US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY 40121

REPORT NO. 756

A CONTINUOUS BODY TEMPERATURE MONITORING SYSTEM FOR UTILIZATION IN PYROGEN TESTING

(Interim Report)

by

Captain Robert W. Bull, VC
and
Captain David K. Hysell, VC

24 October 1967

UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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Acknowledgment

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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Pathology Division
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ABSTRACT

A CONTINUOUS BODY TEMPERATURE MONITORING SYSTEM FOR UTILIZATION IN PYROGEN TESTING

OBJECTIVE

To develop for this laboratory an improved pyrogen testing procedure in rabbits which would be simple and yet effective.

METHODS

A continuous temperature monitoring system was developed in rabbits utilizing a subcutaneously placed copper-constantan thermocouple attached to a direct drive recorder. The results were then evaluated to determine what correlation existed between an injected pyrogen and detectable alterations in body temperature with this system.

SUMMARY

This method had several advantages over the previous method. It was more reliable because the continuous monitoring of the test animals' body temperature was less subject to environmental variations resulting from animal handling and it substantially reduced the technician time required by the other method.

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A CONTINUOUS BODY TEMPERATURE MONITORING SYSTEM FOR UTILIZATION IN PYROGEN TESTING

INTRODUCTION

The beneficial returns from the employment of pharmaceuticals and biological products in medicine can be well documented by the medical literature. Before any of these products can be used by the medical profession, they must be rigorously tested to ascertain any possible detrimental side effect that might result from their parenteral administration to the human body. One such investigation attempts to determine whether a product has a nonspecific pyrogenic or fever producing characteristic. Unfortunately, methods presently used to determine the pyrogenic nature of such substances do not effectively utilize the convenient and efficient technics available in the area of electronic medical technology.

Therefore, it was felt that a more efficient and accurate testing procedure could be developed by this laboratory. Briefly, the old method required that a healthy rabbit, given the test substance, be removed hourly from its cage for a rectal temperature determination. If the temperature of the rabbit rose 0.6°C at any time during the 3-hour test, the compound was said to be pyrogenic in nature. The disadvantages of this procedure were: the continual handling of the animals required too many technical work hours, false tests could easily occur as the result of hyperexcitability stemming from the repeated handling of the animal and there was always the possibility that an acute temperature rise could occur between the hourly readings and be unobserved.

METHODS AND MATERIALS

Thermocouple. The copper-constantan thermocouple utilized was manufactured at this laboratory, being constructed of 36 AWG, one strand of copper and one strand of constantan wire. It had a 0°C reference point, and generated 1.568 MV at 37°C, 1.610 MV at 40°C and 1.652 MV at 41°C (1). The rise in temperature of the thermocouple was linear in microvolt output, being 0.04 MV/°C (Fig. 5A, page 6).

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[1] Roeser, W. F. and A. I. Dahl. Reference tables for iron-constantan and copper-constantan thermocouples. J. Res. Nat. Bur. Standards, 20: 337, 1938.

Recorder. Two different DC direct drive recorders were utilized for the procedures. One, a single channel Honeywell Electronic 19 recorder, and the other a Brown multipoint, multichannel recorder in which each channel was read approximately every 1-1/2 min; both recorders operated equally well.

Rabbit Box. A rabbit restraint box was constructed by this laboratory (Figs. 1, below, and 2, next page). It had a 45° slope to the front of the box, allowing the animal to project its head comfortably through the opening, and movable head restraint brackets, which made possible adjustments to fit each individual animal. A wire mesh floor allowed for adequate disposal of waste material and provided ventilation for the animal (Figs. 1 and 2).

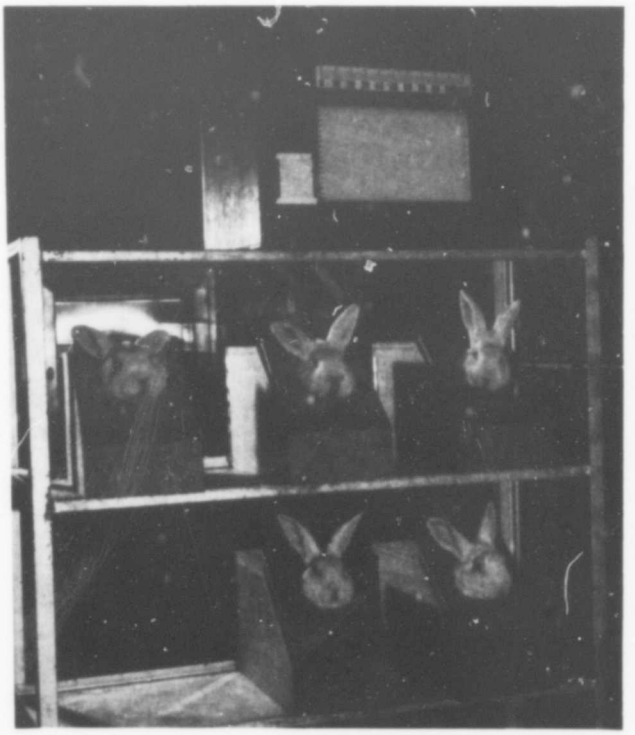

Fig. 1. Rabbits in restraint boxes and thermocouples in place prior to administration of pyrogenic substance.

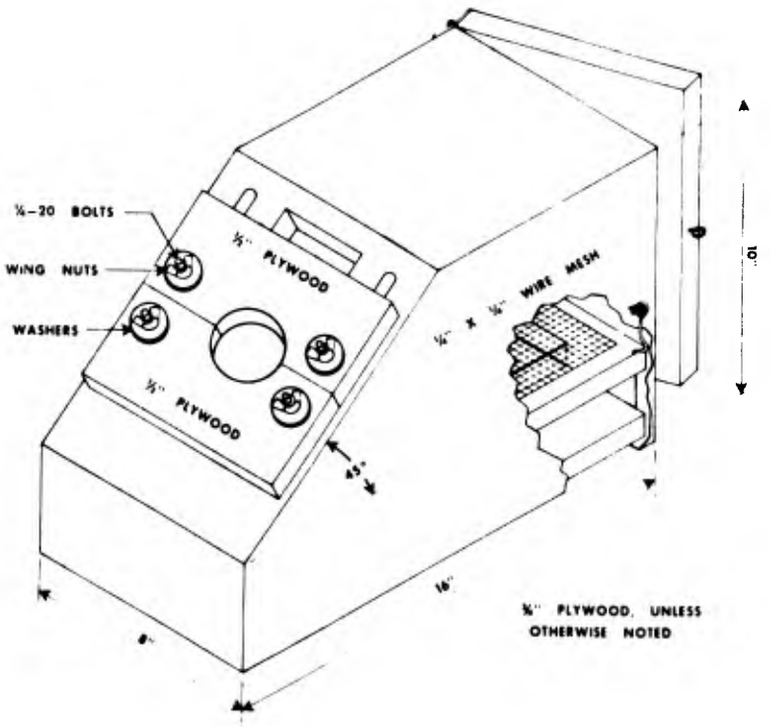

Fig. 2. Design of the rabbit restraint box utilized in this procedure.

Rabbits. The rabbits utilized for the trial runs met the requirements as set forth by the US Pharmacopeia (2). They were healthy animals weighing 1500 gm or more and were maintained for at least one week on a uniform unrestricted diet and lost no weight during this period. During the conditioning period, the animals were placed in the restraint boxes for periods of 15 to 30 min daily in order to reduce the chance of hyperexcitability that might occur during the placement of the animal in the box for the testing procedure. Hemograms and parasite examinations were performed on the animals during the conditioning week.

Pyrogenic Substance. The substance utilized was that which the National Institutes of Health uses in their pyrogen testing procedures; a commercially available product called Piromen, manufactured by

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[2] The Pharmacopeia of the United States of America. 12th Ed., Easton, Pa: Mack Printing Co., 1942, pp. 679-680.

Travenol Laboratories, Inc. (3). It is a sterile, non-protein, non-anaphylactogenic nitrogen containing pseudomonas polysaccharide which, when administered intravenously, produces a fever (4). During the testing procedures, this drug was administered either as the pure drug or diluted in various volumes of saline.

Testing Procedures. On the day of the test, the animals chosen were prepared in the following manner. An area of the skin along the midline of the back at the level of the last rib was cleansed with alcohol. A 16-gauge needle (Fig. 3, below) was placed through the skin and the copper-constantan thermocouple threaded through the needle into the subcutaneous tissue of the animal (Fig. 4, next page). The needle was withdrawn, moved back up the thermocouple wire, and the animal was placed in the restraint box. The thermocouple was then attached to the recorder.

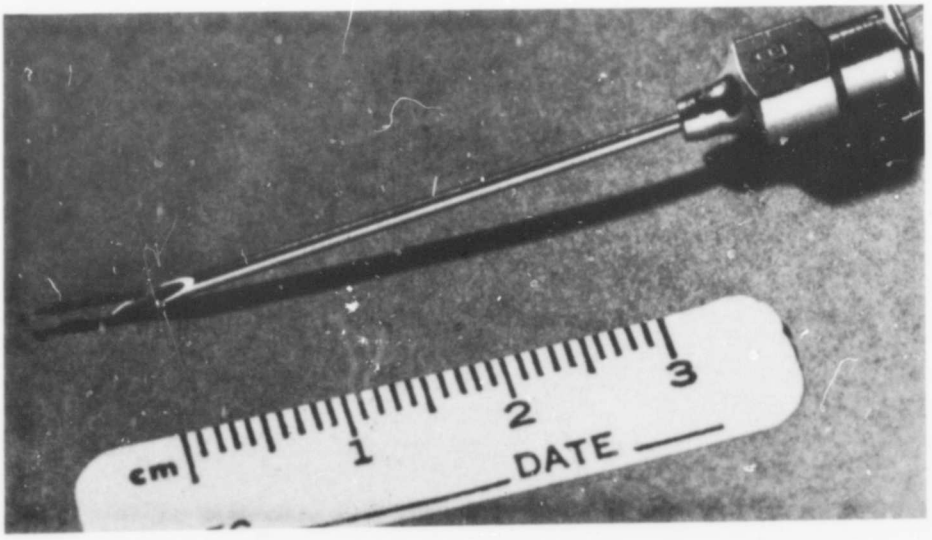

Fig. 3. Copper-constantan thermocouple threaded through a 16-gauge hypodermic needle.

[3] Ashworth, G. Personal communications.

[4] Nesset, N. M., et al. Bacterial pyrogens. I. Pyrogenic preparations from pseudomonas species. J. Amer. Pharm. Ass. 39: 456, 1950.

Fig. 4. Thermocouple inserted subcutaneously in the animal and the needle removed back up the thermocouple wire.

The animals were monitored for 1 hr as a control period. If the animal's body temperature was between 38.8°C and 39.8°C, the test substance was administered without removing the animal from the restraint box. The animal was monitored for a 3-hr period and at the termination of this time, the total temperature rise was determined from the graph.

RESULTS

For the pilot run, a rabbit was prepared with the thermocouple, placed in the box and attached to a Honeywell Electronic 19 recorder and maintained for a period of 4 hr (Fig. 5C, next page). This procedure was repeated the following day utilizing the same rabbit, but 5 μgm of Piromen was given intravenously. The total temperature rise in the animal after administration of the agent was 0.55°C (Fig. 5B). This rabbit was utilized 48 hr later and 7 μgm of the pure drug was given with a resultant temperature rise of 1.2°C (Fig. 5D).

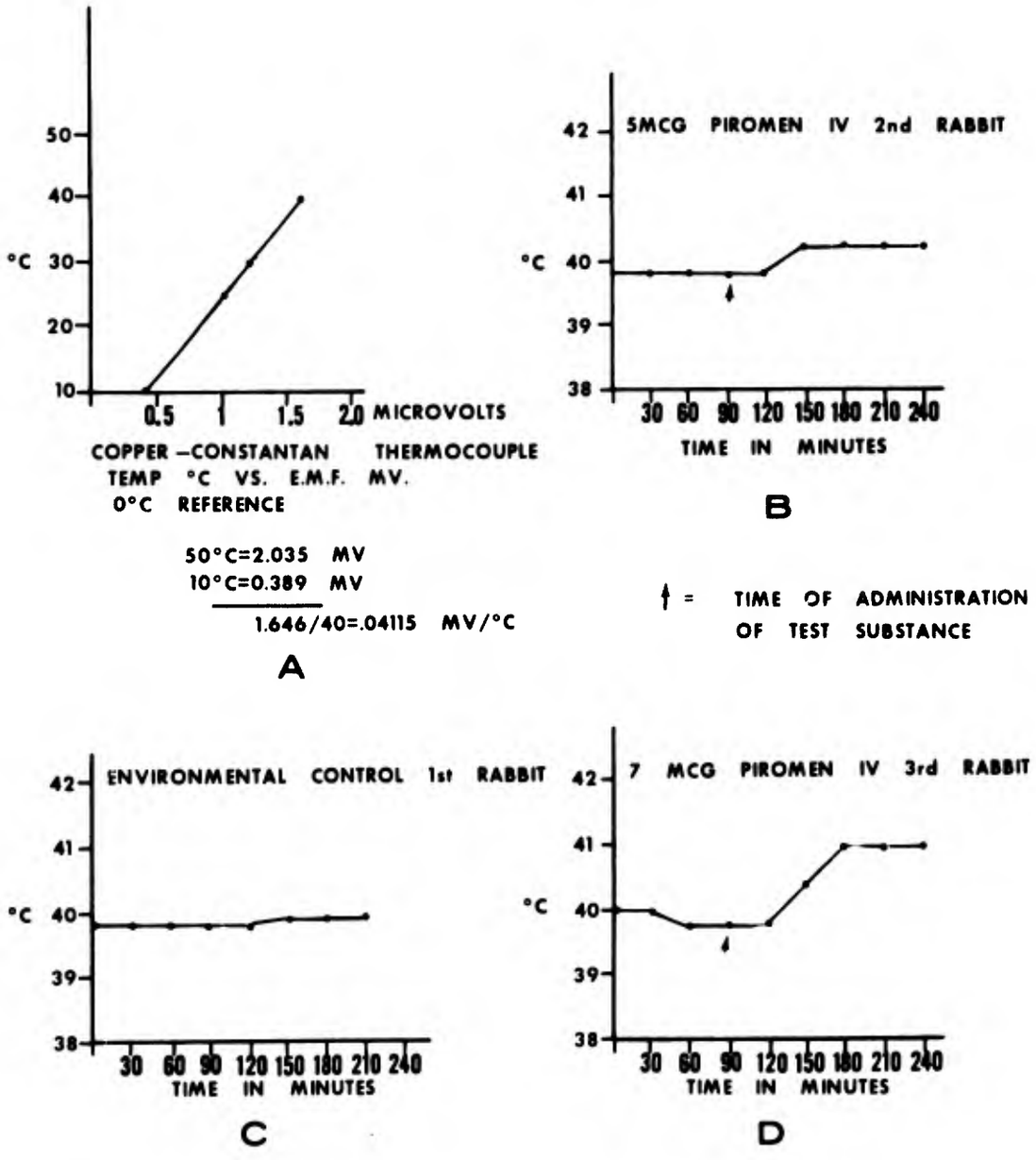
50
40
°C 30
20
10
0.5
1
1.5
2.0 MICROVOLTS
COPPER –CONSTANTAN THERMOCOUPLE
TEMP °C VS. E.M.F. MV.
0°C REFERENCE
50°C=2.035 MV
10°C=0.389 MV
1.646/40=.04115 MV/°C
A
42
41
°C 40
39
38
5MCG PIROMEN IV 2nd RABBIT
30 60 90 120 150 180 210 240
TIME IN MINUTES
B
= TIME OF ADMINISTRATION OF TEST SUBSTANCE
42
41
°C 40
39
38
ENVIRONMENTAL CONTROL 1st RABBIT
30 60 90 120 150 180 210 240
TIME IN MINUTES
C
42
41
°C 40
39
38
7 MCG PIROMEN IV 3rd RABBIT
30 60 90 120 150 180 210 240
TIME IN MINUTES
D

Figure 5

A second run was made utilizing a different type of recorder, a Brown multichannel, multipoint recorder, which checked the channels every 1-1/2 min. This run utilized varying concentrations of Piromen, 2, 5, 7 and 10 μgm, in 10 cc of saline. The control animal was administered 10 cc of saline from the same bottle that was used to dilute the Piromen. As can be seen from the graph (Fig. 6A), the animal to whom saline was given had a decrease in temperature and those animals given Piromen had the desired temperature rise of over 0.6°C (Fig. 6B, C, D, E).

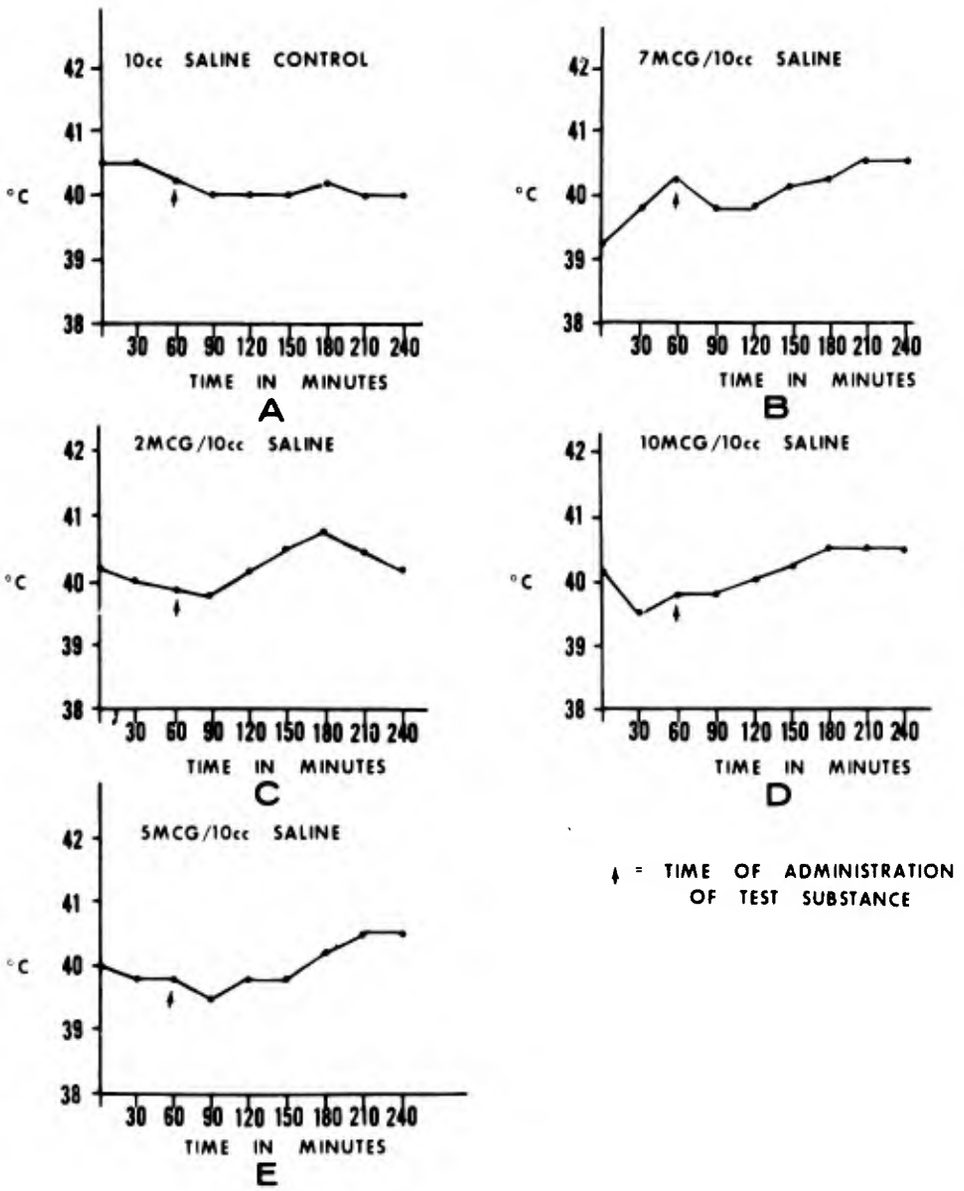

Figure 6

The third run involved a similar protocol as the second run except the animals were given 30 cc of solution to meet the requirement of 10 cc of test substance per kg of body weight (2). These animals received dosages of 50, 30 and 15 μgm of Piromen and the control received the straight 30 cc of saline (Fig. 7B). Two of the three animals given the test substance responded (Fig. 7A and D), while the other animal had a negligible response (Fig. 7C).

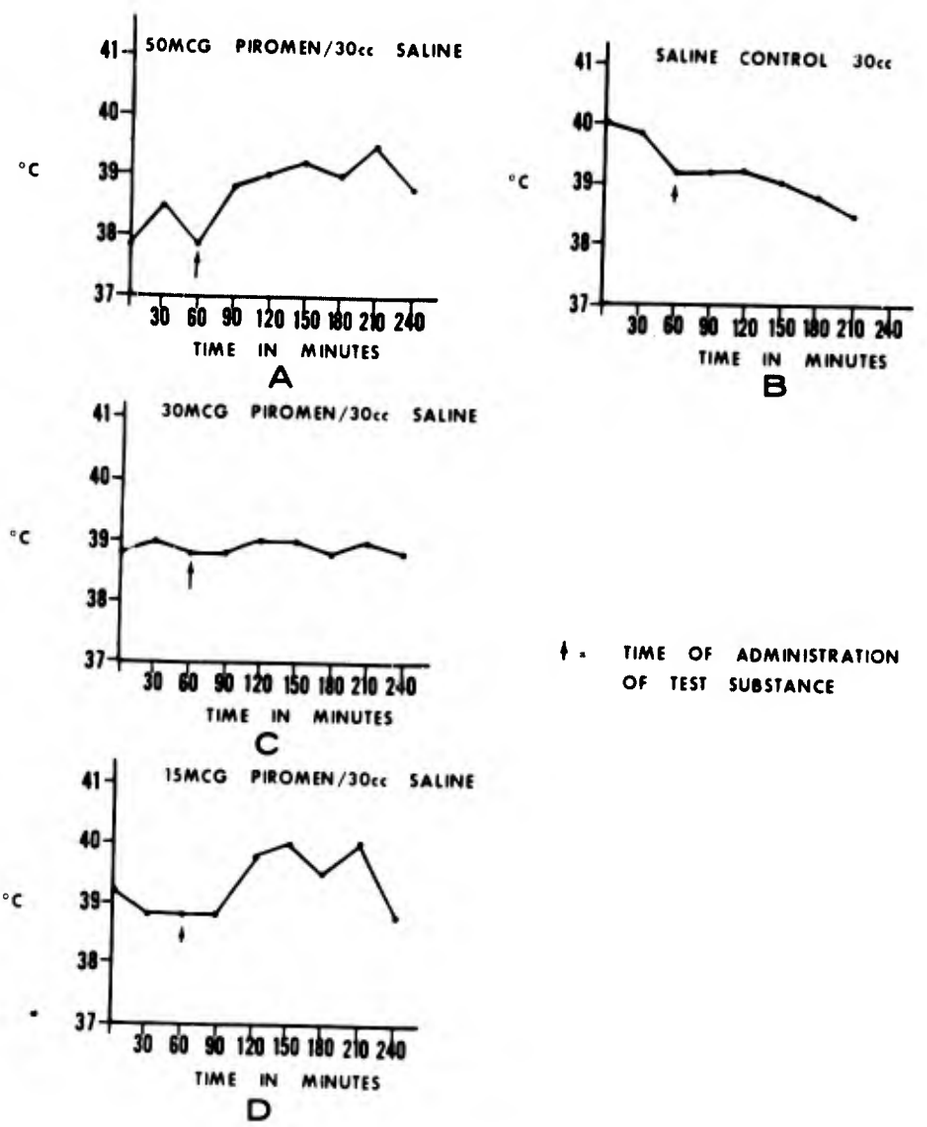

Figure 7

DISCUSSION

The first run (Fig. 5B, C, D) indicated that the thermocouple and recorder were sensitive to small temperature changes in the animal body and that the Piromen was a reliable pyrogenic substance. The second run with the multichannel recorder made possible the monitoring of as many as five animals simultaneously, thereby increasing the efficiency of the testing procedure (Fig. 1). The reason for the high temperature of the rabbit given 7 μgm in 10 cc of saline was that at the time of injection (Fig. 7B), the thermocouple had become detached and had to be reinserted. The third run showed that as much as 30 cc of test substance could be administered to a rabbit. This test also pointed out the biological variability of the animals for there was one animal that did not respond (Fig. 7C). This was still a valid test by the established standards which require that three rabbits be used for each test substance and the test considered positive if two or three show an individual rise of temperature of 0.6°C or more above the normal established for each animal (2).

Only those animals whose body temperature falls within the range of 38.8°C and 39.8°C during the control period should be utilized in the testing procedure (2). Animals with temperatures outside the range had a tendency to give erratic results (Fig. 7B).

By proper utilization of animals that meet the required standards, this testing procedure will reduce many sources of variation in temperature monitoring and should therefore be implemented.

SUMMARY

A continuous temperature monitoring system was employed in rabbits which utilized a subcutaneously placed copper-constantan thermocouple attached to a direct drive recorder. It has the following advantages over the previous method of rectal temperature determinations. It was more reliable, less subject to environmental variations resulting from animal handling and substantially reduced the technician time required by the other method.

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