BE 41

EFFECTS OF 1.07 ghz RF FIELDS ON MICROBIAL SYSTEMS

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ABSTRACT:

Much National discussion of the possibility of long term effects of RF radiation has occured for a number of years. We aver that any such long term event ultimately depends on producing transcription errors in some somatic cells, and thus have subjected microbial models of transcription systems to analysis.

Strain of E. coli C-600 lac II, with a defect in the ton A locus conferring resistance to bacteriophage T5 has been subjected to 1.07 ghz capacitor fed fields during log phase of growth while suspended in Brain Heart Infusion (BHI) with conductivity approximately 1 mho/meter . The system temperature was monitored with a Ramal Liquid Crystal Probe calibrated between each experimental run. Radiofrequency fields were generated by an MCL transmitter with frequency monitored by a Systron Donner frequency counter, input and reflected power monitored by Hewlett Packard 431C power meters coupled via Narda Microline 30db couplers.

It was found that E. coli C-600 became susceptible to infection with non-irradiated bacteriophage T5, as shown both by lysis of E. coli (nearly quantitatively) and production of virus particles, when virus was added immediately post exposure, indicating a membrane receptor site for T5 was uncovered during field application at 42°C.

This strain of E. coli was also more easily inactivated by RF fields than by temperature alone --- 90 minutes incubation at 48°C produced only a minimal reduction of viable C-600.

By incubating bacteriophage with E. coli during radiation it was shown that the bacteriophage was quantitatively inactivated by fields having little or no effect on E. coli. Field exposure of bacteriophage T5 suspended in BHI resulted in 80% inactivation in 2 minutes at 37°C. This bacteriophage is normally stable to temperatures in excess of 48°C.

The fields used in this study were at least $5Vcm^{-1}$ in solution. The data is consistent with data from whole mammalian cell radiations and effects produced in isolated calf thymus RNA.

SUMMARY:

Despite much national discussion of the possibility of long term effects of radiofrequency exposure for several years, there has been only a small amount of effort to define fields necessary to produce effects in known transcription systems which would allow quantitative description of the field amplitudes necessary to produce a biological response propagated in time.

Previous work with microbial systems utilized low amplitude fields, primarily at 2450 mhz, 10 mw cm^{-2}, and were routinely negative. The radiation system used in those experiments makes calculation of absorbed power problematical. Justesen utilized thermophyllus sp. to demonstrate that 2450 mhz radiation caused the appearance of peculiar membrane blebs and could show effects on growth curves. Szmygielski (Szmygielski, S.J., Luezak, M., Janiak, M., Kobus, M., Laskowska, B.: Proc 1976 American Meeting URSI, October 1976) found that herpes viral infection in mice could be attenuated or prevented by irradiation at 3 ghz, a phenomenon he related to attendant hyperthermia at the field amplitudes used.

We have chosen a strain of E.coli C-600 with a ton A locus defect inferring resistance to infection with bacteriophage T_1 and T_5 as a system to test the hypothesis that high amplitude short duration fields can perturb membrane receptor sites.

Bacteriophage T_5 is a virus like particle whose usual infection sequence requires attachment of its tail piece to the bacterial membrane surface, followed by injection of virus DNA into the bacterial cell. This virus DNA is very accurately known for genetic markers and seems characterized by several "nicks", or areas of single strand DNA. Once in the bacterium, a plasmid is formed initiating biochemical synthesis of complementary RNA and reproduction of the plasmid DNA codon.

E. coli C-600 was grown and radiated in Brain Heart Infusion (BHI) medium (Difco) which has a conductivity of approximately 1 mho $meter^{-1}$. While maintained at 37°C in a constant temperature incubator, bacteriophage T_5 was grown on a receptor strain of E. coli.

For radiation, log phase E.coli was suspended in 2 ml BHI at ~ 2 x 10^8 cells/ml contained in a small capped culture tube. Bacteriophage T5 was similarly prepared with approximately equal concentration of virus particles. Bacteriophage assay was performed by plating appropriate dilutions on a 'lawn' of receptor strain E. coli and counting areas of lysis.

Radiation was performed by placing the culture vial in a small capacitor while held in firm contact with a RAMAL Liquid crystal thermometer. The arrangement is depicted in fig (1). The capacitor is coupled to Narda Microline 30db couplers where forward and reflected power is sampled by HP 431C power meters. RF power is supplied by a Microwave Cavity Laboratories transmitter Model 15122 with 1-2 ghz insert Model 6051. Further calibration was performed by measurement of warming and cooling curves of saline solutions whose conductivity was known.

Figure (2) shows the results obtained by exposing E. coli C-600 to 0.245 wg^{-1} absorbed peak power. Individual samples were exposed for the indicated times, plated, and survival was estimated by colony forming events.

Fig (3) shows the results obtained when E. coli C-600 was mixed with equal particle numbers of bacteriophage T_5. No lysis of E.coli was observed, and no multiplication of bacteriophage T_5 occured (top left point in figure).

When E.coli was warmed with 0.245 wg^{-1} 1.07 ghz fields to the final temperature indicated inactivation occured. However, at 42°C, some 60% survival is indicated when radiated in this manner. When E.coli pretreated with 1.07 ghz in this manner was immediately challenged with untreated bacteriophage, nearly complete lysis occurred with production of approximately 200 virus particles per lysed cell. When, however bacteriophage T_5 was added to a suspension of E.coli and the mixed suspension subjected to 1.07 ghz fields, E. coli survival was similar to that found with field application alone, but no viable bacteriophage T_5 particles could be found. Note that the top right datum indicates relative thermal stability for E.coli C-600 for 90 minutes at 48°C when incubated in a warm air incubator in a similar culture tube, though replication is apparently inhibited until plate assays are performed.

Figure 4 shows results obtained when bacteriophage T5 was suspended in BHI medium and irradiated in the absence of E.coli, then plated on the 'receptor strain' lawn for phage assay.

Very short periods of exposure produced dramatic losses of phage replication in this assay system at temperatures shown by separate assay in a warm air oven not to affect this particular strain of bacteriophage T_5 (top right datum in fig 4).

DISCUSSION:

It is apparent that direct field effects can be produced in our microbial system when sufficient field strengths occur in the medium. We do not yet know precisely the lower field limits for detecting these alterations, nor do we yet know the precise relationships of field strength and temperature which allow demonstration of the two phenomena we have examined. It is apparent from the data that rearrangement of the surface of E.coli uncovering a usable bacteriophage receptor site occurs, and that phage replication proceeds reasonably normally once interaction with the bacterium is made possible. It is further evident, however, that replication of bacteriophage is extremely sensitive to field application, a phenomenon we will study much further.

While it is tempting to pass directly to effects on the DNA nucleotide sequence of the bacteriophage, some of which is in single strand form, attention must also be given other components of the suspending medium, since one way to inhibit phage growth would be to cover the foot plate with a media component. thus interferring with injection of phage DNA and RNA dependent replicases into the host bacterium. Quite obviously the event, whether related to either suggested mechanism, cannot be produced by simple hyperthermia alone, though our data do not adequately distinguish the extent of hyperthermia required before this field induced effect can be made evident.

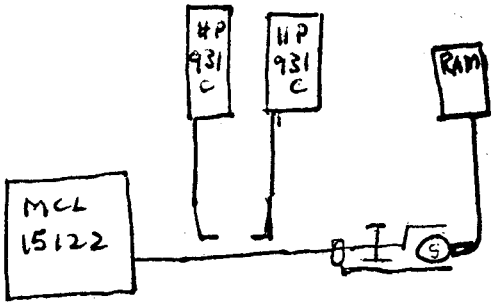

Fig 1

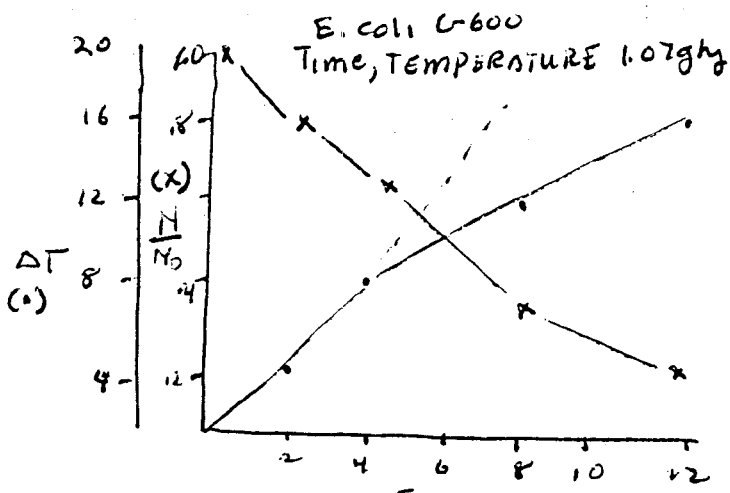

Fig 2

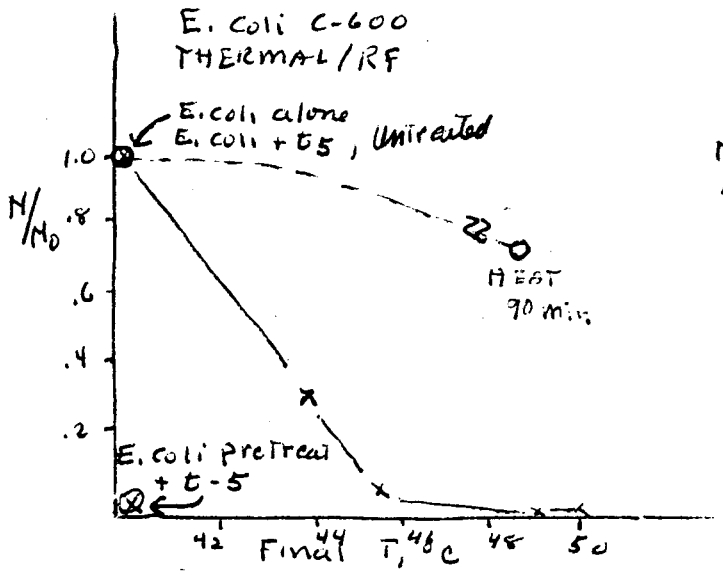

Fig 3

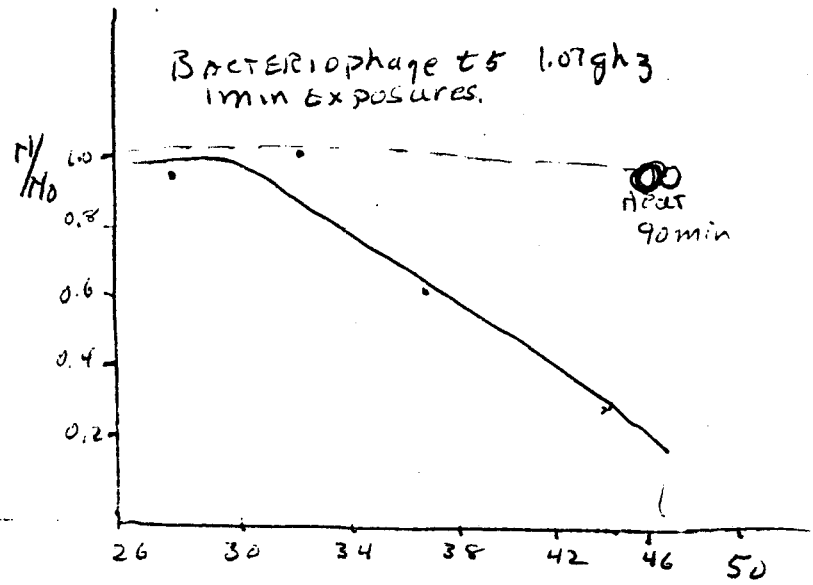

Fig 4