium and not some toxic contaminant. It is also of interest that each of the three levels of H^3TDR resulted in approximately the same extent of inhibition (the difference between the means of experiments 2A and 2B is not significant). These results indicate that thymidine concentration in the medium, at the levels used, exceeds the amount which the cells can accumulate and that the effect is a function of specific activity, not concentration. This is consistent with autoradiographic observations where it appears that, in concentrations between 0.5 and 5 μc/ml, the amount of H^3TDR incorporated per cell is roughly the same.

The results of the last experiment illustrate that growth inhibition by H^3_2O is accomplished only by concentrations in the medium of 5 mc/ml or the order of 1000 times that at which H^3TDR exhibits its effects. However, if the difference in volumes which are under actual irradiation is considered, the dose to the nucleus is probably of about the same magnitude in both cases. To illustrate, in the experiment with 5 μc of H^3TDR per milliliter, only about 20 percent (1

Table 1. Inhibition of HeLa cell growth by inclusion of tritium in culture medium. In all experiments with thymidine, the cells were grown for 24 hours in the presence of H^3TDR, and then in cold medium for an additional 48 hours. In the experiment with H^3_2O, the cells were merely grown for 48 hours in the tritium-containing medium. Each control medium was same as treated except that the tritiated thymidine was replaced by an equal amount of nonlabeled thymidine.

Expt. No.	H^3 vehicle	Medium (μc/ml)	Total cell counts* at end of expt. (cell/μl)		
			Control	Treated	p†
1	Thymidine	2.5	170 ± 12	90 ± 17	< .01
2A	Thymidine	2.5	662 ± 38	357 ± 19	< .01
2B	Thymidine	1.25	662 ± 38	406 ± 43	< .01
3A	Thymidine	5.0	1333 ± 191	747 ± 60	< .05
3B	Thymidine + carrier‡	5.0	1333 ± 191	1124 ± 51	> .3
4A	H^3_2O	5000	696 ± 15	328 ± 65	< .01
4B	H^3_2O	1000	696 ± 15	633 ± 68	> .3

* Indicated values are means ± standard errors.
† Significance level for difference between means of treated and controls.
‡ 17.5 μg of additional unlabeled thymidine per milliliter.

μc) was incorporated. There were about 2.5×10^5 cells per milliliter at zero time in this experiment. The HeLa cell nucleus does not appear to exceed 15 μ in diameter, so that the maximum volume of the nucleus is 2×10^3 μ^3. Therefore, the concentration of H^3TDR would be

$$1\ \mu c/2 \times 10^3 \times 2.5 \times 10^5\ \mu^3$$

or about 2×10^{-9} $\mu c/\mu^3$. In the experiment with H^3_2O, the concentration was 5 mc/ml, or 5×10^{-9} $\mu c/\mu^3$. This twofold difference is less than the error in our estimate of nuclear volumes. Therefore, the inhibition in this case, as in the case of external irradiation, can be reasonably attributed to nuclear damage caused by the β-radiation, and it is unnecessary to postulate special "hot atom" effects as used to explain the toxicity of P^{32} incorporated into the DNA of bacteriophage (*5*) or bacteria (*6*). It is of interest that 5 mc/ml of H^3_2O (the concentration of H^3_2O required for growth inhibition of HeLa cells) was found to reduce the mitotic index in regenerating liver by about 50 per cent (*7*). Water containing 5 mc/ml of tritium irradiates itself at the rate of 65 rep/hr.

These results imply upper limits of labeling for tracer applications of tritium. At the moment these limitations do not appear to be too stringent, for cells labeled at an inhibitory level reduce hundreds of silver grains per week, whereas a few grains per month are quite satisfactory for autoradiography. Nevertheless, the fact that the other radiobiological phenomena, such as chromosome breaks, mutations, and cancerogenesis, occur at much lower radiation levels than needed for gross inhibition of cell division should be borne in mind when interpreting results. The fact that tritium-labeled thymidine concentrates radioactivity in radiosensitive regions where it may remain a long time also demands extra caution in its handling. The radiotherapeutic possibilities of tritium incorporated within the nucleus are being further pursued (*8, 9*).

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8. This research was supported by the U.S. Atomic Energy Commission.
9. We acknowledge the excellent technical assistance of Bernice Giauque.

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Formula for Inferring Atmospheric Density from the Motion of Artificial Earth Satellites

A simple formula for inferring air density from satellite motion may be useful to workers in the field (*1*). To find such a formula, the density, ρ, is approximated near perigee by $\rho_\pi e^{-Kz}$, where ρ_π is the density at perigee, z is the altitude above perigee, and K is the logarithmic gradient of density

$$K = -2.3026\ (d/dz)\ \log_{10}\rho \qquad (1)$$

at perigee. An integral that appears in a basic analysis (*2*) of orbital effects of drag is then evaluated in terms of Bessel functions $I_0(Kae)$ and $I_1(Kae)$, where a is the orbital mean distance and e the eccentricity. The Bessel functions are expanded asymptotically, and there finally results an approximate formula by which ρ_π may be inferred from the rate of change, $\dot{P}$, of the period P and from certain other data. In practical units the formula is

$$\rho_\pi = -4.826 \times 10^{-15} \times \dot{P}\,\frac{m}{AC_D}\,\frac{c^{1/2}}{af(c,e)}\ \mathrm{g/cm^3} \qquad (2)$$

where $\dot{P}$ is in seconds per day; m is the satellite's mass in grams; A is the satellite's area in square centimeters projected on a plane normal to the direction of motion; C_D is the dimensionless aerodynamic drag coefficient, believed to be approximately 2; a is measured in earth radii of 6378 km; c is 6378 Kae if z, in Eq. 1, is in kilometers; and $f(c,e)$ is a function given approximately by

$$f(c,e) = 1 + 2e + (3e^2/2) + \frac{1 - 6e - 10.5e^2}{8c} + \frac{9 + 30e + 85.5e^2}{128c^2} + \ldots \qquad (3)$$

The first two terms alone in Eq. 3 appear to furnish the function to within an accuracy of about 10 percent, and the whole expression, to within a few percent, if e lies between 0.02 and 0.20 and K exceeds 0.01. The mean distance a may be inferred from P, and e and ae from a and r_π, the geocentric perigee distance in earth radii, by the equations:

$$a = (P/84^m.49)^{2/3}$$
$$e = 1 - r_\pi/a$$
$$ae = a - r_\pi$$

The numerical coefficient in Eq. 2 is the reciprocal of $3(\pi/2)^{1/2}$ times the number of seconds in a day times the earth's radius in centimeters. An average value of the A of a nonspherical satellite should be used in Eq. 2, and if all orientations are equally frequent and the satellite is convex, its average A is one-fourth of its total superficial area. The value of K, somewhat dependent on perigee height, can be approximated by applying Eq. 1 somewhat above perigee to a model atmosphere like the ARDC (*3*). Alternatively, K may be determined without reference to assumed models by applying Eq. 2 to two or more satellites with different perigee heights and adjusting K until it is consistent with the resulting perigee densities.

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1. I am indebted to Dr. G. F. Schilling for pointing out the utility of a simple, approximate, formula.
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14 March 1958