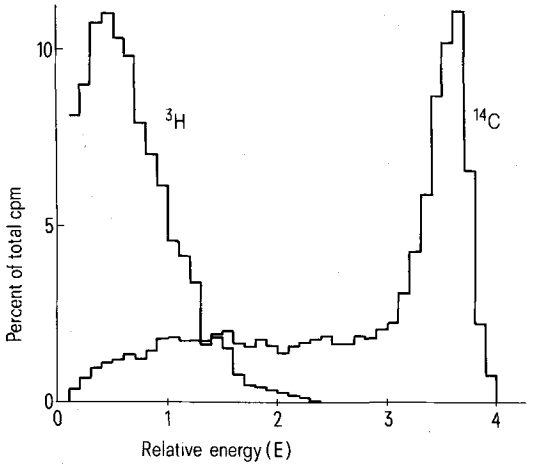
ml Unisolve 294 (Kock-Light Laboratories Ltd., England) in polyethylene vials (W. Zinsser, Frankfurt, Germany) and counted in an automatic beta-gamma spectrometer (Nuclear Enterprise 8312) at room temperature.

As internal standards, 10 µl of [4-^{3}H] toluene (2.13 µCi/g) and [methyl-^{14}C] toluene (0.504 µCi/g) were used, giving 41,009 dpm and 9693 dpm, respectively. All radioactive isotopes were obtained from The Radiochemical Centre, Amersham, England. Spectra were recorded using a fixed window ($\Delta E = 0.1$), on 100 µl of standards added to 1 ml unlabelled tissue digest.



Energy spectra recorded for ^{3}H and ^{14}C in the emulsifying liquid scintillator Unisolve 294. Spectra were recorded in a Nuclear Enterprise 8312 automatic beta-gamma spectrometer, using a window, $\Delta E = 0.1$.

Results. Spectra obtained for ^{3}H and ^{14}C are shown in the figure. Tritium has a remarkably narrow range, being unrecognizible at $E > 2.4$, while ^{14}C has a peak at $E = 3.6$ as well as a plateau at lower energies. Thus, by appropriate choice of windows, ^{14}C can be counted without disturbance from ^{3}H. In the following, the settings given in the table were used.

Miscibility with waterphase: 10 ml Unisolve 294 was found to accept 0–0.1 ml and 0.6–2.0 ml of water, and 0–0.1 and 0.8–2.0 ml 1 M NaCl or tissue solute. Between 0.1 and 0.6–0.8 ml, the mixture becomes cloudy. This is in accordance with the data given by the manufacturer.

Efficiency expressed as percent recovery of internal standard is given in the table.

Storage: Background was negligible even at immediate counting. In the tissue samples, counting efficiency declined linearly with time, reading 80% of the initial value after 5 days at room temperature.

Discussion. The quenching by this method was found remarkably stable and reproducible. Thus, for routine use, quenching correction should be unnecessary. It also gives an unusually good separation between ^{3}H and ^{14}C, which is further improved by using rather narrow channels. Finally, DNA can be measured in the same solute, which implies that weighing errors, which may be troublesome in tissue samples, are avoided.

1 The financial support of the Norwegian Research Council for Science and the Humanities and the skillful technical assistance of Mr A. Vonheim are gratefully acknowledged.

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A simple method for obtaining high plating efficiencies with cultured insect cells

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Summary. A technique has been developed for obtaining absolute plating efficiencies as high as 79% for cultured *Trichoplusia ni* (TN-368) cells. The method involves the use of conditioned medium and MES, BES, or ACES buffers. Cell growth and morphology are not altered under these conditions.

Methods of obtaining high plating efficiencies with cultured mammalian cells are well established[2,3]. Similar techniques for cultured insect cells are not as well defined. Cloning procedures have been developed for insect cells, but these are not useful in plating experiments[4,5]. McIntosh and Rechtoris have devised a method permitting colony formation in soft agar with a plating efficiency of 1%[6]. While this method may be effective for cloning cells, the cell morphology appears to be altered and the method is not readily useful for survival studies. Richard-Molard and Ohanessian have utilized the 'feeder layer' method[3] to successfully clone *Drosophila* cells[7]. In this paper a method is presented for obtaining high absolute plating efficiencies (up to 79%) with *Trichoplusia ni* (TN-368) cells in liquid medium. This method is potentially useful for evaluating the survival of insect cells following various stresses such as exposure to UV, X-rays, or chemicals. A series of cell growth curves (prepared by hemocytometer counts) are compared to a cell survival curve (obtained by colony formation) following X-irradiation of the cells in order to demonstrate the significance of the colony formation technique.

Methods. The TN-368 cell line, derived from the cabbage looper, *Trichoplusia ni*, was used in this study[8]. 5 different variations of TNM-FH medium[9] were tested: 1. Regular TNM-FH (pH of about 6.2), 2. conditioned TNM-FH

Plating efficiencies of *T. ni* (TN-368) cells in modifications of TNM-FH medium

Medium	Plating efficiency (%)
	(n = 19)*
Regular TNM-FH	19.6 ± 1.4**
	(n = 18)
Conditioned TNM-FH	49.8 ± 2.3
	(n = 22)
Conditioned TNM-FH with MES***	57.9 ± 1.8
	(n = 23)
Conditioned TNM-FH with BES***	79.2 ± 2.0
	(n = 17)
Conditioned TNM-FH with ACES***	65.1 ± 2.6

* Total number of dishes from 3 experiments; ** 1 SE.; *** Initial pH adjusted to 6.0–6.2 with 1 N KOH.

(A 3.0 ml aliquot of cells was added to 27 ml of fresh TNM-FH to give a final density of about 2×10^5 cells/ml in a monolayer culture. The medium was collected 24 h later and replaced with 30 ml of fresh medium. The added medium was again removed and collected at 24 h before the cell population reached stationary growth. Medium acquired in this manner was pooled and filtered before use. The pH of the pooled medium was about 6.3.), 3. conditioned TNM-FH with 0.1 M 2-(N-morpholino) ethanesulfonic acid (MES), 4. conditioned TNM-FH with 0.1 M N,N-Bis (2 hydroxyethyl)-2-aminoethane sulfonic acid (BES), or 5. conditioned TNM-FH with 0.1 M N-(2-acetamido)-2-aminoethane sulfonic acid (ACES). The pH of each of the later 3 media formulations containing these buffers was adjusted to 6.0–6.2 with sterile 1 N KOH. An initial medium pH anywhere in the range between 6.0 and 6.3 yields about the same cellular growth rate.

Cells were grown in 30 ml polystyrene tissue culture flasks containing 5 ml of medium. While the cells were in exponential growth, aliquots were withdrawn and counted with a model Z coulter counter. The cells were diluted to a concentration of about 100 cells/0.25 ml in regular TNM-FH. This volume (0.25 ml) was added to 60 mm diameter plastic tissue culture dishes (Lux Scientific) containing 2.25 ml of each of the media to be tested. The dishes were incubated in a humidified incubator at 27 °C and not disturbed for 7 days.

The medium was gently poured from each dish and carefully replaced by 2 ml of a neutral red solution (stock solution of 1 mg/ml H_2O diluted 1:10 in Hanks' BSS containing 15 mg/ml glucose). After 45–60 min, the stain was gently poured off, dishes inverted, and colonies counted with the aid of a stereodissecting microscope. Alternatively, 2 ml of neutral red solution was sometimes added to the medium in the dishes and colonies counted 1 h later by placing the dishes (still containing medium) against a lighted background. The later method insures a minimal amount of disturbance to the colonies.

X-irradiations were performed with a Norelco model 150/300 constant potential X-ray unit (250 KVP, 10 mA, HVL = 10.4 mm Al) at a dose rate of about 340 R/min. Growth curves were prepared by subculturing cells into 30 ml polystyrene tissue culture flasks (Falcon), X-irradiating, and then making cell counts with a hemocytometer every 24 h. Further details concerning this procedure can be found elsewhere[10]. The survival curve was prepared by placing 100–125 cells into 2.5 ml of conditioned TNM-FH containing 0.1 M BES in 60 mm diameter polystyrene tissue culture dishes (Lux), X-irradiating, incubating the cells for 7 days, staining, and counting colonies of greater than 50 cells to determine plating efficiencies.

Results and discussion. Regular medium supports cell growth and colony formation to a limited extent but most of the cell colonies that are present do not attach tightly to the culture dish and are lost when the medium is poured off. Conditioned medium supports colony growth much better than fresh medium. Conditioned medium containing MES, BES, or ACES with the pH adjusted to 6.0–6.2 not only supports cell growth, but also enhances cell attachment. (The use of these buffers in unconditioned medium also increases colony formation nearly as well as in conditioned TNM-FH.) The observation of decreased cell movement and greater cell attachment at acid pH has been reported previously[11]. As shown in the table, reasonably high absolute plating efficiencies are obtained with conditioned medium that has been buffered with MES, BES, or ACES. In addition, cell morphology is not altered by the use of these media (figure 1).

The growth curves obtained by hemocytometer cell counts (figure 2, A) demonstrate that the insect cells undergo a dose-dependent division delay immediately following the X-ray treatment and that the maximum population density decreases with increasing dose[10], but cannot be used to determine cytotoxicity or that percentage of cells which survive each X-ray exposure. The survival curve based on colony formation (figure 2, B) does allow the determination of the surviving fraction of cells after a given X-ray exposure. Roper and Drewinko[12] have shown that colony formation is the only technique which gives a reliable estimate of cell survival. The development of the single-cell

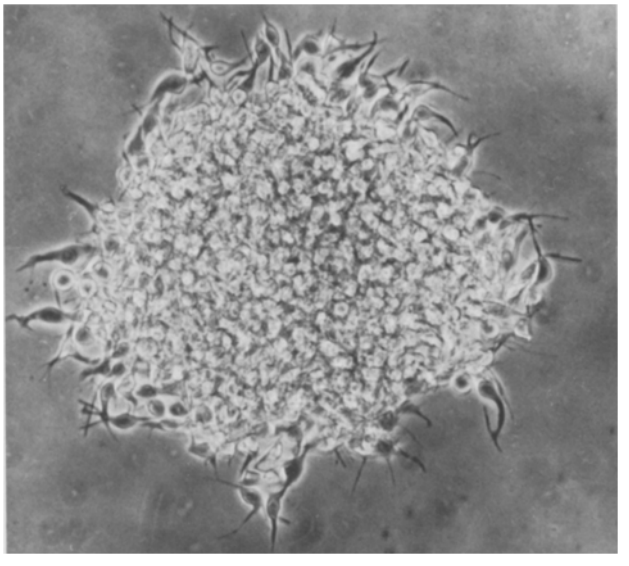

Fig. 1. TN-368 cell colony 7 days after plating in conditioned TNM-FH containing 0.1 M BES. Magnification × 130.

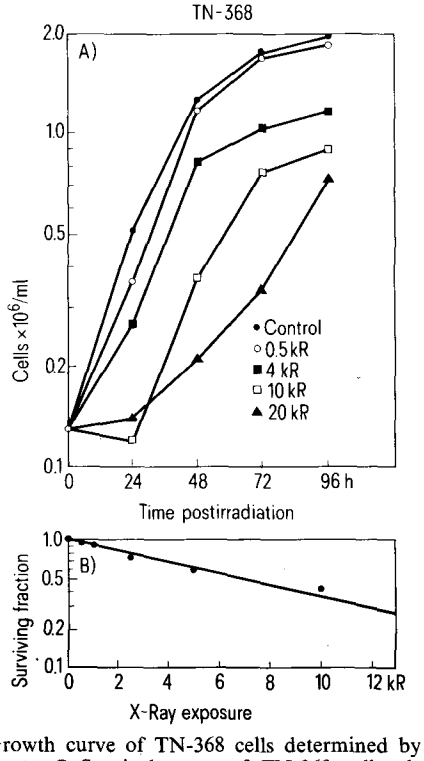

Fig. 2. *A* Growth curve of TN-368 cells determined by hemocytometer counts. *B* Survival curve of TN-368 cells obtained by colony formation technique.

plating technique for mammalian cells was essentially the beginning of quantitative biology using cultured mammalian cells, especially in radiobiology. Therefore, the availability of the presently described technique finally makes possible the same types of quantitative biological studies with cultured insect cells.

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Localization of sound producing animals using the arrival time differences of their signals at an array of microphones[1]

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Summary. This method provides a reliable means to determine distance between vocalizing individuals and their locations through a prolonged period of time without disturbance to the communication process by the presence of an observer. The accuracy of this method was tested by varying the arrival time differences on the microphone grid for known locations in a random fashion. This test shows that a vocalizing animal within an area of 4 ha surrounding the microphone array can be localized with an accuracy of ± 1 m. The accuracy decreases with the distance from the central area and as a function of the geometry of the array. The location of an individual can be determined based on 5 vocalizations given in sequence.

When studying vocalizations used in long distance communication, such as bird song, the location of the communicants and the distance between them are among the most important variables. Although the locations may be determined by personal inspection or in reference to a grid of markers, the necessary presence of the observer is likely to disturb the animals and to influence the communication process under investigation. Furthermore, it is difficult and expensive in terms of time and effort to collect such measurements throughout and extended period of time (days or months) in sufficient number to warrent a quantitative analysis.

As an alternative to such direct observations, an acoustical method for the localization of vocalizing animals which minimizes disturbances in their environment has been designed[2]. It is based on the measurement of arrival time differences of the animal's signals at an array of microphones. Although a similar system has been designed to locate a vocalizing whale in a 3-dimensional space[3], Schleidt's was the 1st attempt to design a system for studies of acoustical communication in ground dwelling animals. Here, an evaluation of the accuracy of the method is presented along with data samples obtained with the array at a study site in Maryland and a determination of the most efficient means of analyzing real data samples.

Materials and methods. The microphone array was used to locate male bobwhite quail, *Colinus virginianus*. The bobwhite call is well suited to test this method since it carries over a considerable distance (several 100 m), it is usually repeated many times from the same location allowing repeat measurements for replications, and particular features of the call are highly individualistic within a population so that specific males can be recognized reliably from sonagrams (figure 1).

4 microphones (Uher M517 with a protective cover and wind screen) were set up in an array at the Chesapeake Bay Center for Environmental Studies. 3 of the microphones were in a straight line, spaced 50 m apart; the 4th was placed on a line perpendicular to the line formed by the 1st 3 and 50 m from the central microphone. The microphones were each connected to an individually shielded, twisted pair of wires in cables which were laid on the ground to a barn approximately 150 m from the nearest microphone. The cable terminal contained the line transformers converting the balanced microphone lines to the grounded input of a 4 channel tape recorder (Sony TC-654-4). The recording was monitored through headphones.

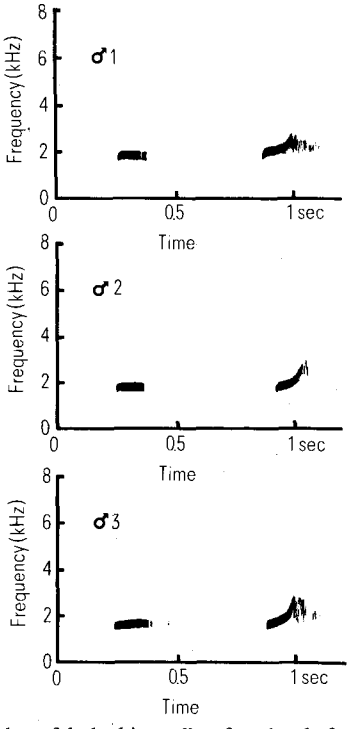

Fig. 1. Examples of bobwhite calls of males 1, 2, and 3 to show individual differences.