Modifiers of Carcinogenesis, T. J. Slaga, Ed. (Raven, New York, in press).
7. M. J. Bissell, C. Hatié, M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 348 (1979).
8. J. S. Brugge and R. L. Erikson, *Nature (London)* **269**, 346 (1977); A. F. Purchio, E. Erikson, J. S. Brugge, R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1567 (1978).
9. M. S. Collett and R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2021 (1978); A. D. Levinson, H. Oppermann, L. Levintow, H. E. Varmus, J. M. Bishop, *Cell* **15**, 561 (1978).
10. M. S. Collett, J. S. Brugge, R. L. Erikson, *Cell* **15**, 1363 (1978); J. S. Brugge, M. S. Collett, A. Siddiqui, B. Marczynska, F. Deinhardt, R. L. Erikson, *J. Virol.* **29**, 1196 (1979); H. Oppermann, A. D. Levinson, H. E. Varmus, L. Levintow, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1804 (1979); R. Karess, W. Hayward, H. Hanafusa, personal communication.
11. A. R. Goldberg and J. G. Krueger, unpublished observations.
12. M. Wigler and I. B. Weinstein, *Nature (London)* **259**, 232 (1976).
13. G. S. Martin, *ibid.* **227**, 1021 (1970).
14. H. zur Hausen, F. J. O'Neill, U. K. Freese, E. Hecker, *ibid.* **272**, 373 (1978); H. zur Hausen, G. W. Bornkamm, R. Schmidt, E. Hecker, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 782 (1979).
15. J. G. Krueger, E. Wang, A. R. Goldberg, *Virology* **101**, 25 (1980).
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Photoluminescent Thermometer Probes: Temperature Measurements in Microwave Fields

Abstract. *Based on luminescence, the photoluminescent thermometer can in principle function in electromagnetic fields without field coupling and perturbation. Several prototypal probes that have been constructed and tested demonstrate the feasibility of the concept. Temperature resolution of approximately 0.3°C has thus far been achieved. The probes are needle-shaped, sturdy and less than 1 millimeter in size. This thermometer system has excellent potential application in monitoring and controlling tissue temperatures when microwaves are used to induce hyperthermia for cancer treatment.*

Hyperthermia is currently being used either alone or in conjunction with radiation and chemotherapy for the treatment of cancer (*1*-*5*). Accurate thermal dosimetry is essential for the objective evaluation and control of this form of therapy (*6*). Electromagnetic (EM) fields (radiofrequency and microwave) and ultrasonic waves are considered practical for inducing local hyperthermia in tissue (*7*, *8*). However, there are unique problems associated with these methodologies with regard to thermometry. This is particularly true with EM fields. Conventional probes (thermocouples and thermistors) give erroneous results in EM fields because of field coupling and perturbations. Temperature measurements with these probes, therefore, require attention with regard to the probe-field orientation and, often, intermittent turning off of the EM fields during therapy while readings are taken (*9*, *10*). Considerable effort has been made to overcome this problem. Several new probes, using liquid crystals, birefringent crystals, optical etalon sensors, fluid viscosity, and so forth, have been explored for specific use in EM environments (*11*).

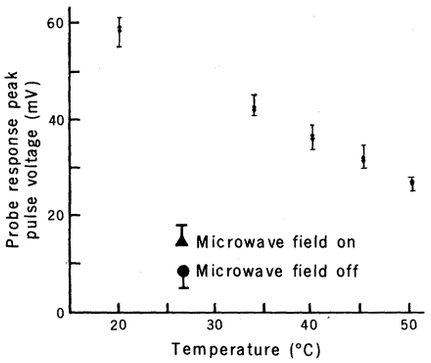

Fig. 1. Temperature response of the calcium sulfide PLT probe with and without 2450-MHz microwave field. Mean response and standard deviations are indicated. The microwave field was generated with a diathermy unit (Burdick MW/225) with a type E corner reflector applicator. The probe tip was positioned in the region of maximum transverse electric field (the transverse plane being that parallel to the plane of the applicator aperture). The probe was inside a glass tube (2 mm inside diameter) through which water from a temperature-controlled reservoir was pumped at a rate of 850 ml/min. The flow was used to keep the probe at the desired temperature while readings were taken. Temperature of the input and output water flow was monitored with thermocouples outside the microwave field region. The estimated power density of the microwave field at the probe was 200 mW/cm^2.

In this report, we introduce the concept of a photoluminescent thermometer (PLT) probe unperturbed by the presence of electromagnetic fields. Figure 1 shows a comparison of temperature measurements with the new PLT probe with and in the absence of 2450-MHz microwave field. Within the current experimental techniques, no perturbation of the probe response as a result of direct coupling with the EM fields can be detected.

The PLT probe is based on the heat-sensitive luminescent response of phosphorescent and fluorescent materials. When excited by light (frequency ν), electrons in these materials can be trapped in higher energy states. The trapped electrons subsequently return to ground states either by producing luminescence (emitting light of lower frequency ν') or by a competing nonradiative process (vibrational quenching). The quantum efficiency (ratio of emitted photons to absorbed photons) depends on the temperature of the material and can be expressed in the form:

$$\eta = [1 + (b/a)e^{-W/kT}]^{-1}$$

where a and b are the radiative and nonradiative transition probabilities, W is the activation energy associated with the nonradiative transition, k is Boltzmann's constant, and T is temperature in degrees Kelvin. In addition, the time dependence of the luminescent response is also temperature-dependent. In a simple single trap model, the lifetime, τ, associated with the rate of luminescent decay is given by $\tau = 1/(se^{-\epsilon/kT})$, where ϵ is the energy associated with the electron trap and s is the frequency at which an electron attempts to escape the trap. This decay time, or lifetime, is an intrinsic property of the luminescent material. Consequently, it allows the measurement of an intensive rather than extensive variable for the purpose of determining temperature. In practice, the temperature dependence of a given luminescent material can be complicated and difficult to pre-

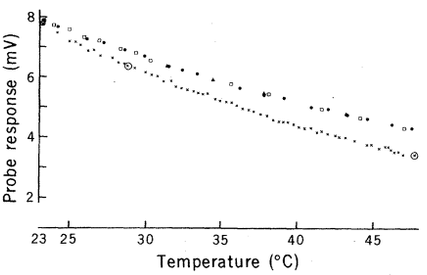

Fig. 2. Temperature calibration curves for prototype probes using different zinc cadmium sulfide phosphors. Xenon light pulses (0.75 msec) were used for excitation, and the time-integrated photoluminescent response was measured. For probe A, the curve of small x's represent individual measurements at the respective temperatures. Approximately 1 minute elapses between each measurement; ⊗ indicates several measurements at selected temperatures. The standard deviation (~ 1 percent) falls within the circle. For probe B, calibration data ● and ▲ were taken 1 and 2 weeks after data □, respectively. Each data point represents the mean of seven separate measurements taken at 1-minute intervals. The standard deviation for each data point is again approximately 1 percent. The resolution for probe A is ~ 0.3° at 40°C, and the resolution for probe B is ~ 0.4° at 40°C.

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dict theoretically. However, by experimentally determining the temperature dependence of either η or τ for a given material, they can be used for measuring temperature.

A practical probe consists of a photoluminescent material attached at the end of a fiber optic bundle. The bundle transmits both the input excitation signal to the photoluminescent material and the luminescent response to a photomultiplier tube light detector.

Figure 2 shows the time-integrated photoluminescent response from two prototype probes, A and B, at temperatures between 20° and 50°C. These probes have an external diameter of 1 mm and use different zinc-cadmium sulfide phosphors as the temperature-sensitive element. The PLT probes were excited with light pulses from a xenon flash. The response was detected with a photomultiplier. The exciting input light signal was separated from the luminescent output signal with appropriate spectral filters. The probe temperature was controlled to 0.1°C through the use of a calibrated water bath. Variations in the response for the system remained within ±1 percent for measurements taken over several weeks. Drift in the probe calibration, if any, remained below this noise level. With the present system, measurement noise of ±1 percent corresponds to a temperature resolution of approximately 0.3°C for probe A and 0.4°C for probe B. Variations in the input light pulse intensity are a major source of fluctuations in the probe response. Methods to eliminate or compensate for these variations are being developed, and we expect that resolution can be improved to 0.1°C. The temperature sensitivity and resolution can also be improved by an optimum choice of photoluminescent material, as is demonstrated by the increased resolution of probe A with respect to probe B (Fig. 2).

Temperature probes designed for use in hyperthermia treatments on humans should be less than 1 mm in size, structurally sturdy, thermally stable, and minimally perturbed in EM fields. These photoluminescent temperature probes demonstrate the potential for achieving the objectives.

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References and Notes

1. R. R. Hall, R. O. K. Schade, J. Swinney, *Br. Med. J.* **2**, 593 (1974).
2. R. R. Hall, V. Wandeara, J. M. Towler, J. R. Hindmarch, P. O. Byrne, *Br. J. Urol.* **48**, 603 (1976).
3. N. B. Hornback, R. E. Shupe, H. Shidnia, B. T. Joe, E. Sayoc, C. Marshall, *Cancer* **40**, 2854 (1977).
4. R. Cavaliere *et al.*, *ibid.* **20**, 1351 (1967).
5. J. S. Stehlin, paper presented at the International Symposium on Cancer Therapy by Hyperthermia and Radiation, Washington, D.C., 28 to 30 April 1975.
6. W. G. Conner, E. W. Gerner, R. C. Miller, M. L. Boone, *Radiology* **123**, 489 (1977).
7. A. W. Guy, in *Proceedings*, International Symposium on Cancer Therapy by Hyperthermia and Radiation, Washington, D.C., 28 to 30 April 1975, pp. 179-230.
8. G. M. Hahn, *Int. J. Radiat. Oncol. Biol. Phys.* **4**, 1117 (1978).
9. T. S. Sandhu, H. S. Kowal, R. J. R. Johnson, *ibid.*, p. 515.
10. J. Mendecki, E. Friedenthal, C. Botstein, F. Sterzer, R. Paglione, M. Nowogrodski, E. Beck, *ibid.*, p. 1095.
11. T. C. Cetas and W. G. Connor, *Med. Phys.* **5** (No. 2), 79 (1978).

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Vertebrate Cells Express Protozoan Antigen After Hybridization

Abstract. *Epimastigotes, the invertebrate host stage of* Trypanosoma cruzi, *the protozoan parasite causing Chagas' disease in man, were fused with vertebrate cells by using polyethylene glycol. Hybrid cells were selected on the basis of* T. cruzi *DNA complementation of biochemical deficiencies in the vertebrate cells. Some clones of the hybrid cells expressed* T. cruzi*-specific antigen. It might be possible to use selected antigens obtained from the hybrids as vaccines for immunodiagnosis or for elucidation of the pathogenesis of Chagas' disease.*

Trypanosoma cruzi, an obligate intracellular protozoan parasite, causes Chagas' disease, an incurable human disease prevalent in South and Central America. Attempts to produce an effective vaccine against infection have not been successful; dead organisms provide only partial protection against subsequent challenge (*1*). The use of avirulent organisms as living vaccines involves the risk of reversion to virulence and subsequent low-level, chronic infection. Selected antigens of the infectious agent would seem more appropriate for the preparation of vaccines. Unfortunately, the isolation and purification of selected antigens from *T. cruzi* as well as other parasites presents serious logistic problems. If somatic cell hybrid technology could be used for the production of parasite antigen, as it has been for the production of monoclonal antibodies (*2*), a major problem in antigen production might be overcome. In this report, we describe fusion between the epimastigote stage of *T. cruzi* and two different mammalian cell types and the production of hybrids that express parasite antigen.

Epimastigotes of the Tulahuen strain of *T. cruzi* were grown at 23°C in liver-infusion tryptose (LIT) medium (*3*) supplemented with 10 percent fetal calf serum and, per milliliter, 20 μg of hemin, 100 U of penicillin, and 100 μg of streptomycin. The vertebrate cells, BESM (*4, 5*), P3-x63Ag8 (*2*), and hybrids were maintained according to routine tissue culture procedures.

Two fusion methods were used to allow studies of both the morphological aspects of the fusion of *T. cruzi* and vertebrate cells and the production of *T. cruzi* antigen-expressing vertebrate cell clones.

A modification (*5*) of the pancake technique of O'Malley and Davidson (*6*) was used to study the morphology of *T. cruzi* and vertebrate cell fusion. This consisted of centrifuging epimastigotes onto monolayers of BESM cells, treating the cells with polyethylene glycol (PEG), and then observing the resultant products by light microscopy (*7*).

In Fig. 1, A and B, the flagellum of a parasite is shown extending from the surface of a BESM cell at approximately 20 minutes after treatment with the PEG solution. Flagella (one to five per heterokaryon) exhibited typical, sinusoidal motion for up to 3 to 4 hours, after which time they became less active and more difficult to observe. The anisotropic nature of the parasite's kinetoplast, an extranuclear DNA-containing organelle, enables it to be detected by polarized light (*8*). During the early stages of fusion, the kinetoplast was observed in the vertebrate cell cytoplasm either close to or some distance from the proximal end of the flagellum (Fig. 1, C and D). Neither the limiting membrane nor the nucleus of the parasite could be visualized.

To identify the parasite nucleus in the heterokaryon, we repeated the above experiments using [^{3}H]thymidine-labeled epimastigotes (*9*). An example of such an epimastigote is shown in Fig. 1E. Figure 1F shows a portion of a binucleate *T. cruzi* × BESM heterokaryon with a labeled parasite nucleus and an unlabeled BESM nucleus. Only the nucleus of the parasite could be observed. The remaining structures of the parasite could not be found.

Heterokaryons remained viable, as in-

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