Oogonia are not found in the parenchyma lobes; rather, they are consistently located around the periphery of the ovule, about 1 mm or less beneath the surface.

Albugo (Fig. 1b) is the infective agent responsible for white rust disease of crucifers. The symptoms are hypertrophy and hyperplasia, resulting in enlarged and distorted organs, particularly flower parts. These symptoms are the same as those exhibited in *Nucellangium*. The fossil oogonia not only are morphologically similar to *Albugo* but apparently produced the same symptoms in the host plant.

Fossil oogonia are spherical (90 to 100 μm in diameter) and usually have darker, thicker walls than the surrounding integumental parenchyma (Fig. 1d). Some have a dark central mass surrounded by a lighter, less dense zone (Fig. 1, f and g). These regions correspond to the egg (oosphere) and peripheral periplasm in *Albugo*. Some appear immature (before delimitation of the egg) and some older, having developed a reticulate structure on the surface of the maturing egg. None show a thick-walled oospore stage. Oogonia may be contiguous with one another or isolated among parenchyma cells (Fig. 1d). In some areas the integument of the ovule is disintegrated and oogonia are found free in the interior of the ovule or in the coal ball matrix outside the ovule. They sometimes occur in pairs (Fig. 1c). Two of the hundred or more oogonia in the ovule contain two to four spherical inclusions (oospheres?) (Fig. 1e).

Antheridium-like structures are in contact with some oogonia (Fig. 1g) but it is possible that these are only fortuitous juxtapositions of adjacent parenchyma cells or partial sections of adjacent oogonia. The largest such structure is shown in Fig. 1g; others are smaller. We have been unable to find fertilization tubes extending inward to the egg.

There are, of course, differences between the fossil and *Albugo* oogonia, one being the larger size of the fossil forms. Nevertheless, the close morphological similarity, together with the symptoms produced in the host plant organs, argues strongly for a close (congeneric) taxonomic relationship between the fossil and extant fungi. If so, this is the first report of *Albugo* from the fossil record.

Nucellangium is undoubtedly a gymnosperm ovule (we have evidence that it was borne on the Paleozoic gymnosperm *Cordaites*) and is now extinct. Extinction of the host necessitated the adaptation of the parasite to a new host group, a transition from a gymnosperm to angiosperms in this case. If our conclusion about the taxonomic affinity of the fossil oogonia (and the parent plant of *Nucellangium*) is correct, it should be possible to find other stages in the life cycle of the fungus on *Cordaites* leaves and stems.

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References and Notes

1. H. N. Andrews, *Ann. Mo. Bot. Gard.* **36**, 479 (1949).
2. We thank D. P. Rodgers and V. K. Howe for their comments on the identity of the fossil oogonia.

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High Speed Scintillation Autoradiography

Abstract. *Impregnation of nuclear track emulsion with liquid scintillator and exposure at –85°C allows rapid autoradiographic labeling. With tritiated thymidine of high specific activity (40 to 60 curies per millimole), exposure time can be shortened to 20 to 60 minutes, allowing complete sample processing within 4 hours. In experiments requiring isotopes with low incorporation rates or low specific activity, exposure time can be shortened from months to several days.*

The time required for standard autoradiography has limited many potential clinical and research applications of the technique. Although in vitro cell cycle analysis could provide useful information for planning chemotherapy for patients with acute leukemia (*1*) or for monitoring response to chemotherapy in patients with solid tumors (*2*), determination of the tritiated thymidine labeling index (percentage of cells going through scheduled DNA synthesis) with conventional autoradiography (ARG) requires at least 6 days to complete (*3*). For other applications in which uptake of tritiated thymidine is low or in which low specific activity is required, or both, ARG exposure times of 6 to 8 months are necessary.

Recent modifications of standard ARG have included the use of tritiated thymidine of higher specific activity (6 c/mmole), which gives results in 24 to 48 hours (*4*). A preliminary report (*5*) has also indicated that the use of a scintillator along with tritiated thymidine of low specific activity can also shorten the process. Using tritiated thymidine of high specific activity (40 to 60 c/mmole), a liquid scintillator, and a low temperature (–85°C) for emulsion exposure, we have developed techniques for very high speed scintillation autoradiography (HSARG). With these techniques, exposure times can be shortened to 20 minutes, and fresh blood, bone marrow, and tumor cell samples can be processed and stained to provide tritiated thymidine labeling results within 4 to 5 hours.

Heparinized cell suspensions (0.5×10^6 to 1.0×10^6 cells per milliliter) are incubated for 1 hour at 37°C with the high specific activity (40 to 60 c/mmole) tritiated thymidine (isotope dose, 5.0 μc per milliliter of cell suspension). (See discussion of effect of isotope dose, below.) Cytocentrifuge smears are then made on gelatin-coated slides and fixed with methanol. In the darkroom the slides are then dipped for 10 seconds into Kodak NTB_3 nuclear track emulsion at 42°C. After drying for 1 hour at room temperature (22°C), the slides are dipped for 10 seconds into the scintillator solution (22°C). The scintillator consists of 35 g of 2,5-diphenyloxazole (PPO) and 100 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl-POPOP) dissolved in 500 ml of dioxane. The scintillator-impregnated emulsion is exposed in the dark for 20 to 60 minutes at –85°C. (See discussion of temperature effect, below.) Slides are then developed at 17°C (*3, 6*). The cytocentrifuge smears are stained through the emulsion with Giemsa stain (*7*).

Multiple experiments were performed with normal, Hypaque-Ficoll-separated (*8*), phytohemagglutinin-stimulated human lymphocytes as a convenient source of cells with a high proportion undergoing rapid DNA synthesis. Five hundred cells were counted per slide, and experiments were done in duplicate or triplicate. Analysis of duplicate samples revealed that a twofold difference in labeling index (percentage) was statistically significant ($P < .05$).

The activation of silver crystals in the photographic emulsion used in autoradiography is dependent upon the number and energy of the beta emissions penetrating the emulsion. With standard ARG the level of beta emission is relatively low. However, with an isotope of very high specific activity (such as the tritiated thymidine used in our studies), there are many more beta emissions per molecule of incorporated isotope. When, in addition, the emulsion is impregnated with scintillator, photons are released as the electrons (beta particles) pass through the scintillator, and this activates even more silver crystals in the emulsion (Fig. 1). Increased sensitivity is accompanied by a slight increase in the scatter of emissions.

In studies with high specific activity (40 to 60 c/mmole) tritriated thymidine the effect of scintillator was tested. Slides were exposed for periods ranging from 10 minutes to 24 hours, with (HSARG) or without (HARG) scintillator. With HSARG using low doses of isotope (0.5 to 1.0 μc per milliliter of cell suspension), close to maximal labeling was reached in 5 to 8 hours; approximately 75 percent of maximal labeling was achieved within 1 hour. Consistently more rapid labeling was obtained when the scintillator was used ($P < .05$ for the 1- and 5-hour time points). When emulsion exposure time was extended to 6 days, the number of cells eventually labeled with the use of HSARG, HARG, and conventional ARG (tritiated thymidine specific activity, 2 c/mmole) was the same and not significantly different from the 5-hour results obtained with HSARG.

However, the number of grains per cell was greatly enhanced by the scintillator. For example, at 5 hours with HSARG, 50 percent of cells had more than 15 grains per nucleus. With HARG, 20 percent of cells had more than 15 grains per nucleus. With standard ARG, labeling was negligible.

Background labeling with scintillator was remarkably low, even when slides were exposed for 6 days. For exposure times of up to 24 hours the mean background count was 38 grains per 100 cells, with a maximum of 65 grains per 100 cells. By Poisson distribution analysis (*9*), there is only a 0.05 percent chance that background could account for more than four grains per cell. Therefore, cells with five or more grains per nucleus were interpreted as being labeled in this and subsequent experiments in which background labeling was comparable or lower.

In animal experiments dealing with hormone distribution and nerve regeneration requiring low specific activity (2 c/mmole) tritiated isotope (1,25-dihydroxycholecalciferol and thymidine) we have been using scintillator with the emulsion and have been able to shorten the exposure time from 6 months to 14 days or less.

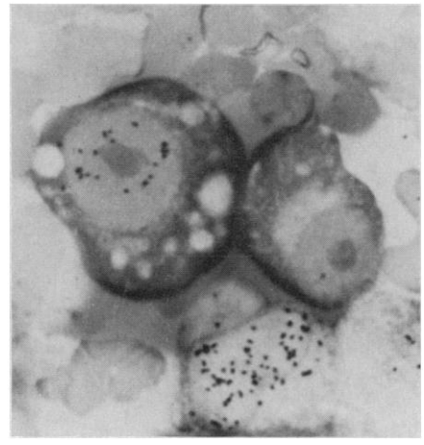

Fig. 1. Myeloma cell (upper left) labeled with HSARG technique (tritiated thymidine specific activity, 40 to 60 c/mmole; isotope dose, 5.0 μc per milliliter of cell suspension; emulsion exposure, 1 hour at –85°C with scintillator). (Oil immersion; reproduced at × 300). Note extremely low background.

Temperature significantly affects fluorography on thin-layer chromatography with tritium compounds. It has been suggested that at low temperatures the vibrational freedom of molecules is "frozen in" and energy which otherwise would be lost in random motion goes into photon emission from the scintillator (*10*). However, as pointed out by Randerath (*10*), this can account for only part of the observed effects. In testing the effects of temperatures between 22°C and –196°C on HSARG, we found that early labeling was enhanced by allowing emulsion exposure at low temperatures between –20°C and –85°C (Table 1). Further cooling produced suboptimal labeling and tended to crack the emulsion. With an isotope dose of 0.5 μc per milliliter of cell suspension, the optimal temperature for labeling was –85°C. At higher doses ($\geq$ 5.0 μc per milliliter of cell suspension) early labeling was slightly (but not significantly) affected by exposure at lower temperatures (down to –196°C). Although this result may appear surprising, it has also been observed in fluorography. The relationship between emulsion characteristics, isotope dose, and temperature has been reviewed (*10*).

The effects of scintillator and temperature were most evident with low levels of isotope incorporation and beta emission (for example, with the 0.5 μc/ml dose; Table 1). By increasing the dose of tritiated thymidine incubated with the cell suspension in vitro to 5 to 10 μc/ml, rapid maximal labeling could be readily accomplished with exposure times of 20 to 60 minutes even at room temperature. Higher isotope doses (up to 100 μc/ml) did not increase labeling. Although grain counts per cell increased beyond 20 to 60 minutes of emulsion exposure, there was only a negligible increase in the number of labeled cells, even when the exposure time was extended to 6 days.

Since one major reason for developing this technique was for clinical applications of autoradiography, we studied a series of patients with leukemia, multiple myeloma, or malignant effusions with HSARG (isotope dose, 0.5 to 10 μc per milliliter of cell suspension) and standard ARG (*3*). Analysis of duplicate samples for each method revealed that a twofold difference in labeling index was statistically significant ($P < .05$). In 52 observations in 40 patients, the mean ratio of labeling with HSARG to labeling with standard ARG was 1.4. Thus, results obtained with the two methods were comparable. With the HSARG method the median tritiated thymidine labeling index of tumor cells for 11 untreated patients with multiple myeloma was 4 percent and for 8 patients in remission it was 16 percent. These data were entirely compatible with our own prior ARG data for patients with multiple myeloma (*11*).

Thus, our technique has several advantages over conventional autoradiography. In circumstances in which intermediate or high doses of high specific activity (40 to 60 c/mmole) isotopes can be used (with resulting high levels of beta emission), emulsion exposure time can be shortened to as little as 20 minutes with no loss in sensitivity, and labeling index results can be obtained within 4 to 5 hours. This dramatic decrease in time required for sample processing is of obvious clinical significance, since knowledge of the tumor growth kinetics of a particular patient can be critical in decisions concerning proper cytotoxic therapy.

For autoradiography in circumstances in which uptake of isotope (particularly tritium) is low and/or low specific activity is required (with resulting low levels of beta emission per cell), the scintillator

Table 1. Effect of temperature upon rate of labeling of phytohemagglutinin-stimulated lymphocytes with two different concentrations of high specific activity (40 to 60 c/mmole) tritiated thymidine.

Emulsion exposure time*	Labeling index (%)†			
	0.5 μc per milliliter of cell suspension		10 μc per milliliter of cell suspension	
	22°C	–85°C	22°C	–85°C
20 minutes	3‡	10	29	22
1 hour	8‡	18	31	37
5 hours	19	26	30	35
24 hours	28	30	35	37

*Impregnated with scintillator. †Percentage of cells significantly labeled (five grains or more per cell). Mean values; 500 cells per slide counted; three slides per experiment. ‡Temperature effect statistically significant ($P < .05$) (that is, twofold or more difference in labeling index percentage; see text).

greatly enhances *early* significant labeling (five or more grains per cell). This effect can be further improved by emulsion exposure at low temperatures (–85°C was optimal in our system) (Table 1). Previously, such experiments have required prolonged exposure times (for example, 6 to 8 months); with our technique results can be obtained in 14 days or less. There are additional potential applications in thin-layer chromatography, paper chromatography, and electron microscopy (*10, 12*).

Because HSARG allows careful analysis of mixed cell populations it has an advantage over other methods of rapid cell cycle analysis (*13*). With Giemsa staining, labeling can be related to morphology, and DNA synthesis rates can also be assessed (*14*). (An example of labeling in a mixed cell population is shown in Fig. 1. Note extremely low background.)

Thus, HSARG provides a rapid, reliable technique which should find wide clinical and research application.

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References and Notes

1. J. S. Hart, E. J. Freireich, E. Frei, III, *Proc. Am. Assoc. Cancer Res.* **15**, 73 (abstr.) (1974).
2. W. K. Murphy *et al.*, *Cancer Res.* **35**, 1438 (1975).
3. H. Coons, A. Norman, A. M. Nahum, *Cancer* **19**, 1200 (1966).
4. R. B. Livingston, U. Ambus, S. L. George, E. J Freireich, J. S. Hart, *Cancer Res.* **34**, 1376 (1974).
5. G. S. Panayi and W. A. Neill, *J. Immunol. Methods* **2**, 115 (1972). In our experience, this technique did not yield consistent results, and further modifications were necessary; see (*6*).
6. The combination of PPO, dimethyl-POPOP, and dioxane was selected after several other combinations were tested. Scintillator could not be premixed with emulsion because this resulted in an opaque gel. Addition of naphthalene (10 g/liter) to the scintillator resulted in significant improvement in the rate of grain development. However, a dense precipitate formed on the emulsion. This precipitate could be removed during processing by dipping the slides into methanol or toluene after emulsion development. If energy emission is very low, this can be of use. Tritiated thymidine was obtained from New England Nuclear Corp., Boston, Mass., NET-027Z; specific activity, 40 to 60 c/mmole. Shandon cytocentrifuge, Shandon Southern Instruments, Sewickley, Pa. NTB_3 nuclear track emulsion (obtained from Eastman Kodak Co., Rochester, N.Y.) is mixed with an equal volume of distilled water and 1.0 ml of Tween 80. PPO and dimethyl-POPOP were obtained from Amersham/Searle, Arlington Heights, Ill. Dioxane (stabilized $C_4H_8O_2$) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Kodak D-19 developer, catalog No. 146-4593; Kodak fixer, catalog No. 197-1746 (Eastman Kodak Co.).
7. The Giemsa stain must be carefully filtered and the *p*H of the citrate buffers checked. We use *p*H 5.75 for 30 seconds followed by *p*H 5.40 for 30 seconds. Giemsa blood stain was obtained from Fisher Scientific Co., G-146; 734319.
8. A. Böyum, *Scand. J. Clin. Lab. Invest.* **21** (Suppl. 97), 77 (1968).
9. W. Feller, *An Introduction to Probability Theory and Its Applications* (Wiley, New York, 1957), vol. 1, p. 146.
10. U. Lüthi and P. G. Waser, *Nature (Lond.)* **205**, 1190 (1965); E. Schram, *Organic Scintillation Detectors* (American Elsevier, New York, 1963); K. Randerath, *Anal. Biochem.* **34**, 188 (1970).
11. S. E. Salmon, *Blood* **45**, 119 (1975).
12. A. T. Wilson, *Nature (Lond.)* **182**, 524 (1958); H. A. Fischer, H. Korr, H. Thiele, G. Werner, *Naturwissenschaften* **58**, 101 (1971).
13. H. A. Crissman and R. A. Tobey, *Science* **184**, 1297 (1974); P. F. Mullaney and W. T. West, *J. Phys. E Sci. Instrum.* **6**, 1006 (1973).
14. S. E. Shackney, S. S. Ford, A. B. Wittig, *Cancer Res.* **33**, 2726 (1973).

15. We thank Linda Vaught for her skilled technical assistance. This work was supported in part by PHS grants CA-14102 and CA-17094 from the National Cancer Institute, National Institutes of Health.

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Nongenetic Variability in Susceptibility to Oncogenesis

Abstract. *Genetically homogeneous mice varied in susceptibility to tumor induction by 3-methylcholanthrene. The early appearance of an induced tumor identified an animal of relatively great susceptibility to tumor induction as compared to other animals of the same genotype.*

Although inbreeding leads to genetic uniformity, it may sometimes result in phenotypic diversity. For example, among inbred multiparous BALB/c mice that carry the mammary tumor virus, approximately half of the animals exhibit the mammary tumor character; the other half do not. Most tumor systems show a similar, though often not so striking, degree of diversity. When a carcinogen is given to inbred mice, especially at near-threshold dosage, there is usually great variation among the animals in how fast they develop tumor and even in whether or not they develop tumor at all. The usual explanation of such diversity in genetically uniform animals is that the genotype is such that the character in question is close to the threshold of penetration. Uncontrollable, minor environmental variables can then determine the presence or absence of the phenotypic character.

In the case of the character, tumor, some of the diversity that is found among genetically uniform, inbred animals might be due to phenotypic variations that affect their susceptibility to tumor induction. Alternatively, tumors are often considered to result from random somatic mutations. Furthermore, there are of necessity small, uncontrolled variations in carcinogen application and the like. Thus, the presence or absence of tumor among genetically identical animals might reflect chance events associated with transformation rather than a predisposing phenotypic variation. The two explanations are not mutually exclusive.

I have attempted to answer the question of whether or not, in an induced tumor system, genetically uniform animals are equally at risk or, alternatively, whether some are more susceptible to tumor induction than others. The relative susceptibility of such animals can be tested, in principle, by determining whether or not multiple primary tumors are more frequent than would occur by chance. However, if multiple tumors are more frequent, the increased susceptibility might be the result rather than the cause of the first tumor. In other words, it would not be clear whether a relative hypersusceptibility was present prior to the appearance of the first tumor or had been caused in some way by that tumor. It is well known that tumor growth can alter mammalian physiology and perhaps influence future oncogenesis. In my work, the possible physiological alterations that might be caused by tumor growth per se were controlled by the use of transplanted tumors.

The mice used were (C57BL/6JNIcr × BALB/cAnNIcr) F_1 hybrid females approximately 2 months old. The animals were produced in the animal production facility of the Institute for Cancer Research (Fox Chase, Philadelphia); the parental strains are maintained by vigorous inbreeding and are routinely monitored for homozygosity by syngeneic skin grafting. Four pellets per mouse of 0.5 per-

Table 1. Number of pairs in which a tumor arose first at a remaining pellet in an experimental (tumor and pellet excision) or a control (pellet excision) animal.

Experiment	Origin of first tumor	Tumor and pellet excision animal	Pellet excision animal	*P* value (sign test)
1	Pellet induced	24	7	.002
2	Pellet induced	19	7	.014
Average latent period*		23.6 ± 12.7	34 ± 23.2	
3	Trocar implantation	18	9	.061
4	Trocar implantation	8	10	
Average latent period*		88.8 ± 19.9	90.26 ± 18.1	

*Latent period is the interval in days between excision of the first tumor and of the pellet and the subsequent appearance of a tumor at one of the remaining pellets.