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Probe Activities
Annual Report
July 1, 1973 - June 30, 1974

by

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PROBE ACTIVITIES

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ABSTRACT

Numerous small scale experiments and feasibility studies were performed for the United States Department of Agriculture. Subjects include: measurement of 7000 R dosimeters for screwworm fly dosage monitoring, tracking of screwworm flies by the use of radar and lasers, development of temperature monitoring transmitters for ear canal and subdermal temperature monitoring, and identifying birds with fluorescent stains.

INTRODUCTION

During FY 1974 interest was expressed in a number of different unrelated topics. Discussions were held with interested staff members of the United States Department of Agriculture (USDA) and the Los Alamos Scientific Laboratory (LASL). Experiments were outlined and funds allocated. The purpose of these experiments was to perform either short-term complete experiments, or to conduct probes into a general area to determine the feasibility of more extensive work. This report describes the experiments in three different areas: screwworm flies, physiological monitoring, and bird tagging.

I. SCREWORM FLIES

Since the increased number of screwworm fly cases has taken place, a renewed interest in the details of this activity has occurred in the USDA staff. Personnel of the LASL have been asked to investigate: 1) do-

simeters for pupae irradiation, and 2) tracking of flies after release.

A. Dosimetry

A trip was made to the USDA, Agriculture Research Service's (ARS), Screwworm Research Laboratory, Mission, Texas, to view the facility and to become oriented with the problems that face the personnel there. It seemed desirable to assure that each canister of pupae had received a sterilizing dose of radiation. Two different approaches have been considered: 1) installing individual dosimeters on the canisters, and 2) instituting process control techniques to assure that the canisters had received the appropriate dose. The Mission facility is quite amenable to the use of dosimeters, but would require extensive modifications for process control techniques to be installed. Therefore, most of the effort has been spent on individual dosimeters.

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Experiments done by Harshaw Chemical Company personnel indicated that a Lithium Borate thermoluminescent dosimeter (TLD) might be suitable to determine irradiation levels of 7000 R. Some experiments performed at Mission indicated a high degree of reproducibility, but the Harshaw personnel were concerned that the dosimeters might not be able to cycle more than 50 times. This fact infers a dosimeter cost of 12 cents per irradiation. The USDA staff estimated that a dosimeter which would cycle 100 times would be acceptable and informed Harshaw of this. Since the dosimeters had never been used in the proposed manner, Harshaw personnel suggested that LASL perform an experiment to evaluate them for the USDA. Such an experiment is being carried out and the incomplete results are reported here.

Approximately 40 cycles have been completed on 100 cards. The following observations have been made:

1. The annealing of radiation damage with time is more pronounced than anticipated by the Harshaw representatives. However, this would not be serious in the screwworm fly program where reading would immediately follow irradiation.
2. The amount of light given off after 7000 R of dose increased by about 50% between the first irradiations and the 37th.
3. All of the individual dosimeters appear to behave in a similar manner so their responses can be predicted.
4. The automatic card reading machine is not reliable enough at present for the Mission, Texas, operation.
5. One card failed on the 37th irradiation.

Figure 1 shows the response of four TLD's in two cards. The light output, which is proportional to current integration from the photomultiplier, is shown as a function of irradiation cycles. Each cycle consists of 7000 R of 250 keV x rays, followed by a reading of the amount of light given off during heating. A 10 s heating cycle was

Fig. 1. The response of four TLD's on two cards. A change in calibration versus the number of irradiation cycles is observed, as well as annealing of radiation damage after irradiation, and before reading.

employed for most of the readings. The scatter in the data prior to run 20 can be attributed to a delay between irradiation and reading, in which the radiation damage appears to be annealed. After run 23, careful attention was given to monitoring the time between irradiation and reading. Where significant delays occurred, these are indicated by the time in minutes. Fifty cards were stacked together for each irradiation. Small variations in dose were observed within the stack.

The experiments are scheduled for completion about August 15, and it will take at least one month to evaluate the data completely. It is obvious that the Harshaw system is not ideal for the irradiation dosimetry, but it may be possible to use it by working around the faults in the system.

B. Fly Tracking

It has been reported by the Mission staff that the only way they have been able to track the movement of plant produced flies on an individual basis is with "day glow" dye powders dusted on the flies and then making the flies visible with a portable UV light source. This was quite unsatisfactory because the weak light had to

be very close to the flies. LASL scientists were requested to investigate an alternate method of tracking which would enable the flies to be tracked at 30-ft distances, or greater. A Doppler radar-type of fly tracking device was constructed which mocked up the beating of a fly's wings by a rotating dipole antenna. This concept of tracking flies was tested briefly. Mock-up dipoles were put on flies to see if they could carry the weight of the antenna and it was concluded that such a method was practical from that point of view. However, the method would only work when the fly's wings were beating and considerable experimentation was needed to do a satisfactory "proof-of-principle" of radar tracking. A simpler and less expensive method was desired.

It was decided to look into replacing the mineral light with a much more intense light source from a laser. "Day glow" powders were brought back to LASL from Mission and tested in argon laser beams to see if this type of light source would be satisfactory. It was found that when certain powders were viewed through "barrier" filter glasses they glowed quite brightly. The "barrier filter" blocks laser light but passes fluorescent light. Since fly release experiments were being performed, flies were collected and examined as a function of time after release to see if enough dye powder remained on the flies for detection with a laser beam. It was found that after as much as four days a sufficient amount of powder remained on many flies and they could easily be detected with a 488 nm wavelength laser beam of about 20 mW and expanded to a 6 in. diameter. Since 20 mW of laser powder is within the realm of air-cooled lasers, it appears feasible to make a "laser flashlight" whose laser power supply could be hauled in a Jeep or pickup. By using binoculars with "barrier" filters to block the laser light it should be possible to see individual fluorescing flies

at 30 ft using a continuous wave laser beam. It was desirable to have the beam spot size larger than 6 in. so the next experiment will be with a beam sweep to see if a spot size as large as 2 ft square can be generated by scanning techniques. It is expected that the eye persistence will make it possible to scan the laser beam at high velocity so a complete scan can be accomplished in less than a 25th of a second. This should keep the average laser beam power at considerably lower levels so the danger of blinding the flies or people would be minimal.

In the course of the experiments with the "day glow" it was suggested by a LASL staff member that tetracycline fed to the larvae of the flies would migrate to the cuticle and the flies might glow in a laser light beam. This would be a very desirable feature since it would be permanent and could be applied to a large number of flies. At the time of writing we understand flies have been fed tetracycline but they have not arrived in Los Alamos.

The dyes and the laser light on the flies are apt to cause some modifications of behavior which are recognized as undesirable. However, it should be possible to obtain useful data that is unobtainable now. With this technique it should be possible to track plant produced flies in the field at ranges of up to approximately 30 ft. These techniques are likely to be applicable to other insects.

C. Fly Drop Distribution

The question of fly behavior after being released from the aircraft has been raised. It has been suggested that flies may fly horizontally until they become exhausted and then fall to the ground. If such is the case, the release of flies at 2000 ft or more would be undesirable. A release at a much lower altitude, but with proper dispersion, would be more desirable.

It was requested that LASL scientists look into the possibility of tracking flies

as they are released from an aircraft to measure the dispersal. Preliminary calculations indicate that "day glow" dusted flies and the laser would not be suitable for this problem. A ten to one hundred-fold increase in laser power over that considered safe would be necessary for the fly drop experiments. However, the radar tracking methods suggested above would likely be suitable for this.

D. Acknowledgments

Dr. Chris Hoffman, of the U.S. Department of Agriculture, Agricultural Research Service's Screwworm Research Laboratory, Mission, Texas, supplied the "day glow" tagged flies which had been captured in release studies.

II. PHYSIOLOGICAL MONITORING

A. Background

Strong interest in physiological monitoring was expressed by numerous USDA personnel and LASL was requested to develop some equipment for active multiple channel telemetry. A "management matrix" was generated to develop a multichannel system and work was commenced. However, experiments by the staff at the USDA, ARS, Plum Island Animal Disease Center (PIADC), Long Island, New York, proceeded at a much faster rate than anticipated, and a request for an operational system was received before a multichannel system could be finished. Therefore, a decision was made to provide a single channel system immediately. This system was first borrowed from the Sensory Systems Laboratory (SSL) and later purchased. It was used in some preliminary experiments at the USDA Animal and Plant Health Inspection Service (APHIS), Veterinary Services Laboratories (VSL), Ames, Iowa; and then sent to Plum Island where it is currently being used. Another priority request for physiological monitoring was received from VSL in Ames so a new and different system was assembled using some LASL circuitry and some commercial circuitry.

The system provided to Plum Island was one-of-a-kind, manufactured by SSL, and LASL has no available circuits or information. However, the system provided to Ames consists of commercial equipment and circuits designed at LASL. This report deals primarily with the latter system and is designed to provide the necessary information needed to duplicate the system provided to VSL.

B. Theory of Operation

A block diagram of the temperature monitoring system provided to Ames is shown in Fig. 2. The portion of the circuit on the animal is indicated in a dashed box. It consists of an antenna, transmitter, pulse rate generating circuit, thermistor, and a battery power supply. The battery provides power to the pulse rate generator and the transmitter. The power to the transmitter is provided in pulses from the pulse rate generator which turns the transmitter on. These power pulses have a frequency that is directly proportional to temperature as measured by the thermistor. Thus, the transmitter sends out a 10 ms pulse of 150 MHz carrier wave every 0.6 of a second at 37°C. The complete transmitter unit is attached to a halter on the animal and the thermistor probe is either inserted in the ear canal or subdermally in the head region. The receiving and recording equipment consists of a 12-channel receiver

Fig. 2. A block diagram showing the essentials of the physiological monitoring system sent to VSL, Ames.

tuned to the transmitter frequency with the aid of an audio feedback signal. The electronic output of the receiver consists of 10 ms bursts of audio signal which are in phase with the transmitter. This signal is conditioned with a pulse generator and shaper circuit of LASL design. The latter circuit generates a square wave signal which is necessary for the period meter. The period meter measures the time in seconds between successive bursts of transmitter signal and displays that time in a decimal digital readout. A digital-to-analog converter provides a dc voltage proportional to the period which is recorded on the chart recorder. Two models of rate generators have been constructed; a single channel that measures the temperature from one probe and a dual channel which will indicate the temperature alternate from two different thermistor probes. It switches from one probe to the other every 80 s. The receiver section is identical with both transmitters.

The transmitter has a useable range of about 30 m with a battery voltage of 5.4 V and 80 m with 9 V. Signal can be detected at greater ranges but the increased noise makes the recorder trace difficult to interpret.

C. Design and Construction

Figure 3 shows a complete single channel transmitter unit ready for mounting on a halter. Figure 4 shows the receiver, pulse shaper, period meter, and a digital-to-analog converter. Figure 5 shows the single channel pulse-rate-generator unit. At the top is the wired unit, in the middle is the printed circuit "mask" negative, and the circuit diagram is at the bottom. From this figure it should be possible to construct the unit. Figure 6 shows a partially completed single channel transmitter unit prior to final wrapping with electrical tape. Figure 7 shows the same items as Fig. 4, but for the dual channel. Figure 8 shows the same items as Fig. 4, but for the pulse

Fig. 3. An assembled transmitter system ready for mounting on a halter. The stainless steel probe must be bent to conform to the animal, and then coated with silastic to minimize tissue response.

shaper. As indicated in the schematic diagram this unit has its own 9 V battery.

Figure 9 shows an exploded view of the external ear canal probe. The exterior ear canal probe is sufficiently flexible to conform to the ear canal. A stainless steel sheath is put over the ear canal probe to make the subdermal probe. The stainless steel is sufficiently thin to allow shaping by hand.

D. Materials and Equipment

This section gives specifications and

Fig. 4. AVM receiver shown on the left, the pulse shaper is in the black box, and the period meter, with Digital to Analog converter, at the right.

Fig. 5. A single channel rate generator, with a wired unit on top, a circuit board layout in the middle, and the circuit diagram at the bottom. From this figure, it should be possible to duplicate the circuitry.

identification of commercial components used in the system supplied to VSL, Ames.

1. Transmitters. The transmitters were acquired from AVM Instrument Company, 808 West Springfield Avenue, Champaign, IL. They are used with AVM portable radio telemetry receivers, Model LA-12. The 150 MHz transmitters cost \$65 each, and were

specified in units of 12, having 25 kHz separation between each transmitter in the series.

2. Receiver. The receiver is Model LA-12 from AVM Instrument Company at \$650 each. The individual receivers are tuned with the aid of a channel selector switch, a fine tuning control, and a gain control. AVM can provide automatic electronic scanning of the 12 transmitters at an additional cost.

3. Period Meter and D-to-A Converter. This unit consists of a Hewlett Packard counter mainframe Model HP ST#5300A, a period meter HP ST#5302-A, and a converter, D-to-A, HP ST#5311A, purchased from Hewlett Packard at \$935 (total). Hewlett Packard's local address is 6501 Lomas Blvd., NE, Albuquerque, NM.

4. Recorder. The recorder used in this work was an Omniscribe Model 5133-1, with 10 mV full scale input and variable speed drive purchased from Houston Instrument at \$600. Houston Instrument is located at 4950 Terminal Avenue, Bellaire, Texas. Any recorder of the proper speed and sensitivity would work.

5. Thermistors. Small 1 MΩ at 25°C thermistors, Model 44015, at \$4 each, were purchased from Bordwick Engineering Sales

Fig. 6. A partially assembled transmitter to show the orientation of transmitter, chokes, antenna, and the silastic potting of the temperature generator. After wrapping with electrical tape, this unit appears as shown in Fig. 3.

Fig. 7. A dual channel temperature rate generator. The wired unit on top, the circuit board layout in the middle, and the circuit diagram on the bottom.

Company, 427 Washington Street, Norwell, Massachusetts 02061. A polyethylene tubing was used to insulate the wires of the thermistor.

6. Wire. Stranded stainless steel electrode wire, part number PN-231-0010, for connecting the thermistor to the circuit board was purchased from Narco Biomedical Systems, 7551 Airport Blvd., Houston, Texas 77017. The leads from the thermistors were silver soldered to the stainless steel wire using a very small acetylene-oxygen torch.

7. Potting Material for Ear Canal

Probe. A vinyl resin, Elvax-260, was mixed with paraffin in a fifty-fifty ratio by weight. The resin was obtained from DuPont Plastics Division, Post Office Box 2749, Los Angeles, California 90028.

8. Stainless Steel Sheath. One-eighth in. o.d. with 0.002 in. wall and 5 in. long, 316 stainless steel tubing was fused on one end and helium leak tested. Manufacture source of the stainless steel tubing is unknown.

9. Batteries. Four penlight AA mercury batteries of 1.35 V each were used

Fig. 8. Pulse amplifier and pulse shaper which is used to condition the output pulse of the receiver for period measurements. At the top is the wired circuit board, middle, the circuit board layout, and at the bottom is the circuit diagram.

Fig. 9. An exploded view of an ear canal probe. At the top is a completely assembled probe, ready for insertion. The various components are shown below.

to power the transmitter. A 9 V "transistor" battery was used for the pulse shaper.

10. Shrink Tubing. Specifications are shown in Fig. 9.

E. Results.

We understand that the first system sent to PIADC has performed well with ear canal probes held in place, using silastic caulking material as a bonding agent in the ear canal. We also understand that similar probes placed subdermally operated well for up to a few days at VSL. However, after that time, there appeared to be body fluid penetration into the probe causing erroneous readings. Stainless steel sheaths were used to cover the ear canal probes. These stainless steel probes were very thin so could be bent easily to conform to the curvature required for the experiments. The probes were covered with silastic to minimize tissue response. We understand the stainless steel probes have been working

for more than a month with no problems. We also understand that the readings obtained annually are significantly different from the continuous monitoring of the active transmitters since no animal handling is required in the latter.

It is necessary to perform a specific calibration for each combination of thermistor and pulse generator because the resistance is slightly different from one thermistor to the other. Figure 10 illustrates a typical calibration curve obtained by placing the probe (which is tied to the bulb of a high precision mercury thermometer) in a vigorously stirred water bath. Neither of the user groups has supplied LASL staff members with data taken using the instruments supplied.

F. Future Developments

An automatically scanning multichannel system would be highly desirable. Two options are available. One is to continue the development of the 10-channel system which was stopped at about one-third completion. This would require approximately six man months to complete. A less expensive option would be to modify

Fig. 10. A temperature-versus-period calibration of a thermistor probe. Temperature is measured by a precision thermometer tied to the probe, which is immersed in a well-stirred water bath. Period is measured on the period meter.

the AVM receiver to automatically scan by electronic means through the 12 channels and make the necessary frequency and gain adjustments. AVM Instruments has submitted a bid for \$1200 to build a unit which would perform this. Such a system would have to be tested and interfaced with the current system, but it appears quite feasible. Other changes in packaging of the transmitters would be highly desirable to make it easier to change batteries and thermistor probes.

G. Acknowledgments

Howard Baldwin, formerly of LASL, originated the circuit designs and concepts which are reported here.

Joseph Woolsey, of LASL, Group A-1, manufactured the stainless steel probe sheaths. Welding such thin walled tubing with a low heat capacity tip requires considerable skill.

III. BIRD TAGGING

A request for identifying birds going through import quarantine stations was received and some preliminary experiments have been performed. The following properties of the system were requested:

A. Requirements

1. The tag should not be visible to the unaided eye.

2. It should be suitable for birds from humming bird to peacock size.

3. Identification should be possible by USDA employees having minimal training.

4. Reading the tag should not harm the bird.

5. It should be possible to encode up to 35 quarantine stations.

6. The identification should be readable for up to 12 months.

Some preliminary experiments have been performed using the fluorescent dyes; acridine orange, fluorescein, and fluran with alcohol, water, and dimethylsulfoxide (DMSO) placed on fingernails and viewing with either black light or 488 nm laser light. The dyes that readily went into the carrier solution seemed to work reasonably well, for at least a day or two. So the approach does appear to have some promise. Inquiries were made of USDA staff to determine the lifetime of the foot epidermis. It was reported that the scales on the feet seem to last the lifetime of the bird so it now appears desirable to go into some longer term and more extensive studies.