# A COMPUTERIZED HYBRID
# RF/WATERBATH HEATER
# FOR MOUSE TUMORS

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RF/WATERBATH HEATER

FOR MOUSE TUMORS

By

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## Abstract

A computer-controlled system, based on the design of S. Brown et al. at the Ontario Cancer Institute, Canada, has been developed for uniform heating of mouse tumors.

A steady hyperthermic temperature was maintained in the tumor by combining waterbath heating with RF-heating. The RF field was provided by a transmitter which was connected to two steel capacitive plates via a matching circuit. 50% isotonic saline kept at a temperature 2°C below that in the tumor was circulated in the waterbath. The saline prevented overheating of the skin and provided coupling between the RF field and the tumor.

A computer program has been written to measure continually the temperature in the tumor with implanted fine thermocouples. The program also controlled the average RF power delivered to the tumor by switching the transmitter on and off at appropriate intervals.

The system has been tested on tumor xenografts growing in the thigh of nude mice. A steady temperature of 42° or 44°C has been maintained in the tumor for up to an hour.

The system could be used to study the effect of hyperthermia on the uptake of radiolabelled tumor-associated antibodies and the treatment of tumors by such antibodies.

## Acknowledgements

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## Abbreviations

| | |
|---|---|
| Ab | antibody |
| AC | alternating current |
| A/D | analogue to digital |
| Ag | antigen |
| CW | continuous wave |
| emf | electromagnetic force |
| ISM band | frequency window for industrial, scientific and medical use |
| LM | LabMate Data Aquisition and Control Station |
| MAb | monoclonal antibody |
| R | resistance |
| RF | radio frequency |
| RIT | radioimmunotherapy |
| RMAb | radiolabeled monoclonal antibody |
| rms | root mean square |
| SWR | standing-wave ratio |
| T | temperature |
| TC(s) | thermocouple(s) |
| TL | transmission line |
| V | voltage |
| VSWR | voltage standing-wave ratio |

# I. Introduction

## I.A. Goal

The goal of this project was to develop a heating system that can be used to study the effect of hyperthermia (HT) on the efficacy of radioimmunotherapy (RIT). Of interest will especially be the change in uptake kinetics of radiolabelled monoclonal antibodies (RMAbs) and the thermal enhancement of radiosensitivity in human malignant melanoma xenografts, grown in the thighs of mice. The effects of HT, i.e. the elevation of temperature locally, regionally, or of the whole body above 37 $^{o}C$, are very sensitive to small changes of the temperature. Joiner and Vojnovic (Joiner 1982) added a RF field to a waterbath to reach a more uniform temperature distribution when heating a tumor in the thigh of a mouse. The waterbath heats the surface of the thigh, which is cooled by bloodflow. This causes a temperature gradient with the warmer region towards the surface. The RF field, due to its greater penetration in soft tissue, results in a more uniform energy absorption pattern that is superimposed on the pattern caused by the waterbath. Furthermore, since tumors have lower bloodflow per unit volume than normal tissue, a temperature gradient with the warmer region towards the tumor is created. This temperature gradient balances off that created by the waterbath heating. An other advantage of the RF field is that it can easily be controlled. The heating system built by S. Brown incorporates a microcomputer and thermocouples (TCs), thus allowing for constant monitoring of temperature. An interface to the microcomputer was used to measure temperatures of the waterbath, different parts of the tumor, and the rectum. A desired steady temperature in the tumor was obtained by manually selecting a suitable power output of the transmitter.

The system developed in this project superseded Brown's design in one main respect. A computer program was used to regulate the temperature in the tumor by switching the transmitter on and off. A wide latitude (from 10 to 100 W) in the output power of the transmitter was allowed.

The basic routines to control a colour monitor and the interface were written by A.D.A. Maidment as part of his thesis (Maidment 1986). They were used as building blocks for the Pascal program developed in this project. The program displayed the temporal variation of temperatures, that have been measured with the TCs on the colour monitor. Thus the heating of the thigh could closely be supervised and the experiment could be stopped should a sudden big change in the temperature make it necessary.

## I.B. Biology of Hyperthermia

### I.B.1. Short History of Hyperthermia

Heat has been used through the ages as a therapeutic agent for various ailments, including cancer. It was reported, by Ramajame (2000 B.C.), Hippocrates (400 B.C.) and Galen (200 A.D.) that ferrum candens (red-hot irons) had been used to treat small, non-ulcerating cancers. After the Renaissance, there were reports of tumor regressions after infectious fever of about 40°C, which lasted for several days.

It was, however, not before 1866 that the first scientific report on the selective effect of HT in neoplastic tissue was published by the German physician Busch. He described the complete disappearance of a sarcoma of the face after prolonged fever caused by an erysipelas infection. But the results of HT treatments, induced by injection of bacterial toxins, were inconsistent due to lack of standardization of the toxins.

Only in the 1960s did a systematic study of the biological effects of HT begin. During the past 15 years knowledge of the biological effects produced by HT, either alone or in combination with ionizing radiation, has grown considerably, although the mechanisms of the effect of HT on a molecular level are still not known.

The main problem in the use of HT was the inconsistency of the results, due partially to the inability to produce a well-defined uniform and constant heating pattern to induce HT. It is now known that HT is strongly temperature dependent in its effective range. Thus it is necessary to develop heating systems that produce such uniform temperature fields.

I.B.2. Rationale for the Oncological Use of Hyperthermia

HT is used in two ways. First it is used in the treatment of specific local lesions with temperatures ranging from 42 to 45°C. Second HT is also used as regional or whole-body heating to treat widespread disease. The temperatures are then in the range of 41 to 42°C and the effect is more likely only palliative. But the second form is still useful, especially when combined with other methods of cancer treatment.

Heat can kill cells directly, especially at temperatures above 43°C, but there is no agreement on whether the effect differentiates between normal and cancerous cells (Streffer 1987). There are some therapeutic benefits, however, since special factors come into play in tumors (Field 1987):

1. The blood supply in some tumors is more sluggish. Therefore some tumors become hotter than the surrounding

tissue in a localized treatment field. There is evidence that this selective heating also occurs in man but the extent of the effect varies from tumor to tumor. The extent of the effect also changes during a fractionated HT treatment. One can speculate that during the course of moderate heat treatment, which is tolerated in well vascularized normal tissue, a number of cells in many solid tumors are killed.

2. Parts of solid tumors are poorly vascularized. Therefore they contain cells which are hypoxic and suffer from nutritional deprivation and acidosis. Hypoxic cells constitute a radioresistant fraction in a solid tumor. This cell population is likely to cause recurrence. In contrast to its effect on the sensitivity to X-rays, hypoxia does not seem to affect markedly the response to HT.

However, experimental investigations show a strong enhancement of the heat-induced cytotoxic effect for cells that have a low pH and/or are nutrient deficient. This effect is enhanced further, since heat above approximately 42°C reduces the blood flow in tumors compared to the blood flow in normal tissue. The reduced blood flow causes more hypoxia and acidosis.

3. Cells in the DNA synthesising phase (S-phase) of the mitotic cell cycle are particularly heat sensitive but

relatively resistant to X-rays. A combination of the two modalities might be advantageous in some circumstances.

4. The combination of HT with chemotherapy might result in an increased uptake of drugs and/or an enhancement of the sensitivity to drugs.

For the use of HT as a radiosensitizing agent, at temperatures below 43°C, there is apparently no differentiation between the effect on normal and on tumor cells. (This is in contrast to the direct cell killing at temperatures above 43°C as outlined above.) The mechanisms are complex and not completely understood, but under some conditions heat synergistically interacts with radiation by inhibiting the repair of radiation-induced sublethal or potentially lethal damage (Field 1987).

There are several reasons to work in the lower temperature range of 42 to 43°C, i.e. the radiosensitizing region. First it is technically less difficult to produce a homogeneous temperature distribution at lower temperatures. Second the risk of side effects in surrounding normal tissue is considerably lower. And third due to modern physical treatment planning the 100% radiation dose is fairly precisely deposited within the tumor volume. The additional moderate heat treatment then preferably radiosensitizes the tumor.

I.B.3. Radioimmunotherapy (RIT)

Antibodies

Ab molecules are very complex protein structures. They can be divided in 5 classes (IgG, IgA, IgM, IgE, and IgD) which differ from each other in size, charge, aminoacid composition, and carbohydrate content. The Igs within each class are very heterogeneous. The basic structure of the Ig consists of 2 identical heavy chains and 2 identical light chains. The chemical details of the subclasses are largely known but many of the physiological functions are not yet fully understood. With the advent of the hybridoma technique, large amounts of MAb of identical properties become available. Advances have been made in the radiochemistry of Abs and the characterization of tumor antigens. Less knowledge has been gathered in the field of pharmacokinetics.

RIT uses Abs directed against tumor associated antigen Ag to deliver cytotoxic radionuclides preferentially to the cells of primary and secondary tumors. Factors that affect the effectiveness of the radiation within the tumor (compared to normal tissue) are the specificity of the Ab, the distribution of the radiation energy within the tumor, and the host's response to injected foreign Abs. The following factors affect RMAb accumulation in the tumor (Goodwin 1987 and Cobb 1986):

Blood flow

Tumors grow radially. Tumor cells form a shell around a hypoxic core. If they outgrow the blood supply, a necrotic central nest is formed that may contain some viable cells which are very resistant to radiation. Since the blood flow is very low, the delivery of drugs or radiopharmaceuticals is very difficult.

Permeability

Abs injected to the cardiovascular system have to cross the capillary wall and to diffuse through the interstitial tissue to reach the tumor cells. An intact Ab molecule is large. Thus its rate of diffusion is slow. Such intact molecules normally take several days to attain maximum level as had been shown in human scintigraphic studies (Goodwin 1987). Ab fragments Fab and $(Fab')_2$ with 1/3 and 2/3 the weight of whole IgG Abs diffuse more rapidly into the tumor (Goodwin 1987).

Concentration gradient

The concentration of Abs in tumor increases with the time integral of blood concentration. Ab fragments diffuse more rapidly, but they also disappear more rapidly from the blood.

Receptor (antigen) binding

Unique specificity is the reason for the application of Abs. The binding to the tumor is noncovalent. The affinity constant of the Abs to the Ag is normally of the order $10^9$ $M^{-1}$ or greater. With an Ag concentration of about $10^5$ molecules per cell in the tumor (e.g. $4 * 10^5$ molecules per cell for the Ag p97 which occurs in more than 80% of human melanoma) and a very low concentration in normal adult tissue, preferential binding of the Ab in the tumor can be achieved.

Factors affecting RMAb background

In man more than 90% of the injected Abs are in the blood pool and only less than 1% is in the target tumor within a few days of injection (Goodwin 1987). As far as the purity, the affinity, radiolabelling, and the specificity of MAbs are concerned, the optimum has been approximated. In order to reduce the background different strategies have been used.

Using fragments of Abs increases the maximum target to background ratio but also reduces the absolute uptake in tumor. $F(ab')_2$ fragments could be a compromise between whole Abs and Fab fragments. Second Abs can be used to clear the first Abs from the blood. The problem that arises are the higher concentrations of immunocomplexes in the liver. Abs can be delivered locally. However this is limited to accessible areas.

Other strategies of reduction of background include the use of metabolizable chelate linker, reversible Ab hapten complexes, or pretargeted immunoscintigraphy.

I.B.4. Radioimmunotherapy and HT

Disseminated melanoma is virtually incurable by conventional therapeutic methods. The median survival is a few months to a year. The antigenic composition of melanoma cells has been studied extensively. Abs have been used in clinical studies. These studies show that even g quantities of Ab can be given safely and tumors larger than 1 - 1.5 cm diameter can be detected.

Rationale for the use of HT in combination with RMAbs

The rationale for the use of HT in this project differs somewhat from that given above for the use in general of HT in the treatment of tumors with conventional radiotherapy. The system that has been developed will be used to combine radiolabelled monoclonal antibodies with HT. It will be used to study the effect of HT on the uptake and efficacy of RMAbs in tumors in the treatment of malignant melanoma xenografts.

A significant limiting factor in the potential use of RMAbs is the inadequate blood supply at the site of solid tumors. Generally the tumor blood flow per unit mass is reduced when the tumor mass increases. It has been shown that a relative increase in blood flow within some tumors could be achieved by moderate temperature elevation (Song 1984). The increase may be due to dilation of vessels, increased blood pressure, or recruitment of capillaries after stimulation with vasoactive substances.

A study (Stickney 1987) onhuman melanoma xenografts in nude mice showed a distinct increase in Ab uptake after the induction of HT. The concentration of Ab in the tumor 24 h and 48 h after HT (with 41 $^{o}C$) were 48% and 71%, respectively, higher than in the control group.

The improvement of the efficacy with HT is better with RIT than with conventional radiotherapy since the percentage of cells with only sublethal damage is higher when RIT is used.

## I.C. Physics of Hyperthermia

### I.C.1 RF Power Dissipation in Tissue

The power dissipation is related to the specific absorption rate (SAR) by:

$$SAR = \frac{P_L}{D} = c \times \frac{dT}{dt} \qquad (1\text{-}1)$$

where $P_L$ is the power density [$Wcm^{-3}$] across the loaded capacitive plates,

$D$ is the density [$gcm^{-3}$] of the tissue,

$c$ is the specific heat [$Jg^{-1}{}^{o}C^{-1}$] of the tissue,

$dT/dt$ is the rate of temperature rise [${}^{o}Cs^{-1}$].

The power across the plates is given by Joules law (edge effects ignored):

$$P_L = |I|^2 R_L = (V_S)^2 R_L \,/\, |Z_L + Z_S|^2 \qquad (1\text{-}2)$$

where $|I|$ is the magnitude of the current [A] in the circuit,

$V_S$ is the voltage [V] produced by the source,

$Z_S$ is the complex impedance [Ohm] of the source,

$Z_L$ is the complex impedance [Ohm] of the loaded capacitor,

$R_L$ is the real impedance [Ohm] of the heated tissue.

When the circuit is matched, i.e. $(Z_S)^* = Z_L$, the maximum power is delivered. The complex impedance of the loaded capacitor, $Z_L$, is given by:

$$Z_L = -j / (\omega C) \qquad (1-3)$$

where $\omega = 2\pi \times 13.56$ MHz is the angular frequency,

$j = \sqrt{-1}$

The capacitance, C, for a parallel plate capacitor, edges neglected, can be expressed as:

$$C = A \varepsilon^* / d \qquad (1-4)$$

where A is the area of the plates,

d is the distance between the plates,

$\varepsilon^*$ is the complex permittivity or dielectrical constant.

The complex permittivity is:

$$\varepsilon^* = \varepsilon (1 - j / (\rho \omega \varepsilon)) \qquad (1-5)$$

where $\rho$ is the resistivity [Ohm x cm] of the material between the capacitor plates,

$\varepsilon$ is the permittivity of the load which is given by: $\varepsilon = \varepsilon_r \times \varepsilon_o$

where $\varepsilon_r$ is the relative permittivity,

$\varepsilon_o = 8.85 \times 10^{-14}$ $\mathrm{Fcm}^{-1}$ is the permittivity of free space.

Manipulation of the equation yields:

$$C = (A \varepsilon / d) \times (1 - j / (\rho \omega \varepsilon)) \qquad (1-6)$$

This is the general case for the complex impedance of the capacitive load. Using the values from table 1-1 (Brown

1986) at 13.56 MHz tissue and distilled water act as insulators, while isotonic saline is a conductor ($1 / (\rho \omega \varepsilon)$ $2.2 \times 10^{-1}$, $3.4 \times 10^{-1}$, 6.6, respectively). Now the complex impedance can be given as a function of resistivity and permittivity:

$$Z_L = (d / A) \times ( \rho / (1 + j\rho\omega\varepsilon ) ) \qquad (1\text{-}7)$$

I.C.2. Influence of coupling medium on power deposition

To discuss the influence of the coupling medium on the power deposition, a model developed by S. Brown (Brown 1986) is used. The discussion follows closely his paper.

It is assumed, that the space between the plates is either completely occupied by the leg (region 1) or the leg fills 1/3 of the distance between the plates with the rest filled by a dielectric (region 2) (as shown in figure 1-1).

First the current I is determined when the space is completely filled with the dielectric (i.e. tissue, distilled water, or isotonic saline). The circuit is matched for tissue:

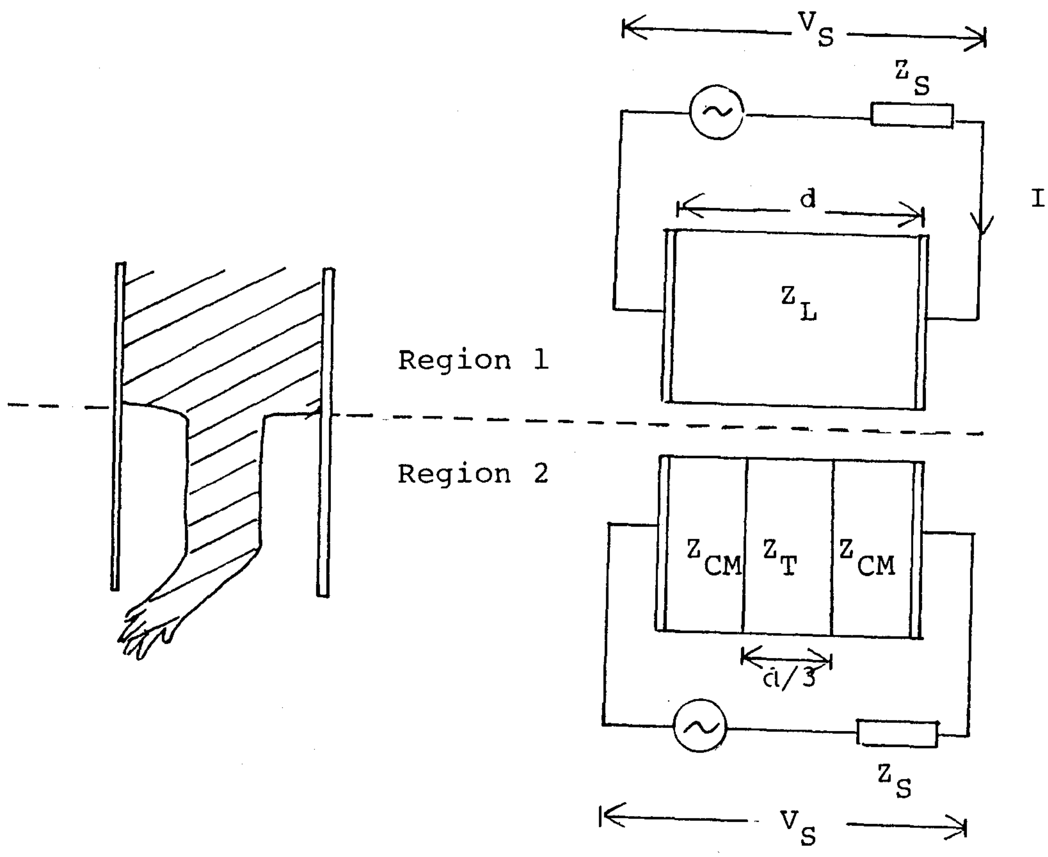
$$Z_S = Z_L^* \text{ (tissue)} = (249 + 113j) \text{ Ohm } d/A \qquad (1\text{-}8)$$

$Z_S$ is not changed when tissue is replaced as the dielectric by saline or water. The magnitude of the current $|I|$ can be found in table 1-1. It was calculated from:

$$|I| = V_S / |Z_S + Z_{L2}| \qquad (1\text{-}9)$$

where $Z_{L2}$ is the complex impedance in region 2 with the

Figure 1-1 Model for Influence of Coupling Medium on Power Deposition




Source: S.Brown (Brown 1986)

Table 1-1 Electrical Parameters of Muscle and two Coupling Media

| dielectric | tissue | distilled water | isotonic saline |
|---|---|---|---|
| $\rho$ [Ohm x cm] | 300 | $5 \times 10^4$ | 2.5 |
| $\varepsilon_r$ | 200 | 78 | 80 |
| $Z_{L1} \times d/A$ [Ohm] | 249 - 113j | 0.6 - 1700j | 2.5 - 0.004j |
| $Z_{L2} \times d/A$ [Ohm] | 249 - 113j | 83.4 - 1171j | 84.7 - 37.7j |
| $\lvert I\rvert = V_S/\lvert Z_L+Z_S\rvert$ [A] | $V_SA/498d$ | $V_SA/1108d$ | $V_SA/342d$ |
| $P_L \times V_S^2 \times A/d$ [W] | $3.35 \times 10^{-4}$ | $6.76 \times 10^{-5}$ | $7.10 \times 10^{-4}$ |
| $SAR_2/SAR_1$ | 1 | 0.2 | 2.0 |

Notes:

$\rho$ is the resistivity of the dielectric.

$\varepsilon_r$ is the relative permittivity fo the dielectric.

$Z_{L1}$ is the complex impedance in region 1 (dielectric occupies the full distance between the plates).

$Z_{L2}$ is the complex impedance in region 2 (1/3 of the distance is occupied by tissue and 2/3 by the dielectric).

$\lvert I\rvert$ is the current between the plates.

$P_L$ is the power delivered to the tissue occupying 1/3 of the distance between the plates imbedded in the dielectric.

$SAR_2/SAR_1$ is the ratio of the SAR in tissue surrounded by a dielectric relative to the SAR in tissue surrounded by tissue.

Source: S. Brown (Brown 1986)

dielectrics tissue, water, or saline.

Next the ratio of the power delivered to region 2 to the power delivered to region 1 is determined. Since D is about 1 $gcm^{-3}$ this ratio is about the same as $SAR_2$ / $SAR_1$.

The following calculation of the power delivered to the thin part of the leg $P_t$ is given for a dielectric (tissue, water, or saline) surrounding tissue in region 2.

$$P_t = |I_t|^2 \times R_t \qquad (1\text{-}10)$$

where $I_t = I \times a/A$ is the current through the thin part of the leg (depends on dielectric used)

a is the cross-sectional area of the volume of the heated tissue

$R_t = 1/3 \times R_L = 1/3 \times Re(Z_L)$ is the real part of the impedance of the target. (The thin part of the leg occupies only 1/3 of the distance between the plates.)

$Z_L$ is $Z_L$ for tissue

As can be seen in table 1-1 where the ratios $SAR_2$ / $SAR_1$ are listed distilled water, chosen as coupling medium, would result in excess heating in the thicker part of the leg. More current would flow through the thicker part, because the complex impedance of the tissue is substantially smaller than that of distilled water (see table 1-1). The ratio of the SAR of region 1 to that of region 2 is 5 (table 1-1).

If, however, isotonic saline is chosen, the opposite effect would occur and the thinner part of the leg would be heated excessively. The ratio $SAR_2$ / $SAR_1$ would then be 0.5.

The shape of a mouse leg in reality is somewhat tapered. By changing the concentration of the saline the profile of the power deposition along the leg can be changed.

From table 1-1 it can also be seen, that it is the resistivity which is primarily responsible for the differences in the SAR, since it changes rapidly, while the dielectric constant is approximately the same. It can also be seen that the ratio $SAR_1$ / $SAR_2$ is about 10 times as great when the coupling medium is water as when the coupling medium is isotonic saline.

I.C.3. <u>Excessive heating of the subcutaneous fat layer</u>

The major disadvantage of the configuration with the parallel capacitor plates is the high dissipation in the resistive subcutaneous fat, which in series may induce burnings and the possibility of bypassing targets if they have low conductivity. In the following it will be shown that fat is heated ca. 4.5 times more than muscle.

As the electric field E is perpendicular to the interface between fat (f) and muscle or tumor (m), the boundary conditions for E are given by:

$$\mathcal{E}_f^* \times E_f = \mathcal{E}_m^* \times E_m \qquad (1\text{-}11)$$

where $\mathcal{E}^*$ is the complex dielectric constant for m or f

$$\mathcal{E}^* = ( \mathcal{E} - j \times \sigma / ( \omega \mathcal{E} ) ) \qquad (1\text{-}12)$$

is the total conductivity

The power absorption per unit volume P is given by:

$$P = 1/2 \times \sigma \times |E|^2 \qquad (1\text{-}13)$$

where E is the electrical field

Therefore, the ratio is given by:

$$P_f/P_m = (\sigma_f/\sigma_m) \times (|E_f|^2 / |E_m|^2)$$

$$= (\sigma_f/\sigma_m) \times (|\mathcal{E}_m^*|^2 / |\mathcal{E}_f^*|^2) \qquad (1\text{-}14)$$

With the following values for $\mathcal{E}_r$ and $\sigma$ (from Pethig 1987):

| | fat | muscle |
|---|---|---|
| $\mathcal{E}_r$ | 38 | 152 |
| $\sigma$ | 0.21 | 0.74 |

$P_f/P_m$ is found to be 4.5.

Thus, while the conductivity $\sigma_f$ is lower than $\sigma_m$, $P_f$ is higher than $P_m$ because of the high value of $E_f$. The excessive heating is pronounced, because of the low specific heat and thermal conductivity of fat due to less vascularization of fat, by comparison with muscle.

This is a basic flaw of the configuration where parallel capacitor plates cause an electric field perpendicular to the skin and can not be avoided. However, the leg is immersed in a water bath to cool the skin and thus to prevent damage to the tissue.

I.C.4. Energy balance in Hyperthermia

Here only the basic energy equation which governs the rate of heating $d(\Delta T/dt)$ of biological tissue is briefly discussed. It can be expressed as:

$$d(\Delta T)/dt = (1 / (4186 * c)) * (W_a + W_m - W_c - W_b) \quad (1-15)$$

where $W_a$ is the SAR of RF energy [W/kg]

$W_m$ is the metabolic heating rate [W/kg]

$W_c$ is the power dissipated by thermal conduction [W/kg]

$W_b$ is the power dissipated by blood flow [W/kg]

$\Delta T = T - T_o$ is the difference between the tissue temperature $T$ and the initial temperature $T_o$ prior to treatment [°C]

$c$ is the specific heat [$J \times kg^{-1} \times {}^oC^{-1}$]

Thus it can be seen that the excessive heating of the subcutaneous fat layer is enhanced by reduced blood flow in fat.

It also can be seen that $W_a$ has to be changed when $W_b$ or $W_m$ changes during the heating. Especially the blood flow and thus $W_b$ may have strong fluctuations. Therefore it is not possible to determine the power required to heat a tumor at a steady temperature prior to the actual treatment. Hence a controlling mechanism had to be developed to keep the tissue at a constant temperature.

In chapter II. of the main section the hardware of the system is described, while chapter III. deals with the software. The results of the preliminary test runs are presented in chapter IV. . Chapter V. concludes the report. The complete listing of the program is given in the appendix. It is, due to its length, bound in a separate volume.

# II. System Hardware

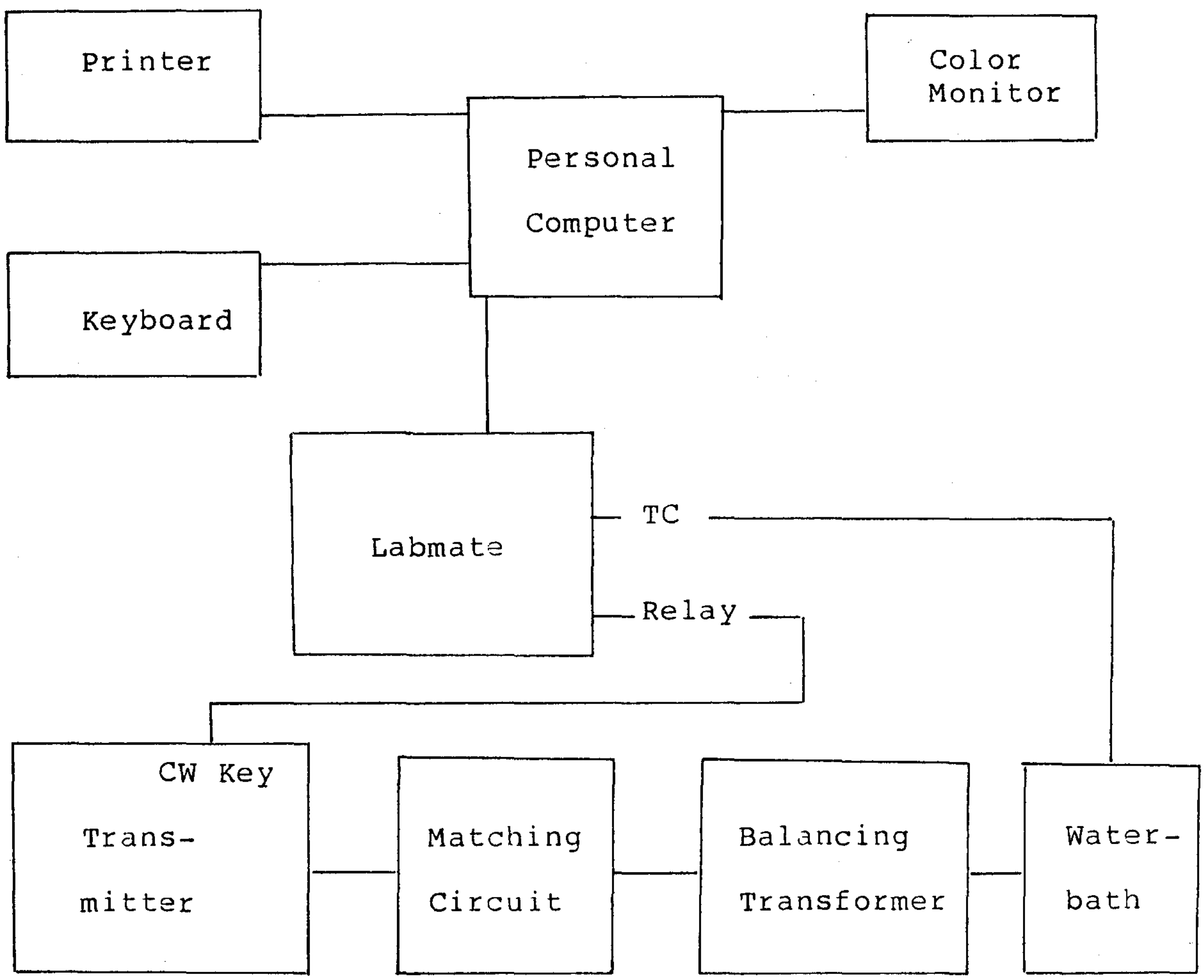
## II.A. Introduction

The heating system that has been developed in this project performed several tasks. It could be used to heat up, monitor and control the temperature of a tumor in the leg of the mouse. An overview of the system is shown in figure 2-1. The system could roughly be divided into a 'heating' and a 'controlling' part.

The heating part included a waterbath to warm up the leg to about 2 $^{o}C$ below the desired temperature and a capacitive RF heating circuit. To keep the temperature constant at the desired level, the RF field was switched on and off by the controlling part. The switching relay was part of the Labmate (see chapter II.B.), which together with a personal computer and its peripherals, formed the control part.

The rest of this section contains a description of the Labmate, the RF circuit, the waterbath and holder for the mouse. It ends with a description of the production of the TCs, and of the physical principles that underlie their use as thermometers.

Figure 2-1 Overview of the Heating System



## II.B. Personal Computer and Labmate

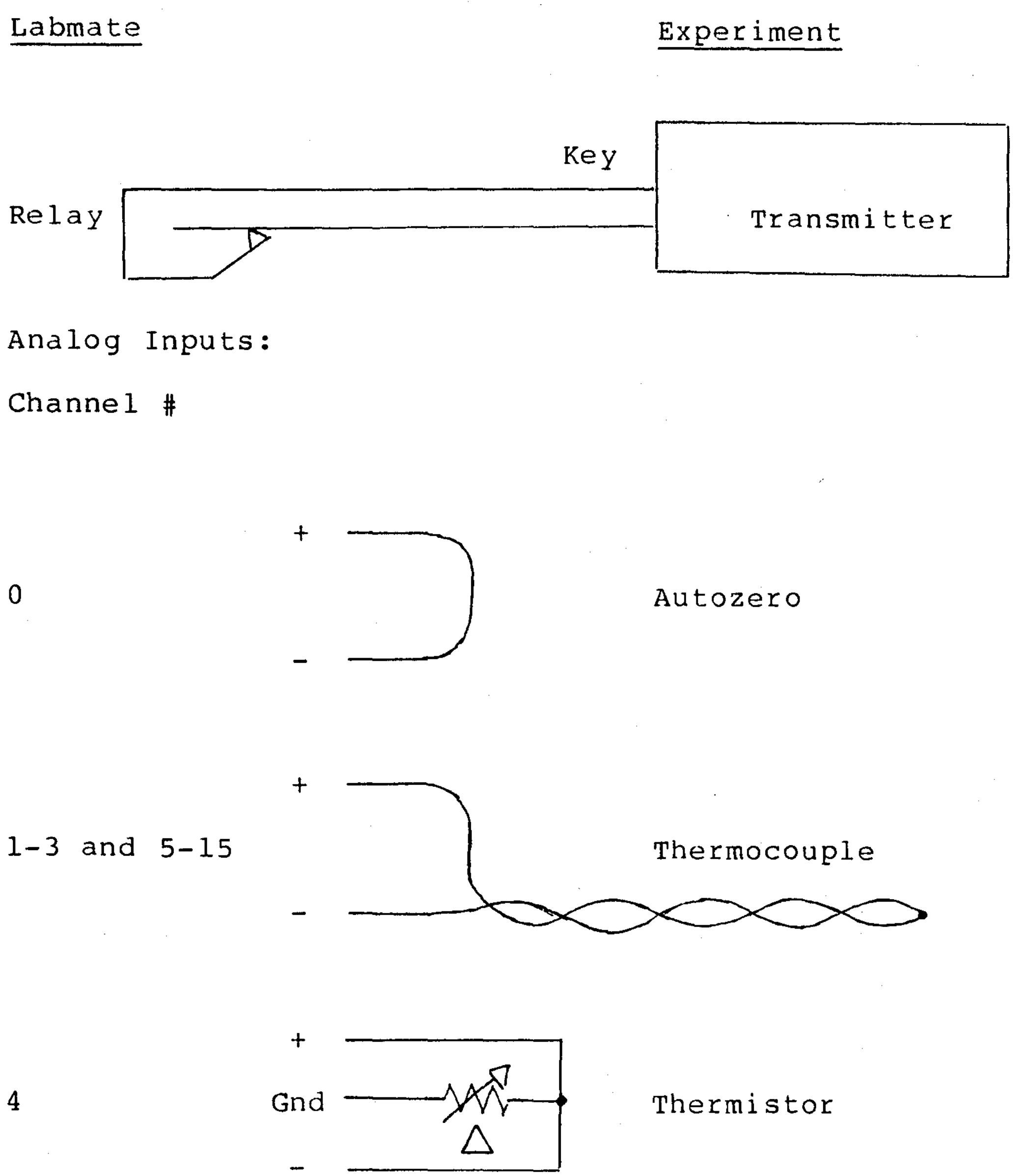
### II.B.1. Configuration of the Personal Computer (PC)

The PC used, was an IBM compatible AST 826 with 1 MByte of RAM and a 40 MByte hard disk. The main output device was a colour monitor (NEC Multisync Monitor, NEC, USA) that was controlled by an EGA card (Spectra EGA Card, Model 4800, Genoa). The computer had also a double and a high density floppy disk drive. The Labmate was connected to the computer using a dedicated interface card (IBM PC interface series 801, Sciemetric Instruments, Manotick, Ontario, Canada).

### II.B.2. The Labmate

The LabMate Data Acquisition and Control System (Sciemetric Instruments, Manotick, Ontario, Canada), in the following referred to as Labmate (LM), was a general purpose laboratory control and measuring system. It was used to connect the PC, the TCs and the RF transmitter. Among its different input and output functions, the following were required by the present work: 16 channels for differential analog inputs, and one of two SPDT relay outputs (see figure 2-2). The system used up to 14 of the 16 channels for analog inputs (A/D conversion channels) of voltages of the TCs and one of the two relay outputs to control the transmitter. In

Figure 2-2 Labmate Functions



the next section the different functions of the Labmate that were used in the experiment are described in detail.

II.B.2.a. Measurement of the reference temperature

The temperature of the connector block which housed the cold junctions of the TCs must be known accurately (see chapter II.E.1.). It was measured with a thermistor (44005 thermistor (3000 Ohm at 25 $^{o}$C), Yellow Springs Instrument Co.) which was connected to one of the A/D channels (software programmable). The measured temperature was accurate to 0.2 $^{o}$C over the range -30 to 150 $^{o}$C and was thus more accurate than this amount at or around room temperature. To convert the thermistor resistance R (in Ohm) into temperature T (in $^{o}$C) the software uses the equation:

$$T = \frac{1}{a + b \times \ln(R) + c \times \ln^2(R) + d \times \ln^3(R)} - 237 \qquad (2\text{-}1)$$

Where: ln = natural logarithm

a, b, c, d = curve fitted constants

For the thermistor that was used the values are as follows:

$a = 1.403 \times 10^{-3}$ $\quad$ $b = 2.375 \times 10^{-4}$

$c = -3.188 \times 10^{-8}$ $\quad$ $d = 1.006 \times 10^{-7}$

II.B.2.b. Auto-zero channel

Another channel, also software programmable, was used for software auto-zeroing. This technique measures periodically the voltage present on a short-circuited channel, and subtracts it from all subsequent voltage measurements to compensate for the error, induced by resistances inherent in the Labmate and the related systems. The channel number given to the auto-zero channel was 0.

II.B.2.c. A/D conversion

The LM used a multiplexed 12 bit-plus-sign A/D converter to perform the analog measurements. Hence its resolution was 0.024% of full scale (or 1 in 4096). The converter was of an integrating type with a period of integration of 33.33 ms. The differential voltage, V, was passed through a variable gain (software programmable) differential amplifier. The resolution of the measurement was then $2.4\ 10^{-6}$ V.

The software used least-square fitted equations for the thermoelectric voltage as a function of the temperature (as given by standard tables (e.g., ANSI)), assuming that the reference junction temperature is 0 $^{o}$C. Since the reference temperature in the Labmate was not 0 $^{o}$C, the forward as well as the inverse equations of T vs. V are used. Forward equation:

$$T = a_0 + a_1 \times V + a_2 \times V^2 + \ldots + a_n \times V^n \qquad (2\text{-}2)$$

Inverse equation:

$$V = b_0 + b_1 \times T + b_2 \times T^2 + \ldots + b_n \times T^n \qquad (2\text{-}3)$$

The second equation allows one to correct for the voltage induced at the reference junction. The software calculated the values for V and T to the third order. The coefficients for the E Type TCs that were used are:

$a_0 = -0.0264$ $\qquad a_1 = 17.07668$

$a_2 = -0.23082$ $\qquad a_3 = 0.00538$

$b_0 = 2.5577 \times 10^{-4}$ $\qquad b_1 = 0.05855$

$b_2 = 4.9214 \times 10^{-5}$ $\qquad b_3 = -3.0384 \times 10^{-8}$

II.B.d. <u>SPDT relay</u>

The output power of the transmitter could only be changed manually and could not be controlled automatically. However, the transmitter could be switched on and off by shorting its continuous wave (CW) key with the Labmate's relay.

## II.C. RF circuit

### II.C.1. Introduction

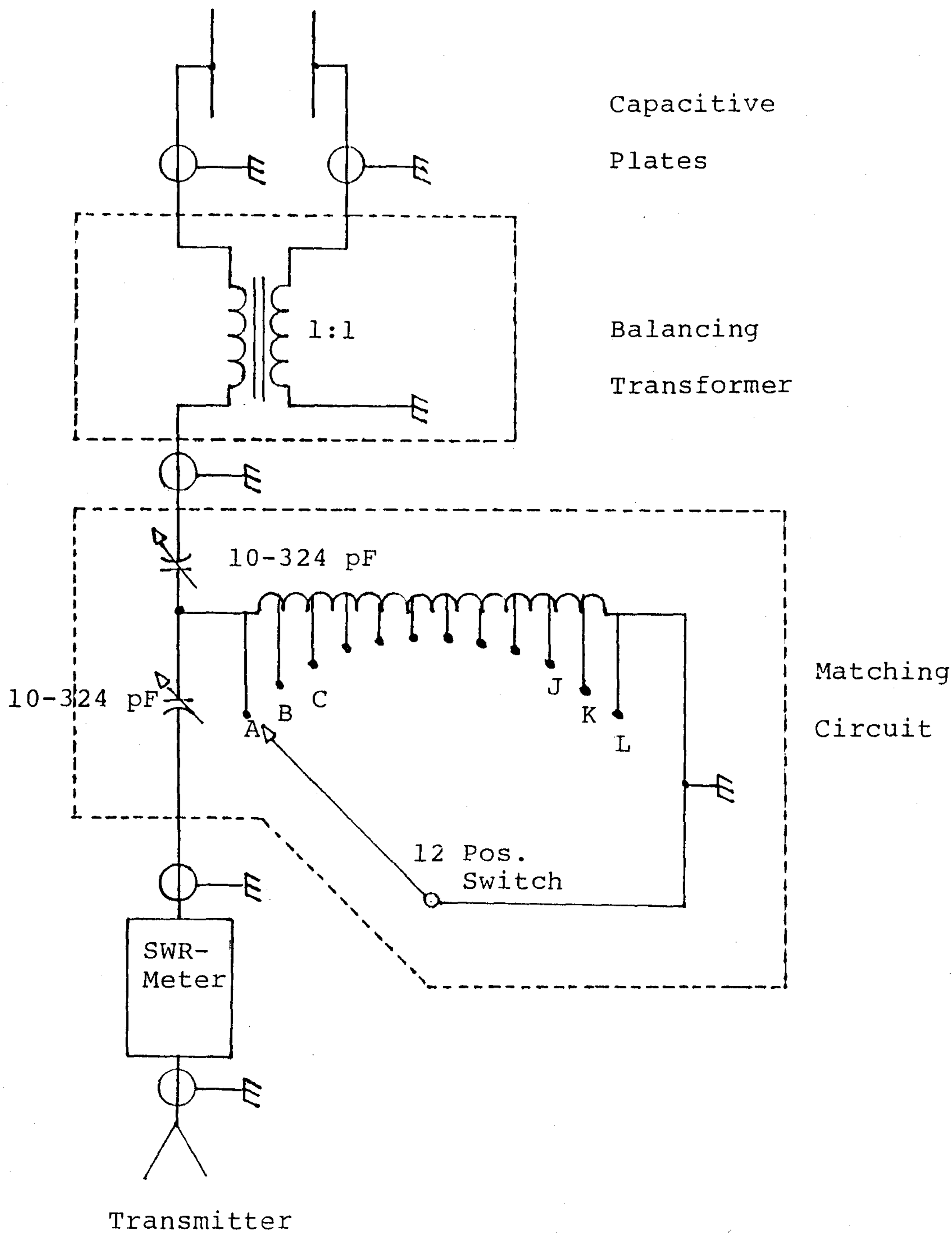
The leg of a mouse was heated up by a waterbath and by the energy absorbed from an alternating electromagnetic field. The frequency of this field was 13.56 MHz. Hence a radio frequency (RF) circuit was designed to transfer the energy from a transmitter to a pair of capacitor plates in the waterbath. This circuit included a transmitter, connecting coaxial cables (i.e. the actual transmission line (TL)), a SWR-meter, a matching circuit, and a balancing transformer. The circuit is shown in figure 2-3.

### II.C.2. Transmitter

The system used a commercially available transceiver (Model IC-735, ICOM Inc., Osaka, Japan) as the source of the RF-field. The transceiver obtained its power from an AC power supply (Model PS-55, ICOM). The power supply could deliver a maximum of 20 A at 13.8 V to the transceiver. The power input to the transceiver was 200 W at its maximum output power level.

The output power of the transceiver was continuously adjustable to a maximum of about 100 W in the continuous wave mode (CW-mode). The antenna impedance required was 50 Ohm, unbalanced.

Figure 2-3 RF Circuit



The system was operated at 13.56 MHz in the ISM frequency bands. These bands include those frequencies that are allocated exclusively to industrial, scientific and medical uses. The transceiver kept the frequency stable within a range of +/- 200 Hz after 1 min and the frequency remained within +/- 500 Hz in the temperature range 0 to 50 $^{o}C$.

II.C.3. Transmission line

A TL is a conducting system used to guide electrical energy from one point to another. Every TL has an input (or generator) end, i.e. the end connected to the transmitter, and a load end. The characteristic impedance of the TL has to be matched to that of the transmitter and to that of the load. When the characteristic impedance of the line and the impedance of the transmitter are not the same, part of the outgoing RF waves are reflected directly at the cable and are not transmitted. Similarly, when the load is not matched to the TL, the waves are partly reflected and hence not the maximum energy is transferred.

The system used coaxial cables of the type RG-58A/U. The advantages of such coaxial lines compared with two wire lines are their perfect shielding and their minimum radiation loss. Both the transmitter and the coaxial cables had the same impedance of 50 Ohm. Hence only the load and the balancing transformer had to be matched to the rest of the circuit.

Since the attenuation coefficient of the line was very small (0.3 dB = ca. 8 ft. * 3.3 dB / 100 ft. at 50 MHz) the RF circuit could be treated as a loss-less TL.

The coaxial cable that connected the transmitter with the capacitor plates was interrupted by a SWR meter, a matching circuit and a balancing transformer.

II.C.4. SWR-meter

Wave interference, due to reflection, creates standing waves of voltage and current on a TL. Measurement of these waves provide useful information concerning the electrical conditions of the line. The conditions may be defined in terms of the reflection coefficient, k, i.e. the ratio of reflected to incident voltage, $V_r$ and $V_i$ respectively:

$$k = \frac{V_r}{V_i} \qquad (2-4)$$

and the standing-wave ratio (SWR), r. The SWR is defined as the ratio of maximum root mean square (rms) voltage, $E_{max}$, or current, $I_{max}$, to minimum rms voltage, $E_{min}$, or current, $I_{min}$:

$$r = \frac{I_{max}}{I_{min}} = \frac{E_{max}}{E_{min}} \qquad (2\text{-}5)$$

It is related to the reflection coefficient by the following equation:

$$r = \frac{(1 + |k|)}{(1 - |k|)} \qquad (2\text{-}6)$$

The reflection coefficient itself is determined by the characteristic impedance of the line, $Z_0$, and the load impedance, $Z_1$, in the following way:

$$k = \frac{(Z_1 - Z_0)}{(Z_1 + Z_0)} \qquad (2\text{-}7)$$

The voltage standing-wave ratio was measured with a SWR-meter (Cross needle SWR & Power Meter, Model CN-410M, Daiwa Industry Co. Ltd., Japan) and used to determine the line performance. For the incident, reflected and transmitted powers, $P_i$, $P_r$, and $P_t$ respectively, the following relations hold true:

$$\frac{P_r}{P_i} = |k|^2 = \frac{(r - 1)^2}{(r + 1)^2} \qquad (2\text{-}8)$$

and

$$\frac{P_t}{P_i} = 1 - |k|^2 = \frac{4 \times r}{(r + 1)^2} \qquad (2\text{-}9)$$

Maximum power is transferred when the SWR is equal to 1, i.e. at its minimum. In this case no reflection occurs ($k = 0$ and $P_r = 0$) and, assuming the attenuation is negligible, the incident power is completely transferred ($P_t = P_i$).

II.C.5. Matching circuit

The matching circuit (200 Watts Versa Tuner, Model MFJ-901, MFJ Enterprises, Inc., Starkville, Mississippi, USA) permits matching the impedance of the load and the line to the impedance of the transmitter. The circuit is made of two continuously variable capacitors in series and a variable inductance with discrete values (see figure 2-3). The capacitors and the inductance were chosen to be variable because the impedance of the load, i.e. the impedance of the system capacitor plates, saline, and leg of the mouse, could change from experiment to experiment.

II.C.6. Balancing transformer

The RF circuit was balanced, w.r.t. the ground, by a transformer in order to reduce the RF pick-up of the TCs. The transformer provides a 1:1 matching of the signal from the transmitter to the loaded plates. The transformer itself has an iron-powder toroidal core (T-106-2 red, Amidon

Associates Inc., North Hollywood, California, USA) with a frequency range from 1 to 30 MHz.

A highly accurate ratio for a 1:1 ratio transformer can be achieved using a bi-filar winding. This gives a voltage ratio that is the same as the turns ratio to a higher accuracy than any other form of winding. Because the voltages of two completely symmetrical windings are affected identically by the presence of inductance, resistance and capacitance, their ratio is precisely equal to the ratio of the electromagnetic forces (emfs) induced in the windings. To achieve this, a pair of twenty-two gauge enamelled magnet wires, were twisted together sufficiently tightly so that they lay in contact throughout their length. The twisted wires were encapsulated in a Teflon tubing.

One wire was grounded on the input side of the transformer and the other was connected to the matching circuit. At the output of the transformer, since the connecting lines were identical, the signals were $180^{o}$ out of phase , i.e. they were balanced. This reduced signal pick-up between the plates, since the signals from each plate were exactly equal in magnitude and of opposite phase. A TC that is positioned halfway between and parallel to the plates will thus have only a very small pick-up.

## II.D. Waterbath and Holder

### II.D.1. Introduction

A waterbath was used to augment the RF field in heating the tumor. This was done by immersing the leg in approximately 50% isotonic saline, which was kept at a fixed temperature of approximately 2 $^{o}C$ below the desired temperature, so that the skin suffered negligible thermal injury.

In the following the terms 'water' and 'saline' are used as synonyms, but both refer to the 50% isotonic saline.

The design of the hybrid waterbath/RF-heater was based on the work of Joiner et al. and Brown. The waterbath and the holder have been modified such that the mouse sat above the surface of the water with only one leg immersed. The bath served several purposes. First, it helped to warm up the tumor. Second, it provided good coupling between the RF field and the leg. Third it prevented overheating of the skin, which was caused by the higher absorption of the energy of the alternating em-field in the subcutaneous layer of fat. The last two aspects have been discussed before (see chapters I.C.2. and I.C.3.). If the leg were only heated by a waterbath, cooling by the flow of blood would result in a temperature gradient from the warmer skin to the cooler inner regions of the leg. A more uniform temperature distribution is achieved by combining the waterbath with a capacitive RF heater.

II.D.2. Water-Circuit

The water-circuit consisted of a waterbath for the mouse, a reservoir, a circulating heater and connecting tubes. Saline was circulated from the reservoir to the waterbath and back to the reservoir by a pump of the circulating heater.

The reservoir contained about 6 litres of saline. The circulating heater, equipped with a thermostat, was used to maintain the saline temperature at a constant level above room temperature. The temperature of the saline was continually monitored with thermocouples placed near the leg of the mouse. For a schematic diagram of the flow of water and a view of the bath see figure 2-4.

II.D.3. Holder for the mouse

The mouse was held in a small box made out of lucite. The box, as shown in figure 2-5, sat on top of a plate which was above the surface of the water. The box had drilled holes at the top, front and rear ends and the sides, to allow air to circulate around the mouse. A fan was used to help cooling the body of the mouse, should its body temperature exceed the critical temperature of 40.5 C. The box also had an opening in the rear bottom through which the

Figure 2-4 Water circuit (diagram)

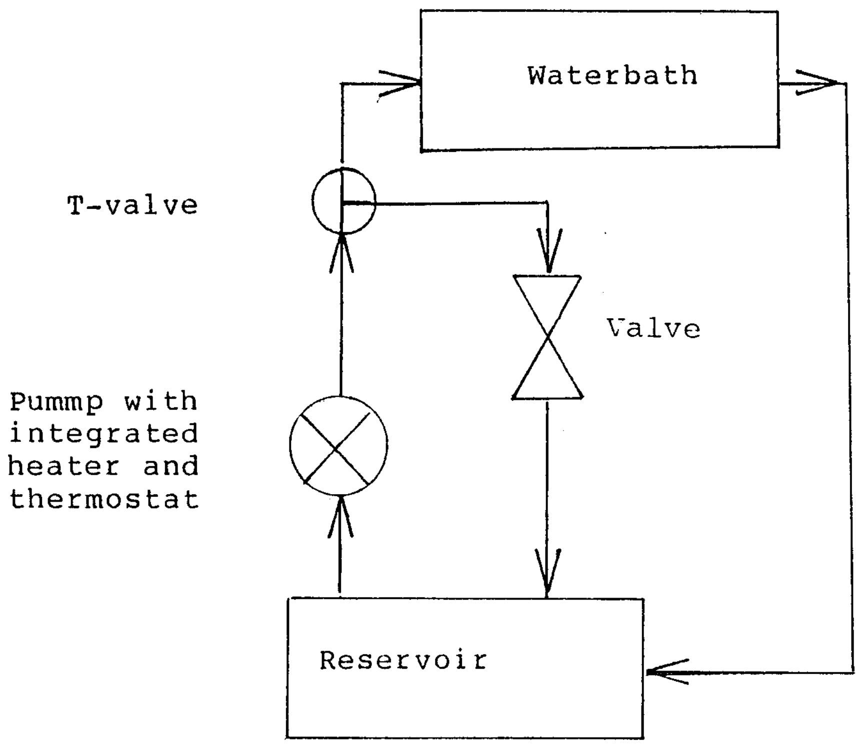
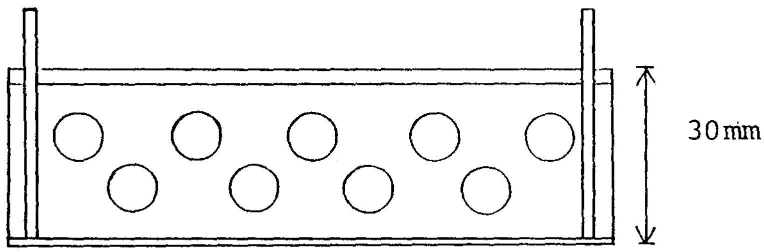
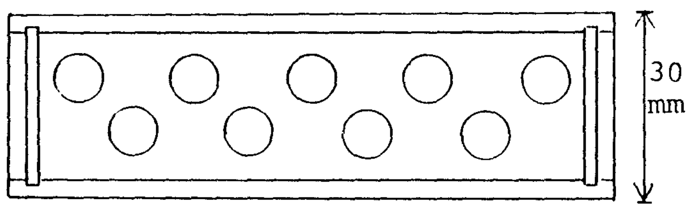


Figure 2-5 Box for Mouse

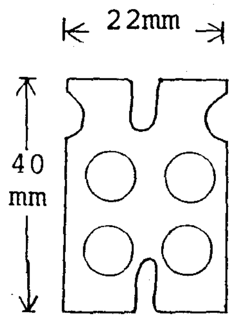
Side View:



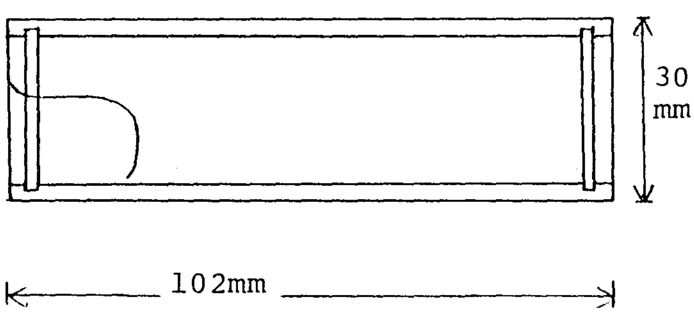

From Top:




Rear:




From Bottom:




Front:

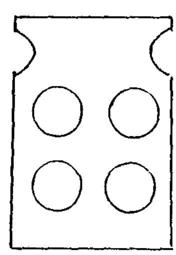


Figure 2-6 Top View of the Waterbath

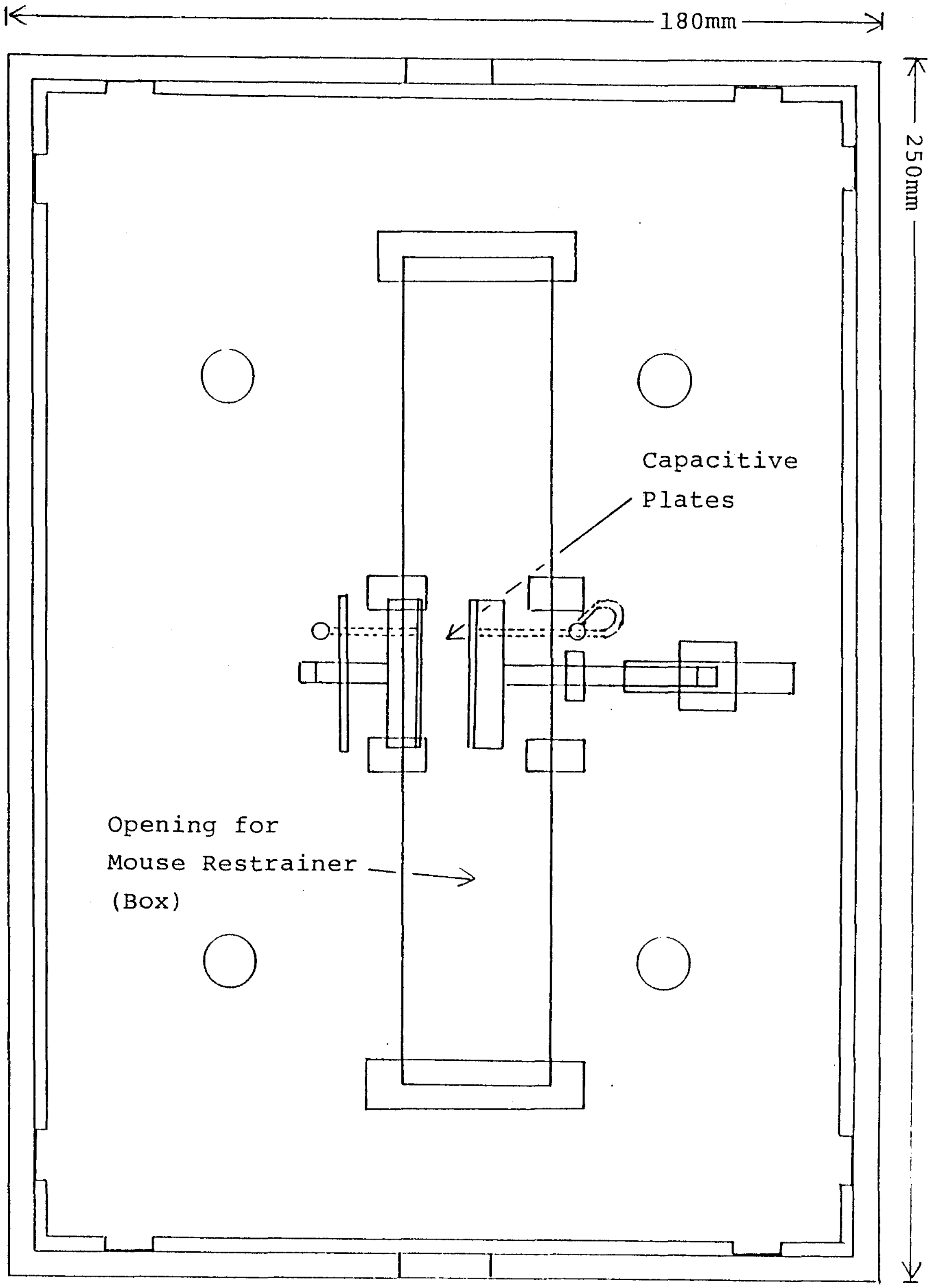


Figure 2-7 Side View of the Waterbath

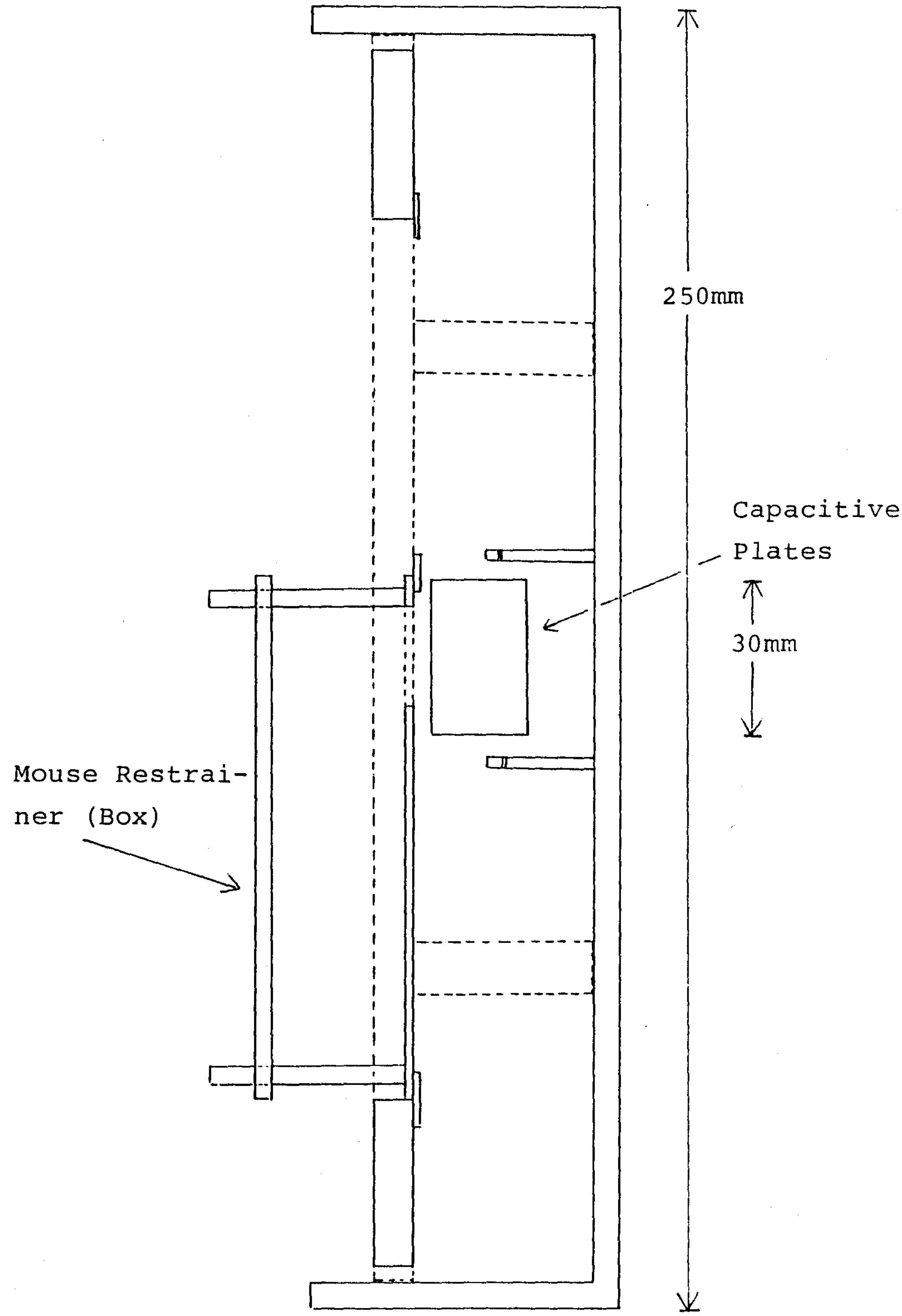
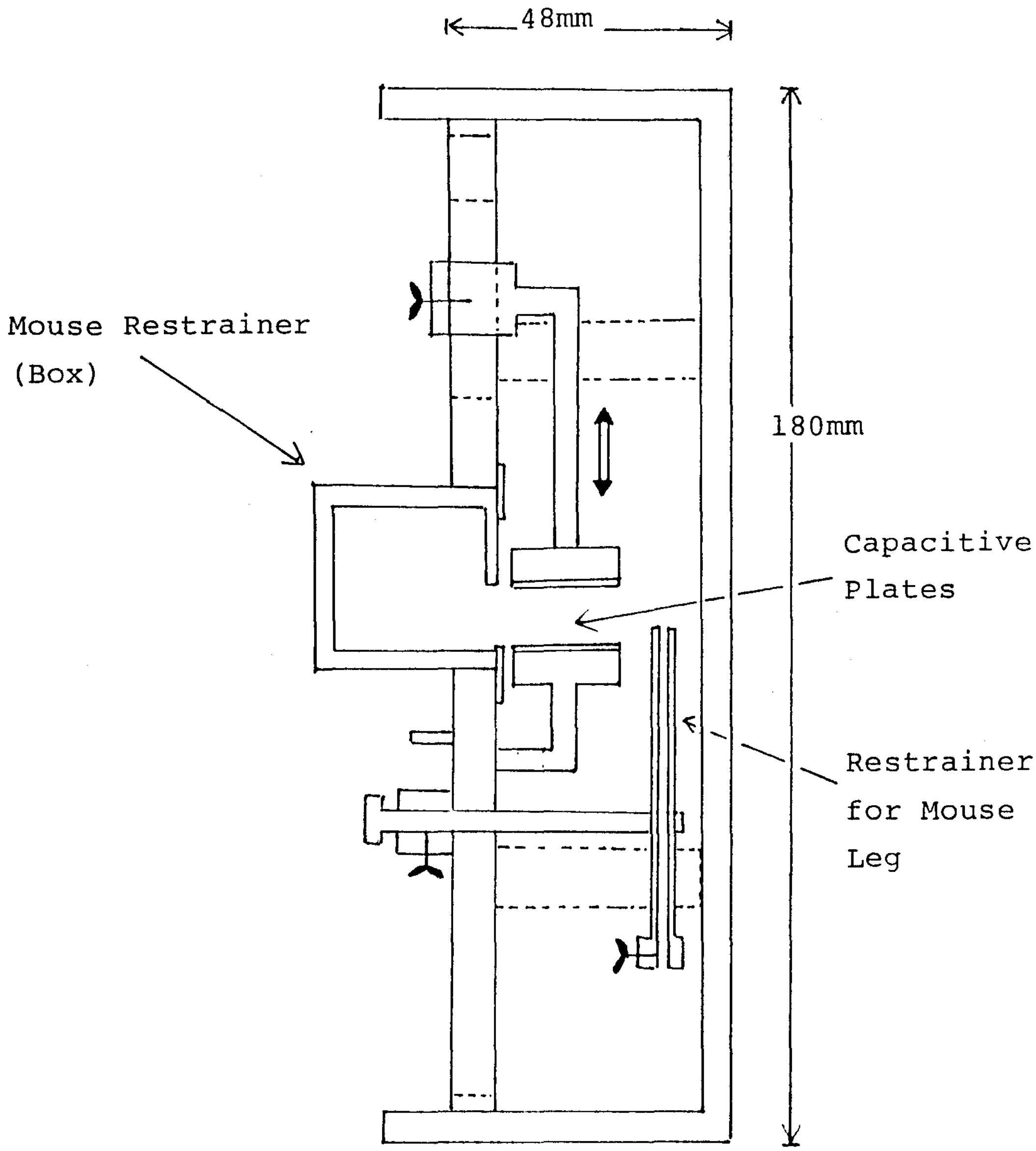


Figure 2-8 <u>End View of Waterbath</u>



leg was pulled down into the water. The leg was held between the plates by a small loop made out of plastic tube and fitted just above the knee. Figures 2-6 to 2-8 show the top, the side, and the end views of the waterbath, respectively.

II.E. <u>Thermocouple thermometry</u>

In order to keep the temperature of the tumor constant, its temperature had to be measured. In addition, the temperatures of the waterbath, the surrounding air, and the rectum were also measured. The system used TCs for all these measurements. Since TC wires of the required thickness (2.5 mil diameter) are not robust a "welder" for TCs was built. Thus they could be manufactured locally and their configuration and length could be customized. Should they fail, they could be replaced immediately.

II.E.1. <u>Physical principles of TC thermometry</u>

The measurement of temperatures with TCs is based on the Seebeck effect. This effect occurs when two wires, composed of different metals, are joined at both ends and the ends of the wires are kept at different temperatures. Under these conditions a continuous current flows through the wires, driven by the thermoelectric force.

When the wires are only joined at one end and the loose ends are kept at a temperature different from that of the junction, also called the hot junction, a voltage in an open circuit is created. The loose ends are normally called the reference junction or the cold junction. The temperature difference between the two ends of the TC is determined once the voltage is measured. If the temperature of the reference junction is known, the unknown temperature at the hot junction can then be calculated. The calculation is facilitated by calibration tables, set up for the internationally standardized TC types.

Temperature in the present work was measured with Type E TCs. This is one of the internationally adopted types of TCs. Its negative arm is made of constantan (TM), a copper-nickel alloy (45% Ni, 55% Cu). The positive arm is made of chromel (TM), a nickel-chromium alloy (90% Ni, 10% Cr). The wires were obtained from California Fine Wire Co., USA. They had a diameter of 2.5 mil and were insulated with polyimide.

The decision to use TCs of Type E, and not those of the more widely used Type K, was based on the fact that Type E TCs have the highest emf output of common TCs. They have a thermopower, i.e. a ratio of the change in voltage to the change in temperature, of 58.5 $\mu V/^{o}C$ at 0 $^{o}C$ rising to 81 $\mu V/ ^{o}C$ at 500 $^{o}C$. At temperatures below 800 $^{o}C$ this type also has a lower drift rate than the Type K. Other reasons to choose the Type E are the low thermal conductivity of both its arms and the fact that the alloys, chromel and constantan, can be manufactured with good homogeneity.

It should be noted, that the thermoelectric effect is a bulk property of the material and not a junction effect. Hence it only depends on the types of metal or alloy used and the temperature gradient from the hot to the cold junction. The only function of the hot junction itself is to provide electrical continuity.

II.E.2. The TC-welder

The welder was the device used to produce the junctions for the thermocouples. When TCs were made, one end of the wires had to be joined together. Here this was done by melting them together. The rapid discharge of a capacitor delivered the energy, which was necessary to heat the wires up to their melting point.

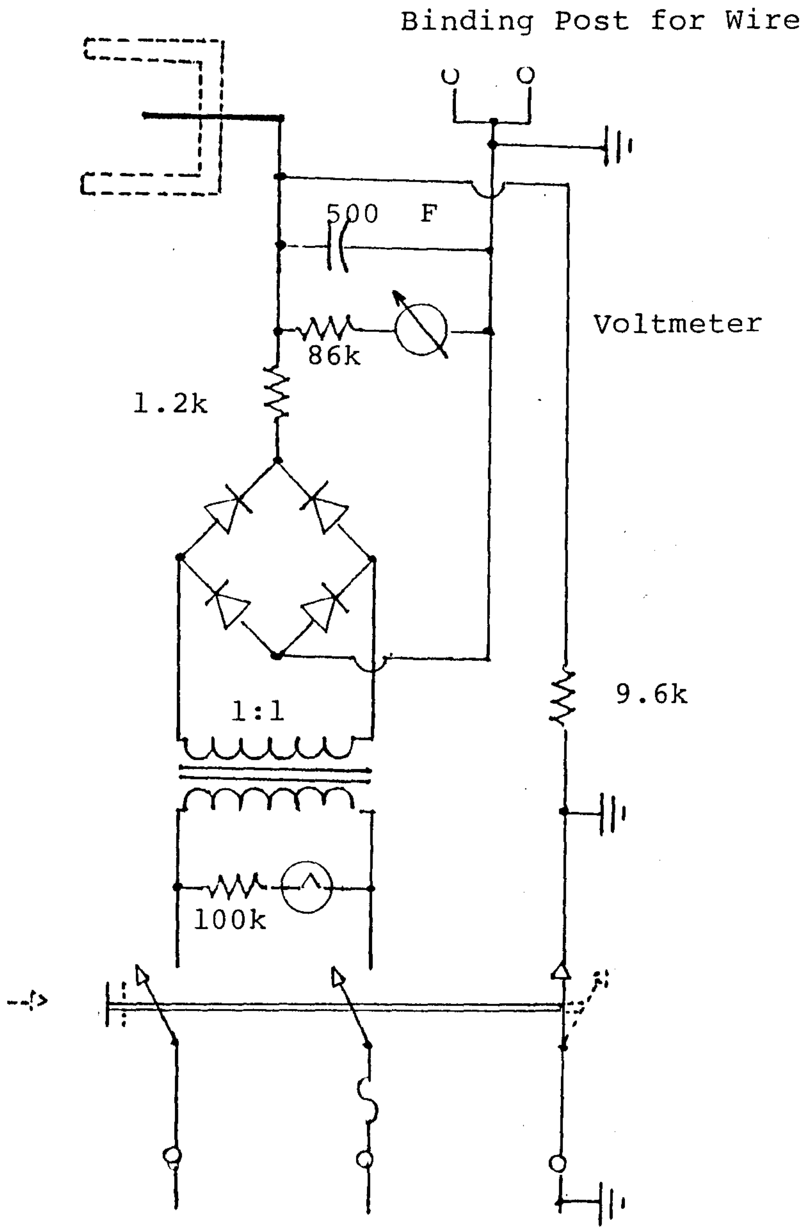
Circuit of the welder

For the circuit of the welder see figure 2-9. The main elements of the circuit were a transformer, a rectifier bridge and a capacitor. The 1:1 transformer separated the rest of the circuit from the power main (110 V). This was necessary since the insulation of the TC-wires is too thin to withstand the high voltage of the power main. The bridge consisted of 4 diodes that rectify the alternating current. The capacitor ($5 \times 10^{-4}$ F) was charged up by the bridge and was connected to the posts and to the carbon electrode. A voltmeter was connected parallel to the capacitor and measured the rate at which it was charged up. A fuse protected the circuit against a short-circuit. A resistor (9.6 kOhm) was connected parallel to the capacitor to discharge it after switching off the power. The part of the circuit that was connected to the posts was grounded. This should further increase the safety of the user of the device. In addition a small pilot lamp indicated whether the power was switched on or off.

II.E.3. Operation of the Welder

First the reservoir around the electrode was filled with glycerol. A piece of chromel wire and a piece of constantan wire of the desired length were twisted together with the help of an electric machine drill. The insulation

Figure 2-9 Electric Circuit of the Welder



of one end of the wires was removed by burning. Then these ends of the wires were connected to the posts. The insulation was also removed from the other ends of the wires for a length of 1 mm. Those ends were then twisted together. The welding was done by a spark to the twisted end of the wires. The capacitor was discharged by bringing the twisted ends close to the electrode. The electrode was submerged in a glycerol bath. The immersion of the wires should prevent the oxidation of the alloys. After the junction has been made the wires were wound around a suture to strengthen them. The TC was ready to be connected to the Labmate and to be calibrated. The calibration is described in chapter IV.A..

## III. Software

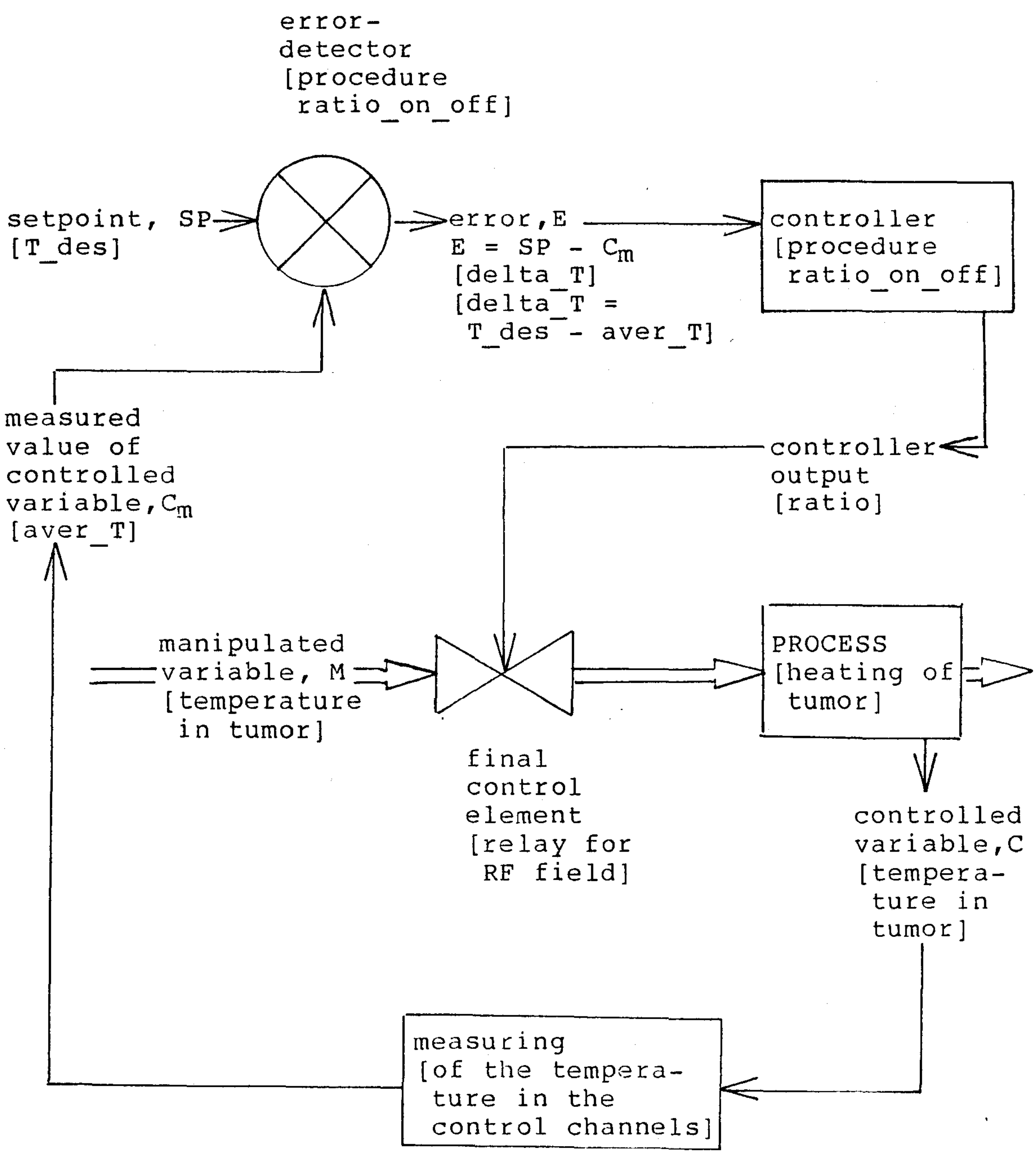
### III.A. Introduction

The hyperthermia system was controlled by a computer via the Labmate. The computer controlled the measurement of the temperature in the tumor, displayed temperature versus time curves, and used the information to switch on and off a relay that in turn switched on and off the output of the transmitter, thus maintaining a steady temperature at the desired level. Different functions of the Labmate were controlled by the software provided with the interface. The interface procedures to access them were originally written in IBM PC BASIC Version 2.0 and were delivered with the Labmate. They were translated by A.D.A. Maidment into Turbo-Pascal Version 3.02A. These Pascal procedures were the basic units of the program written to control the system. Maidment also wrote the procedures that were used to control the EGA-card of the colour monitor, which is not supported by Turbo-Pascal Version 3.02A.

### III.B. Theory of temperature control

In order to maintain a desired steady temperature, a feedback loop was installed. First, a description of a generalized loop is presented, followed by the one

Figure 3-1 Blockdiagram of a feedback loop



implemented. Both are shown in figure 3-1. The terms in square brackets refer to the implemented loop, those without to the generalized version.

The generalized feedback loop works in the following way: A setpoint, SP, is given which is compared with the measured value of the controlled variable, $C_m$, in the error detector. The output of the error detector, error, E, is converted by the controller into the controller output which in turn controls the final control element. This element influences the process itself thus changing the manipulated variable, M, and with it the controlled variable, C. The variable C is measured and the result of the measurement is the measured value of the controlled variable, $C_m$, that has been mentioned above.

The system implemented gives those quantities specific meanings: The setpoint is the desired temperature, T_des, and the error is now the temperature difference, T_delta. The error detector and controller are both implemented in the procedure ratio_on_off which returns the ratio of time on to time off, ratio (as the controller output). The final control element is a relay in the Labmate that switches the power on and off. The manipulated variable, M, is the temperature in the leg of the mouse whereas the controlled variable, C, becomes the temperature in the tumor at the site of the Tcs. The temperature measurement with the TC results in the average temperature in the control channels, T_aver, which is in the generalized model the measured value of the controlled variable, $C_m$. The T_aver is then compared with the T_des and the resulting T_delta determines the output, ratio, of the procedure ratio_on_off.

## III.C. The program

The program listing can be found in (Schaarschmidt 1988). It is mostly self-explanatory. In the following section the structure is outlined and the procedure ratio_on_off is described in detail. At the end a list of all the procedures used is given together with a short description of their functions.

### III.C.1. Structure of the main program

The main program had three parts: the initial part, the feedback loop that controlled and monitored the temperature, and the final part that wrote out the data and plotted them again, if desired. Figure 3-2 and 3-3 show its block and its flow diagram, respectively. It was highly modularized, to make it more readable and thus more verifiable. Another advantage of the structured program is the fact, that it is also easier to change, should changes become necessary in the future. Pascal, the programming

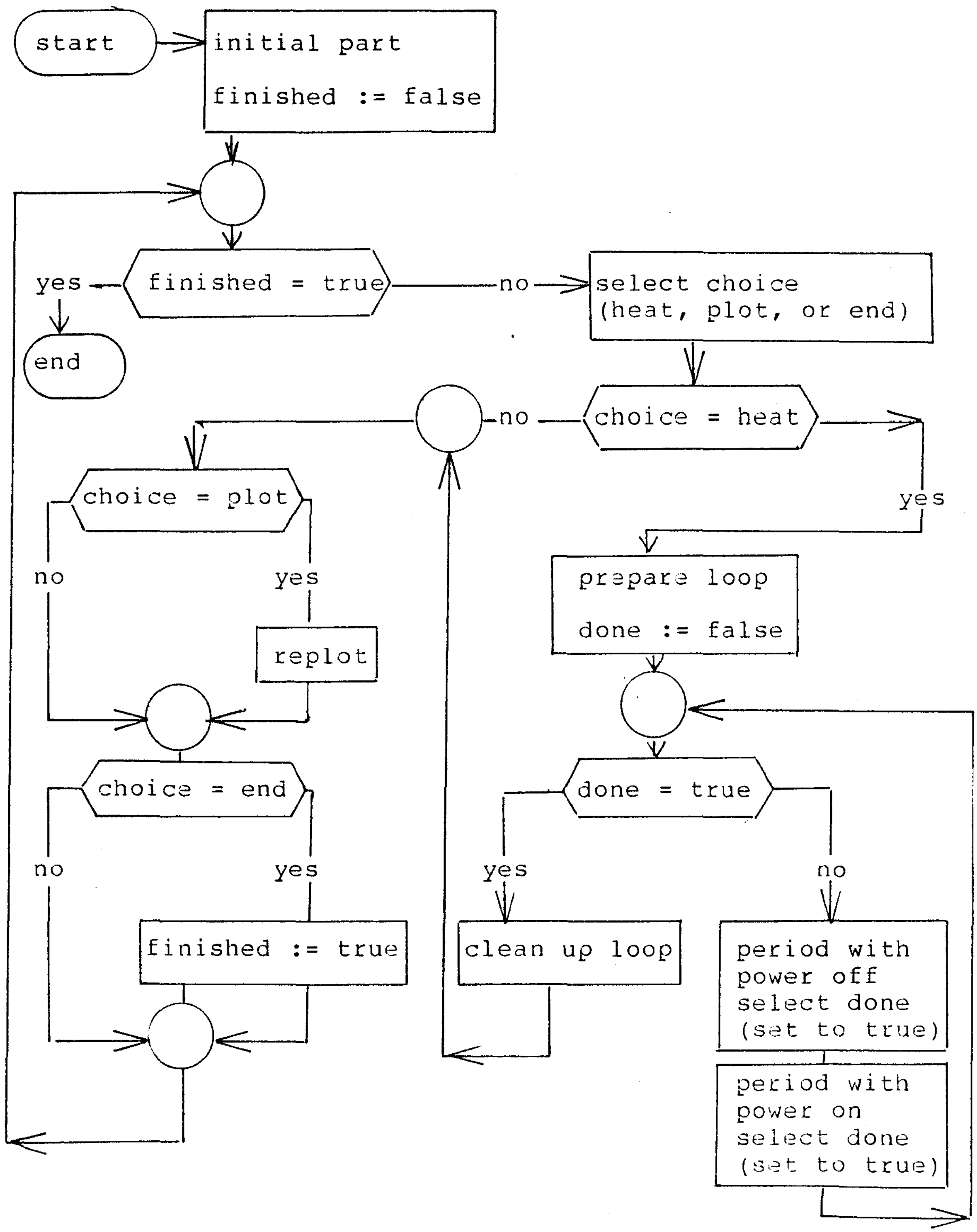
Figure 3-2 A block-diagram of the main program

```
(begin of program)

1.INITIAL PART
  switch RF power off
  assign variables
  initialize screen
  read file labmate.par
  initialize labmate

2.CHOICE OF OPTIONS

  WHILE NOT FINISHED

    START OF NOT FINISHED LOOP

    select (HEAT, PLOT, or END)

    if HEAT then
       input user parameter
       write user parameter to file
       initialize variables for loop

       START OF LOOP

       PERIOD  WITH RF POWER OFF
       wait settling time
       measure reference temperature
       sample temperature with TC
       calculate ratio time-on/time-off
       wait for rest of time-off
       check for ending

       PERIOD WITH RF POWER ON
       switch RF power on
       record temperature
       plot temperature vs time curve
       wait for rest of time-on
       check for ending
       switch RF power off

       END OF LOOP
```

(continued on next page)

```
    (from previous page)

        FINAL PART OF HEAT
        switch RF power off
        write out data

    if PLOT then
      replot

    if END then
      quit WHILE NOT FINISHED

    END OF NOT FINISHED LOOP

(end of program)
```

Figure 3-3 Flow Diagram of Main Program



language used, supports modules, due to the structures it provides. Its strong typing of variables made it also easier to debug the program. The program was stored on separate files to make the handling easier. These 'include files' were imported into the main program when it was compiled.

III.C.2. Algorithm for procedure ratio on off

This procedure, the most important part of the program, calculated the ratio of the times the power was switched on and off. The procedure first took the average, aver_T, of the temperatures of the control channels. Then it compared this average temperature with the one desired, T_des. It used the temperature difference, delta_T, later to determine the ratio of time with power on to time with power off. In the following the value of this variable is referred to as ratio, r. Delta_T was defined as:

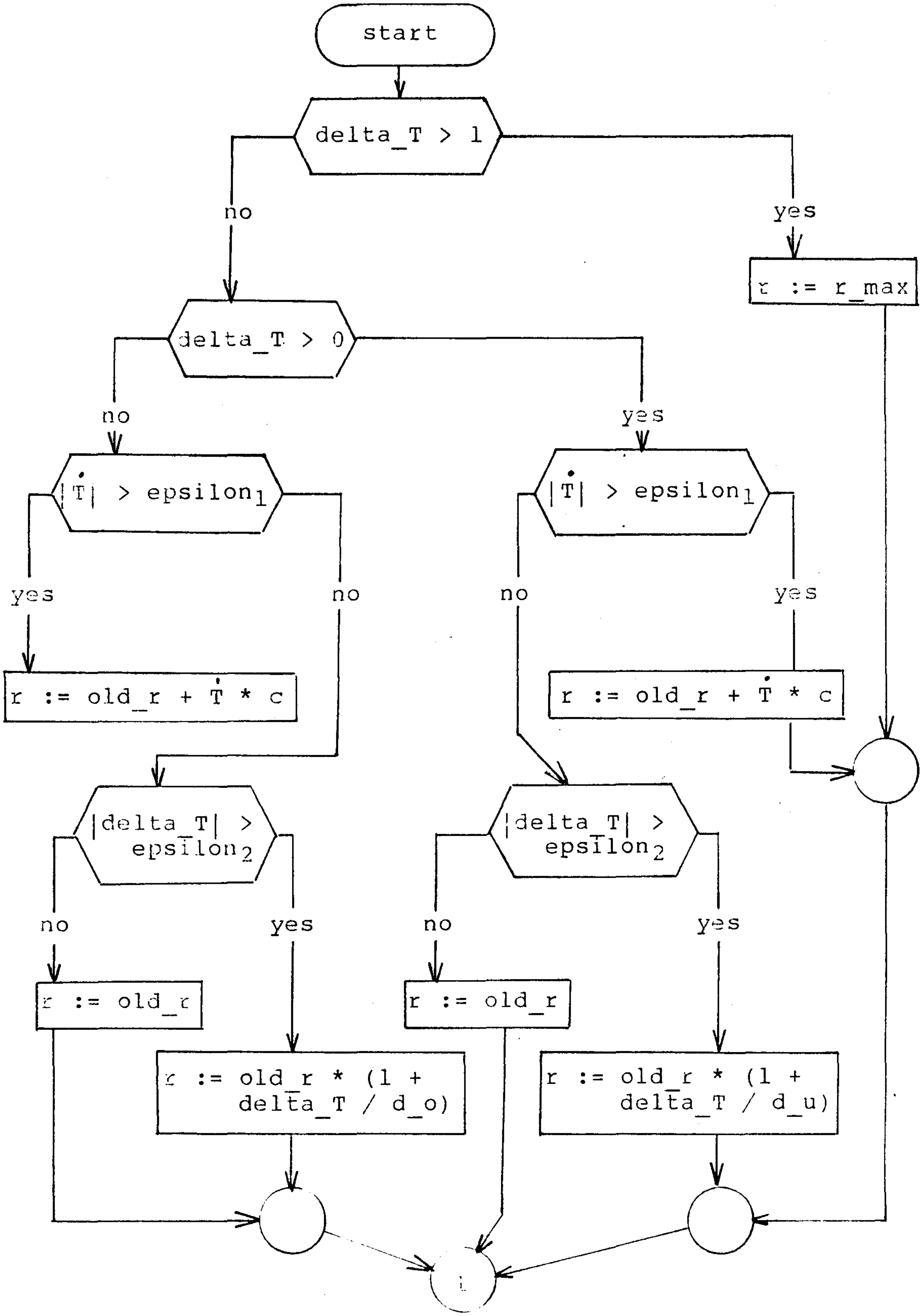
delta_T = T_des - aver_T (3-1)

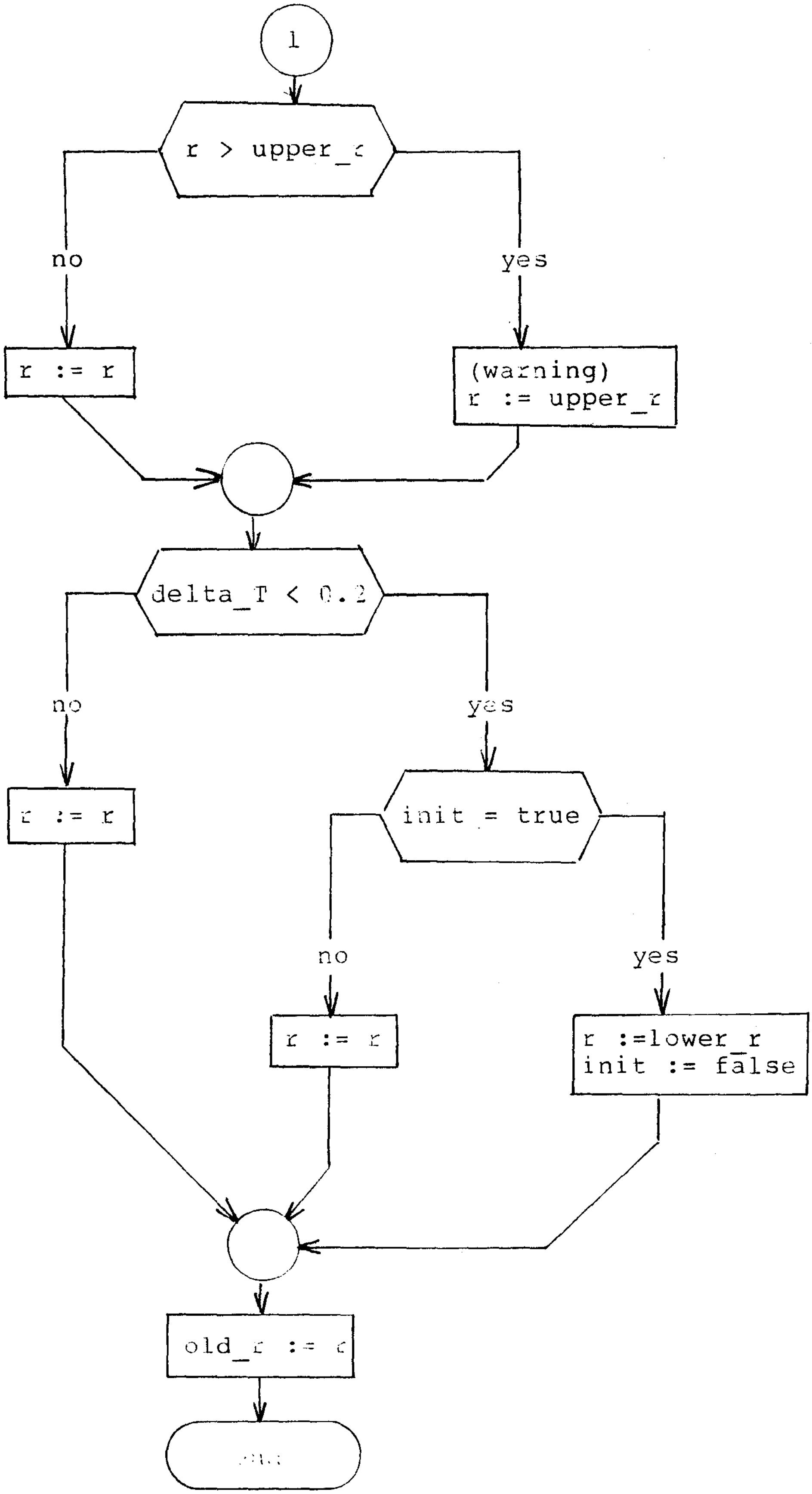
A flow diagram of the procedure, starting with delta_T, is given in figure 3-3. To avoid oscillations due to the inertia of the system, a parameter that was related to the rate of change of delta_T, T_dot, was introduced:

T_dot = delta_T - old_delta_T (3-2)

where old_delta_T was the delta_T of the previous cycle. In the flow diagram $\dot{T}$ is used instead of T_dot, although this quantity is not the derivative of delta_T w.r.t. time. Where

Figure 3-4 Flow-diagram of procedure ratio-on-off




1
r > upper_r
no
yes
r := r
(warning)
r := upper_r
delta_T < 0.2
no
yes
r := r
init = true
no
yes
r := r
r :=lower_r
init := false
old_r := r

necessary, the resulting ratio is set to its maximum upperlimit, the maximum ratio, r_max, so that heating proceeded most quickly.

Calculation of r

The algorithm first applied the highest permitted ratio, r_max, which was limited by the performance of the transceiver used, until delta_T has dropped to 1 $^{o}$C.

As the next step, the sign of delta_T was determined. Even though the following steps were the same for the aver_T below or above T_des, a distinction was made, so that the constants used to calculate the ratio could be set differently for the two ranges.

Before the temperature was brought closer to T_des the rate of change was slowed down in order to reduce oscillations. Here the value of ratio, r, was determined by T_dot in the following way:

$$r = old\_r + T\_dot * c \qquad (3-3)$$

where c was a constant factor.

As soon as the temperature was stable, i.e. the absolute value of T_dot was within the specified range, $epsilon_1$, the absolute value of delta_T was compared with $epsilon_2$, which was the permitted range of delta_T. The ratio was changed in such a way that delta_T was reduced, should delta_T be outside that range. The ratio, r, was then set to:

$$r = old\_r * ( 1 + ( delta\_T / d ) ) \qquad (3\text{-}4)$$

where d was a constant.

Should the ratio r as calculated exceed an upper level, r_up, which was set to less than or equal to r_max, r was set to r_up.

To avoid a large overshoot in the initial heating phase, the ratio was set back to a small value when delta_T has fallen to 0.2 $^{o}C$ for the first time. The logical variable init was introduced to keep track of it. At the start of the experiment it was set to true and was changed to false after the first overshoot. This interruption prevented the initial overshoot to be larger than 0.5 $^{o}C$ (see chapter IV).

III.C.3. Other routines

As the system will be used under different conditions, with different parameters, emphasis has been placed on the input of these parameters. The user has the choice to define all the parameters or only some of them new. He can also use the parameters, that were used in the previous experiment.

The number of samples that could be stored was limited to 500. Therefore the procedure recording stores the sampled data only every 1/500 of the total time the experiment was run. The file that the data were written to, after the heating was finished, was formatted in such a way, that it could be read into a spreadsheet (Symphony or Lotus 1-2-3, both Lotus products) or into a graphics program (DataTap, Temple University)

EGA routines

These routines were used to control the Genoa EGA card that drove the NEC Multisync Colour Monitor. They provided the basic graphics functions.

**HiResMode** - sets the screen into the high resolution mode (16 colours and 350 x 640 pixels).

**HiResScreen** - switches between the two screen pages 0 and 1.

**HiResXY** - sets the cursor in the text mode (25 lines and 80 characters) to the position provided.

**HiResPut** - prints a character n times in the specified colour starting from the current cursor position.

**ConWrite** - replaces the CON: device interface in Turbo Pascal. It is used by the compiler to print characters to the screen.

**ConInit** - initializes the vector pointing to the CON: device service routine (above).

**ScreenColor** - sets the colour of the text, when it is printed by the CON: device handler.

**HRWrite** - can print a line of text.

**HRPlot** - plots a line from x1, y1 to x2, y2 in the specified colour.

**HRRead** - returns the colour of the pixel specified in input.

**DrawBox** - draws a box with the upper left corner at x1, y1 and the lower right corner at x2, y2.

<u>Labmate routines</u>

The following subset of routines was needed to control the Sciemetric Instrument Labmate. The code was in part translated from the BASIC programs, that were provided with the Labmate.

**viainit** - issues a hardware reset to the VIA. It is used after the detection of errors and during the initialization procedure.

**errorhand** - handles the various error conditions that may occur in general usage of the Labmate. The error is printed at the top of the second screen (screen 1).

**relay** - sets the desired relay states and turns the RF-power on and off.

**ad2** - samples the output of the A/D-converter. It is called exclusively by ad1 and should under no circumstances be called directly by the user.

**ad1** - is the main A/D conversion control procedure. It performs all the primary measurements.

**gpvolts** - is a general purpose voltage routine and can be called with several parameters. It returns the differential voltage across the high and the low voltage inputs, when the parameter func is set to 0.

**thermocouple** - tests the temperature on an analog channel, containing a J,K, E, or T type thermocouple.

**w3ohms** - determines the resistance on a specified channel, using a three wire impedance measurement technique.

**thermistor** - returns the temperature measured by a thermistor in a specified channel.

**reftemp** - determines the temperature of the cold junction, and the backfit coefficients to correct for the temperature of the isothermal block in TC measurements.

**bitloop** - cycles through each bit of the specified output register of the Labmate for error testing.

**regtest** - is a general purpose Labmate register test.

**init** - initializes the operation of the Labmate.

Labmate parameter input

This routine reads parameters that are not to be changed, unless a different model of the Labmate is used or the system is changed.

**parameter_labmate** - reads and displays the parameters that are used to control the low level functions of the Labmate. These parameters are not changed by the user.

User parameter input

These procedures allow the user to set the parameters used in the program to control the experiment. The user has three main options (see description of the procedure choices_of_parameters).

**display_parameters** - displays the chosen parameters onto the screen.

**parameter_input** - allows the user to change the user parameter. It calls the following routines, which have not been listed separately, to enter the individual parameters: **inp_per_tot_time, inp_settl_time, inp_per_toff_min, inp_per_ton_max, inp_np, inp_tc_type, inp_tc_labels, inp_temp_des, inp_contr_chan, inp_name, and inp_repetition.**

**predefined_default_parameters** - allows to use parameters that are set by the programmer.

**write_default_to_file** - saves the parameters that have been chosen into a file, named 'default', on the disk.

**read_default_from_file** - reads the content of the file written by the procedure above.

**choice_of_parameters** - allows the use of the values of the last run, by calling read_default_from_file, to take the previously defined values, by calling predefined_default_parameters, or to set new values for the parameters by calling parameter_input. It also includes a conditional call of display_parameters to enable the user to check the values entered.

Sampling and measuring routines

These routines are of a higher level than those of the section 'Labmate routines' and are called by the main program.

**sampling_temperature** - returns the temperature of the channels and the time of the measurement. It calls the procedures thermocouple and time2.

**set_variables** - sets the variables and flags for the loop and the procedure thermocouple.

**ratio_on_off** - returns the ratio of time with power on to the time with power off. Its structure has been explained in chapter III.C.2. above.

**recording** - this routines determines which of the data collected are stored and which are discarded.

Program control routines

These two routines allow the user to stop heating and to select one of the options listed in the description of select.

**decision_to_end** - ends the heating cycles when a maximum number of cycles has been reached or the time that HT is applied has expired. It also allows the user to stop the heating.

**select** - allows the user to choose one of the following options: Run the experiment, plot a graph again, or end the program.

Graphing routines

These procedures are used to plot the graph during the heating as well as to plot a graph again.

**axis** - draws a grid on the screen and labels its axes.

**permanent_label_screen** - writes text on the screen that does not change during the execution of the program.

**prepare** - prepares the execution of the loop by calling several procedures (set in cursive brackets). It sets variables (set_variables), switches to the first screen in the high resolution mode (HIResMode, HiResScreen), draws the grid (axis) and labels it (permanent_label_screen), and draws on the second screen (HiResScreen) the box for the error messages (Draw_Box).

**temp_plot** - is called during the heating to plot the temperature curves on the screen and to display the actual time and cycle number.

**read_in_data** - reads the data that have been previously sampled from the disk.

**decision_to_end_replot** - allows the user to end plotting of the graph before the whole graph has been drawn.

**temp_replot** - draws the curves of the graph again. It calls decision_to_end_replot to allow the user to end the plotting.

**replot_graph** - is the main routine for plotting a graph again. It calls the last three procedures as well as the procedures axis and permanent_label_screen.

Data storing routine

**write_out_data** - stores the data used and collected during the heating onto the file 'name' on the disk.

## IV. Results

After the system had been assembled, it was tested to make sure that the different components worked together well. The testing included calibration of the thermocouples, as well as running heating experiments with nude mice. Some of the legs heated had tumors and some, used as control in the studies to follow, were without tumors. Before the system was tested on mice, initial tests were run with a phantom. In these preliminary experiments the constants for the procedure ratio_on_off of the program were determined.

### IV.A. Calibration

The accuracy of temperature measurement with TCs is determined by the properties of the TCs itself as well as by the uncertainty in the temperature of the cold junctions. The temperature of these junctions was measured with a thermistor located in the isothermal block of the Labmate. To minimize systematic error induced by an uneven temperature distribution in the isothermal block, i.e. the part where the TCs were connected to the Labmate, the mean temperature of the block was measured by placing the thermistor in the middle of the block. The measurements with TCs of the temperature of a well mixed waterbath were

Table 4-1 Calibration of Thermocouples

| Temperature of Thermometer [°C] | Temperature [°C] of Thermocouple Number: 1 | 2 | 3 |
|---|---|---|---|
| 34.90 +/- 0.02 | 34.31 +/- 0.46 | 34.86 +/- 0.05 | 34.88 +/- 0.05 |

Note: This is just an example of a batch of 3 TCs. TC Number 1 was discarded, TCs number 2 and 3 were used.

compared, at different temperatures in a waterbath, with those done with a total immersion thermometer (Fisher Scientific Co.) whose temperature was known with an accuracy of 0.02 $^oC$. Since the relationship between the temperature and the voltage induced by the thermoelectric effect is well known, e.g. from standard tables, it was only necessary to compare the measurements done with the TCs at some, fixed temperatures with a standard. Table 4-1 indicates the systematic error induced by selected TCs was less than 0.1 $^oC$. The variations in the measurements may be due to fracturing of the TCs, caused by the stress applied to them during their production. In order to avoid those large systematic errors, it was important to calibrate each TC before it was used.

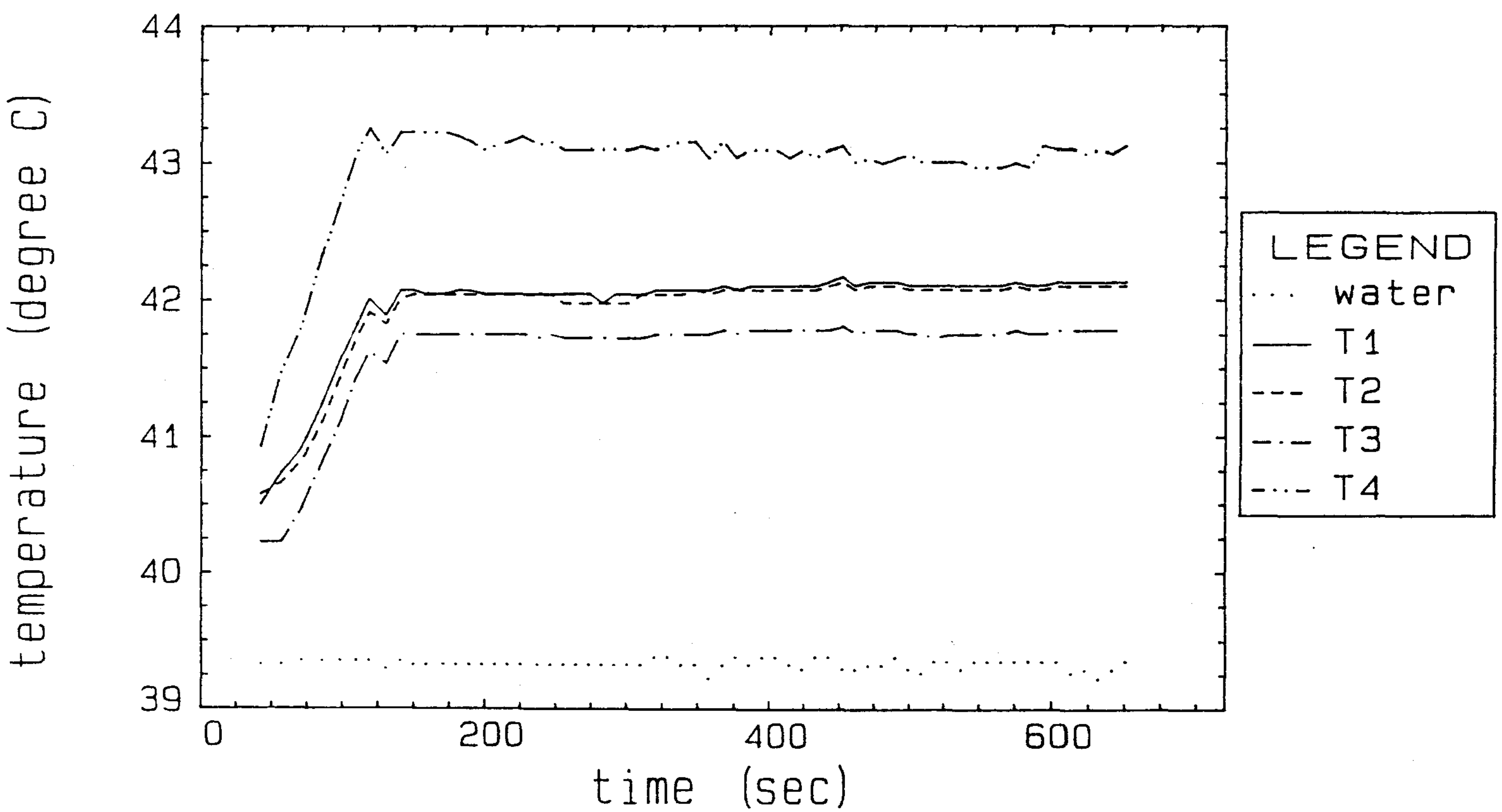
IV.B. <u>Heating of phantom</u>

The phantom, a piece of sausage (frankfurter) of 1.5 cm diameter, filled the space between the capacitor plates completely. About 2.5 min after the start of the experiment, an equilibrium was established in the phantom between the absorption of electromagnetic energy and energy dissipation into water. Since there was no blood flow, excess heat absorbed by the phantom, was dissipated only through the water bath. Hence the heating of the phantom was faster than that of the mouse. The transmitter RF output power required in experiments #1 and #2 for the phantom to maintain a temperature difference of 1.5 $^{o}C$ at 42 and 44 $^{o}C$ was 15 W. The RF circuit was matched manually in all experiments by varying the capacity and inductance of the matching circuit. The circuit was matched, when a SWR of approximately 1 was obtained (see chapter II.3. to 5.). In this case the total power was transferred. The power was measured with the same instrument.

Experiment #1

This experiment was run to test the system with the phantom before inducing HT to the leg of a mouse. The fact that the specific absorption coefficients of the RF waves in the phantom were different from those of tissues in the leg of a mouse were not of particular concern, since only the effectiveness of the algorithm in maintaining a stable temperature was tested. The water temperature was set at 39.3 $^{o}C$ and with a RF output power of 15 W the phantom could be heated up to an average temperature of 42 $^{o}C$ in 2.5 min. without problems. The variation of the temperature in the phantom with time can be found in figure 4-1. Due to the temperature difference of 2.7 $^{o}C$ between the phantom and the surrounding water, a temperature gradient could be observed in the temperature versus time curves. Three TCs, T1, T2, and T3, were placed 3 mm below the surface of the phantom,

Figure 4-1 Phantom at 42 degree C

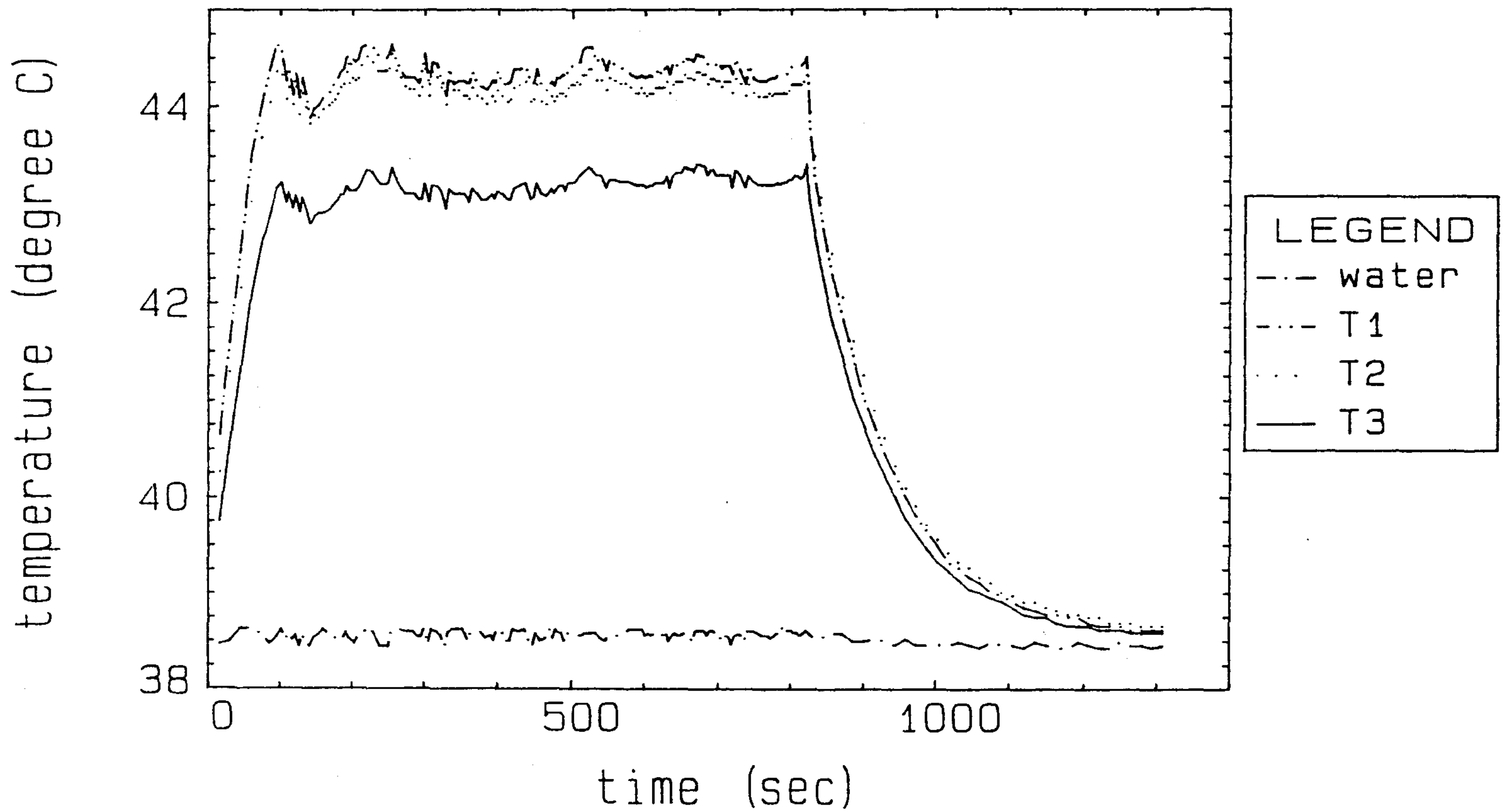


the other, T4, at a depth of 6 mm. The average of the temperatures, measured with these four TCs, had been used to control the heating. The graph does not show the beginning of the initial period of heating, since the temperature of the phantom, which had been heated in a previous experiment, did not drop to the temperature of the surrounding water before the experiment was started. However, the experiment shows that the temperature of the phantom could be raised more than 2 $^{o}C$ above the temperature of the water. The temperature was very stable within 0.25 $^{o}C$, once an equilibrium had been established. After 10 min the experiment was stopped.

Experiment #2

The same phantom was used. But now, to have conditions closer to those of the experiments with mice, the water temperature was lowered to 38.5 $^{o}C$ and the phantom heated up to 44 $^{o}C$. The RF output power was still 15 W. However, the average power transferred to the system was higher, since the periods during which the power was switched on were longer. The system used 3 TCs to control the temperature. Figure 4-2 shows both the initial phase of heating up and the drop of the temperature of the phantom to that of the surrounding water, after the power was switched off at the 820th second. Again it can be seen that a

Figure 4-2 Phantom at 44 degree C



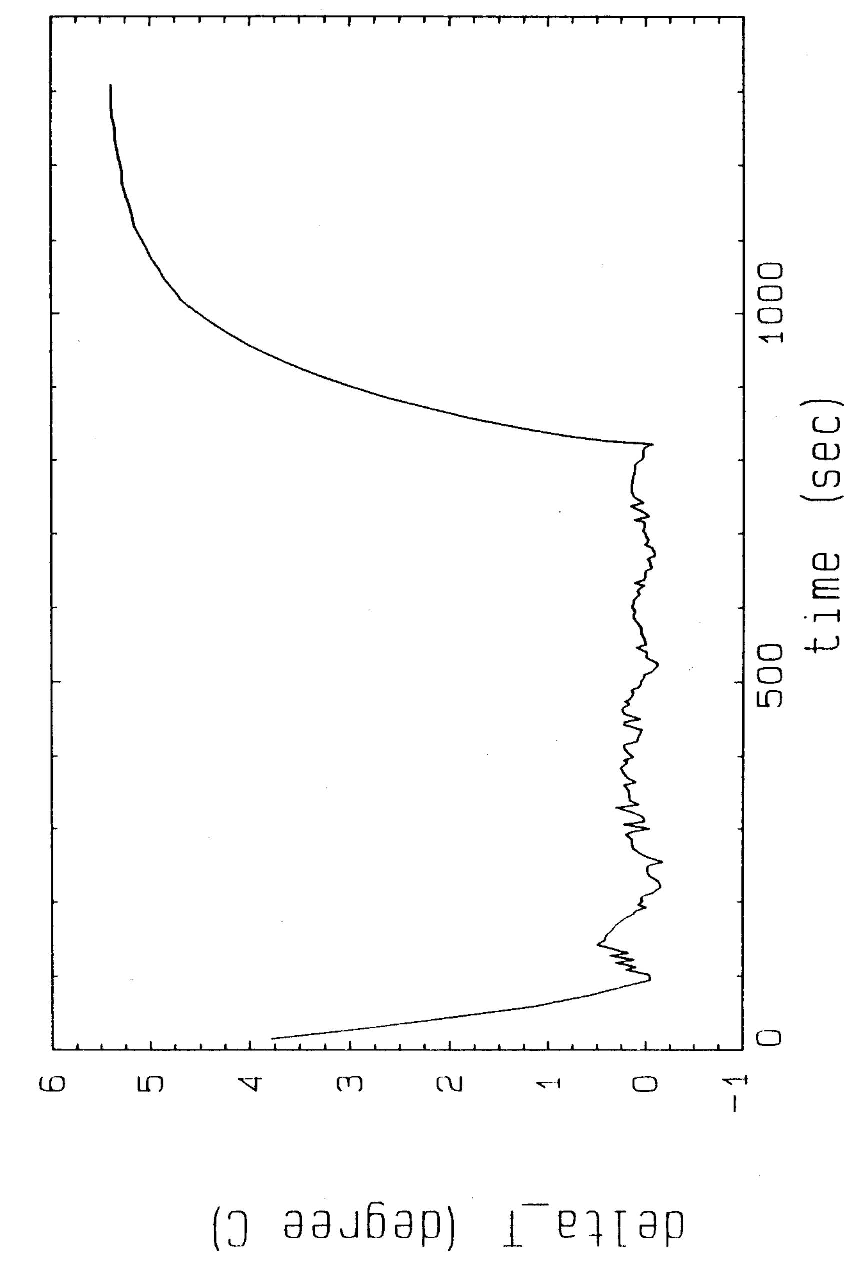
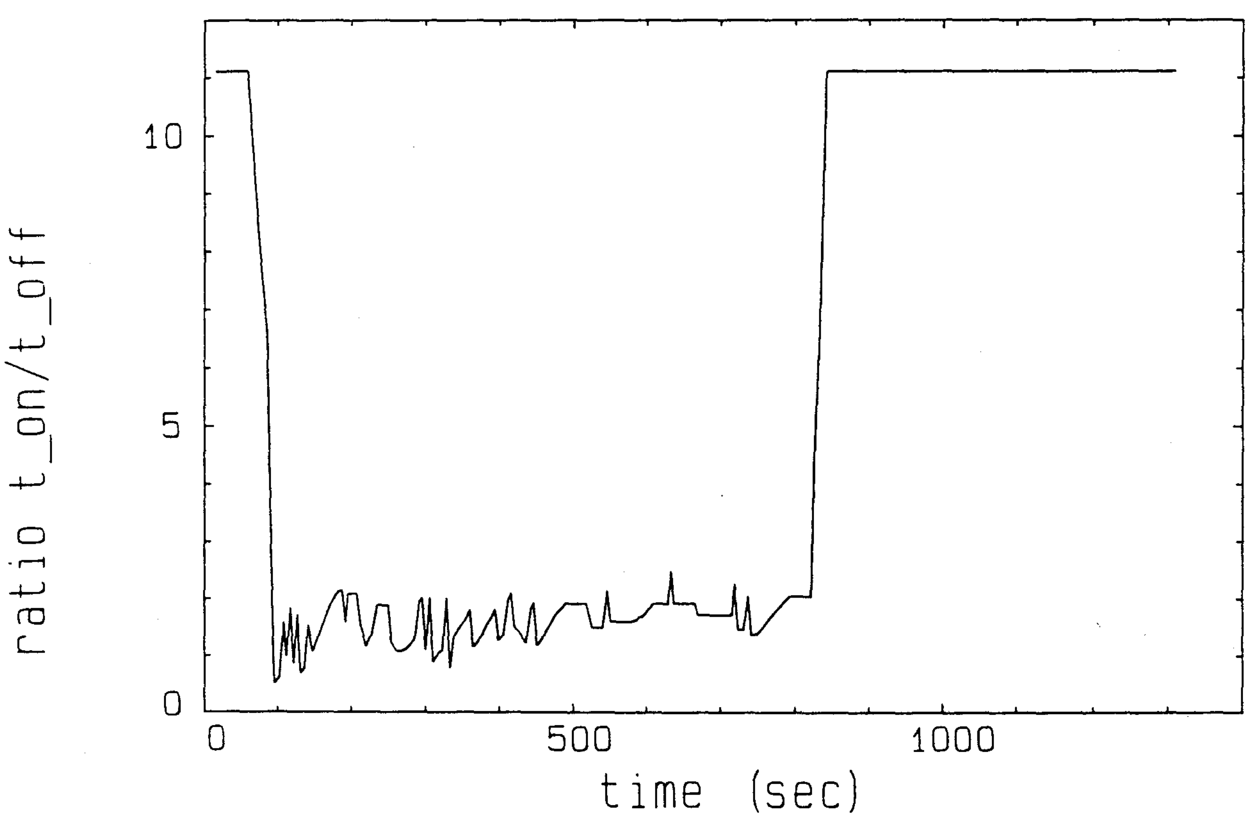



Figure 4-3 Phantom at 44 degree C

Figure 4-4 Phantom at 44 degree C



temperature gradient exists in the phantom. The probes T1 and T2 were placed 6 mm below the surface, whereas T3 was positioned at a depth of 3 mm. The temperature in T3 was lower. This would be expected, since a uniform energy absorption in the phantom together with a heat sink, the surrounding waterbath, had a lower temperature and would thus cause a temperature gradient in the phantom.

Figure 4-3 and figure 4-4, drawn for the same experiment, show the temporal variations of the deviation (called delta_T in the program) of the average of the temperatures, sampled in the control channels, in the phantom from the desired temperature and the ratio (called ratio in the program) of the time with "power on" to that with "power off" respectively. Chapter III.C.2. describes how the ratio, which is the output of the procedure ratio_on_off, responded to the temperatures measured.

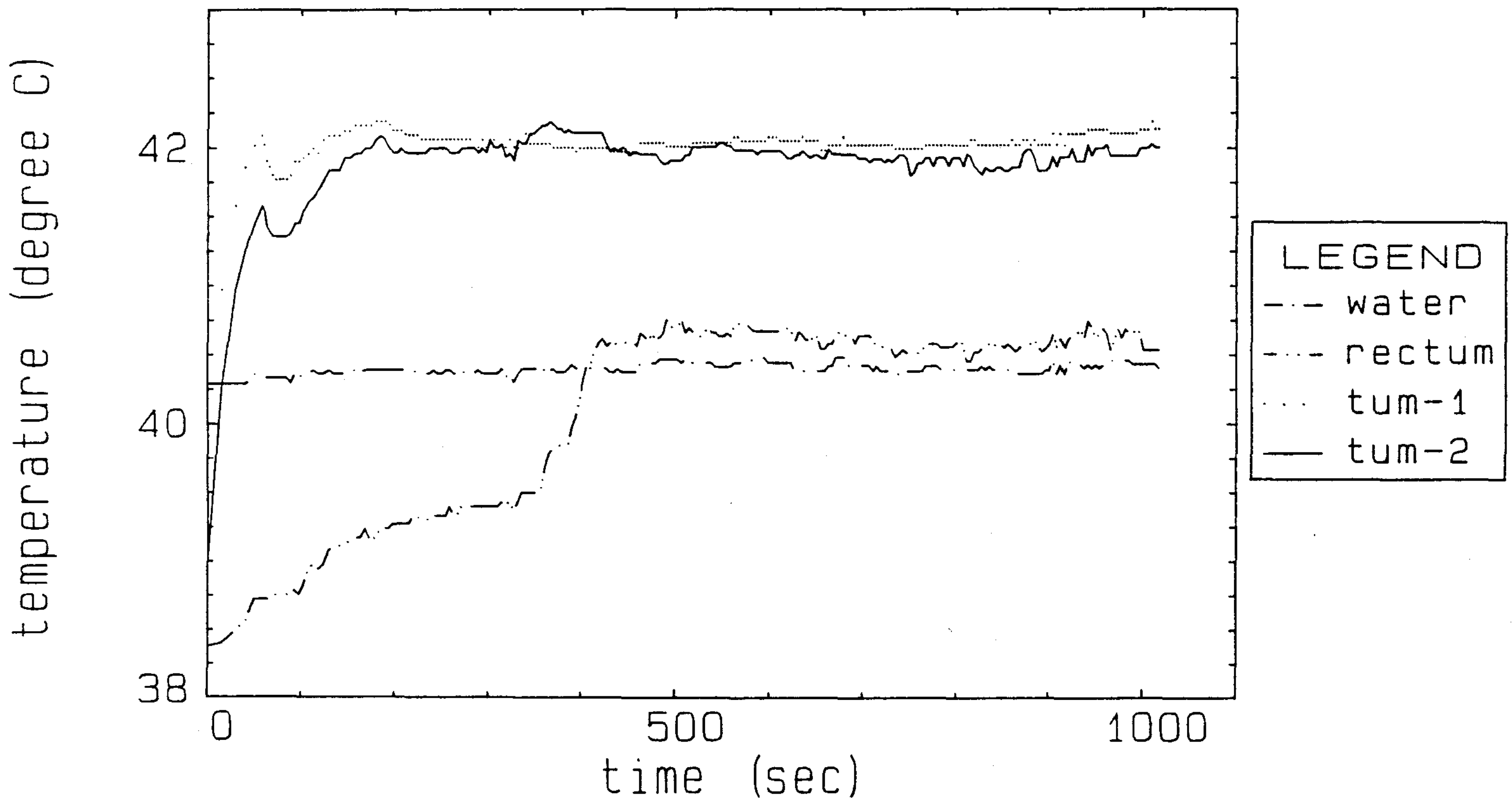
## IV.C. Heating of a leg of a mouse with and without tumors

When the heating experiments were done with animals, blood flow, in addition to the cooling by the waterbath, reduced the rate of heating to the target, which was a tumor or muscle in the thigh of a mouse. Hence in this set of experiments the RF output powers, used to keep the temperatures at the elevated levels, were higher than in the experiments where phantoms were heated. The system was used to heat up the target to 42 $^{o}C$ and 44 $^{o}C$. Thus HT above and below the threshold value of 42.5 $^{o}C$ (see chapter I.B.) was induced.

Experiment #3

The aim of this experiment was to demonstrate the feasibility of heating the thigh of a mouse to 42 $^{o}C$ or above in the presence of blood flow. The water was kept at 40.3 $^{o}C$. Two TCs T1 and T2 were inserted into the thigh, which had a diameter of 6 mm, at depths of 3 mm and 2 mm respectively. In this and the following experiments, the temperature of the body of the mouse was measured with a separate rectal probe. The body temperature of the mouse must not exceed a critical value of 40.5 $^{o}C$. The experiment was run only for ca. 17.2 min, because the rectal probe accidentally fell out ca. 5.5 min after the start of the experiment. Figure 4-5 shows the time course of the experiment. With the RF output power set to 50 W, a steady temperature of 42 $^{o}C$ in the thigh could be maintained. The difference in the temperatures of the probes in the thigh (ca. 0.3 $^{o}C$) was considerably smaller than that in the phantom (ca. 1 $^{o}C$) in experiments # 1 and 2. The main reason might be the smaller distance between the probes. But this may also be due to the blood flow in the animal, which would reduce the temperature gradient in the normal thigh.

Figure 4-5 Leg of normal mouse at 42 degree C



However, this explanation might not be applied to experiment #4, in which a tumor in the thigh with reduced blood flow was heated.

It was not possible, with the temperature of the water set even at 43 $^{o}$C and the RF output power increased to its maximum level of 100 W, to heat the normal thigh of a mouse to 44 $^{o}$C without exceeding the critical body temperature. Blood flow in the heated tissue must have been increased so much that the attempt to cool the mouse with a fan was not successful. The experiment was consequently stopped before the desired temperature was reached.

<u>Experiment #4</u>

Here the ultimate purpose of the system was tested to see whether it could be used to heat up a tumor in the thigh of a mouse. Two TC probes Tum-1 and Tum-2 were placed 2 mm and 5 mm respectively below the skin of the thigh bearing a tumor of 11 mm diameter. The temperature of the water was kept at 40.3 $^{o}$C. Time course of the experiment is given in figure 4-6. RF output power of 50 W was high enough to maintain a desired temperature of 42 $^{o}$C.

After ca. 20 min the two temperatures, measured in the tumor with probes Tum-1 and Tum-2, converged, to the same value, probably because of changes in blood flow in the tumor. At the 40th min the experiment was stopped. During

Figure 4-6 Leg with tumor at 42 degree C

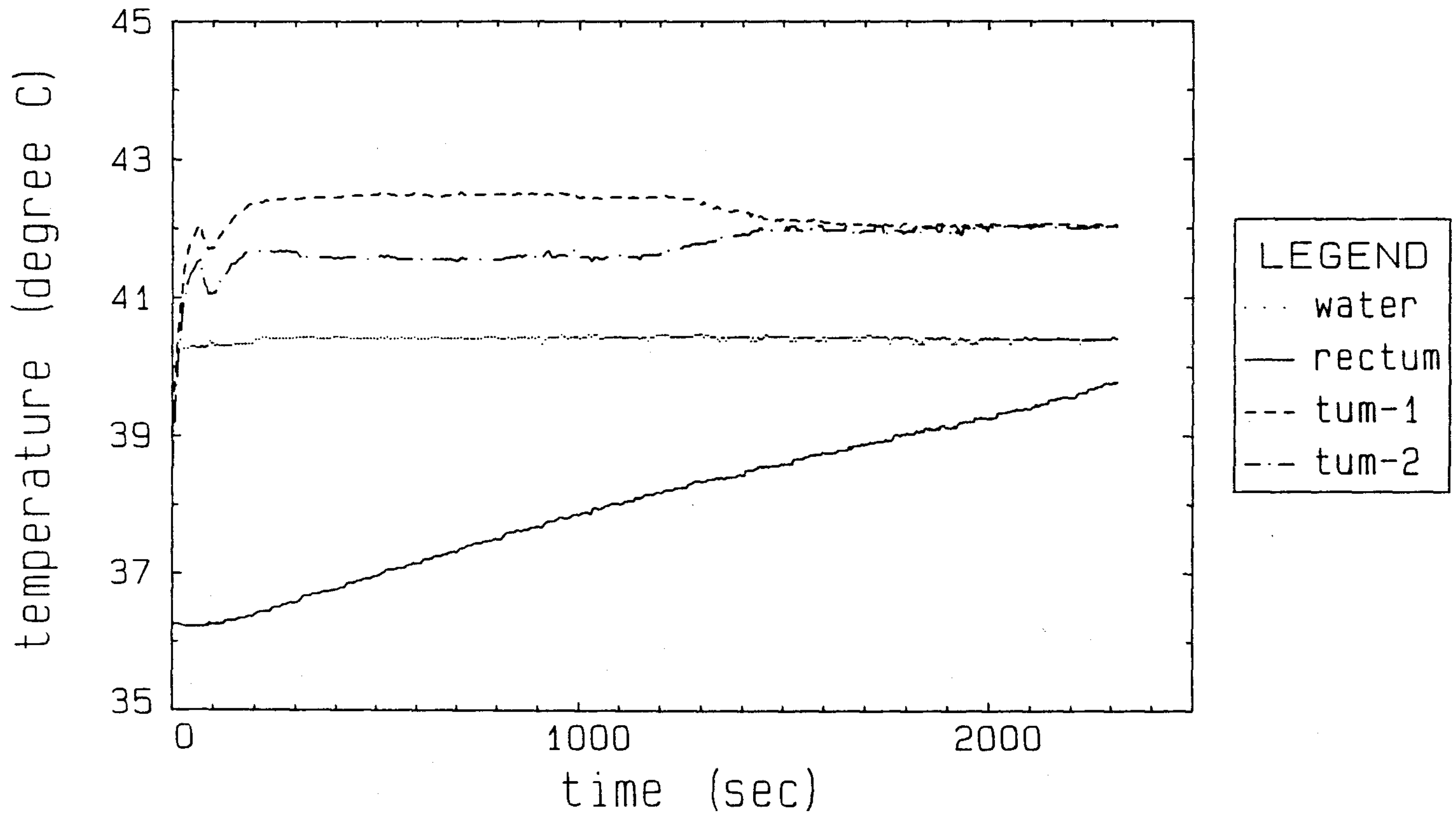


Figure 4-7 Leg with tumor at 42 degree C

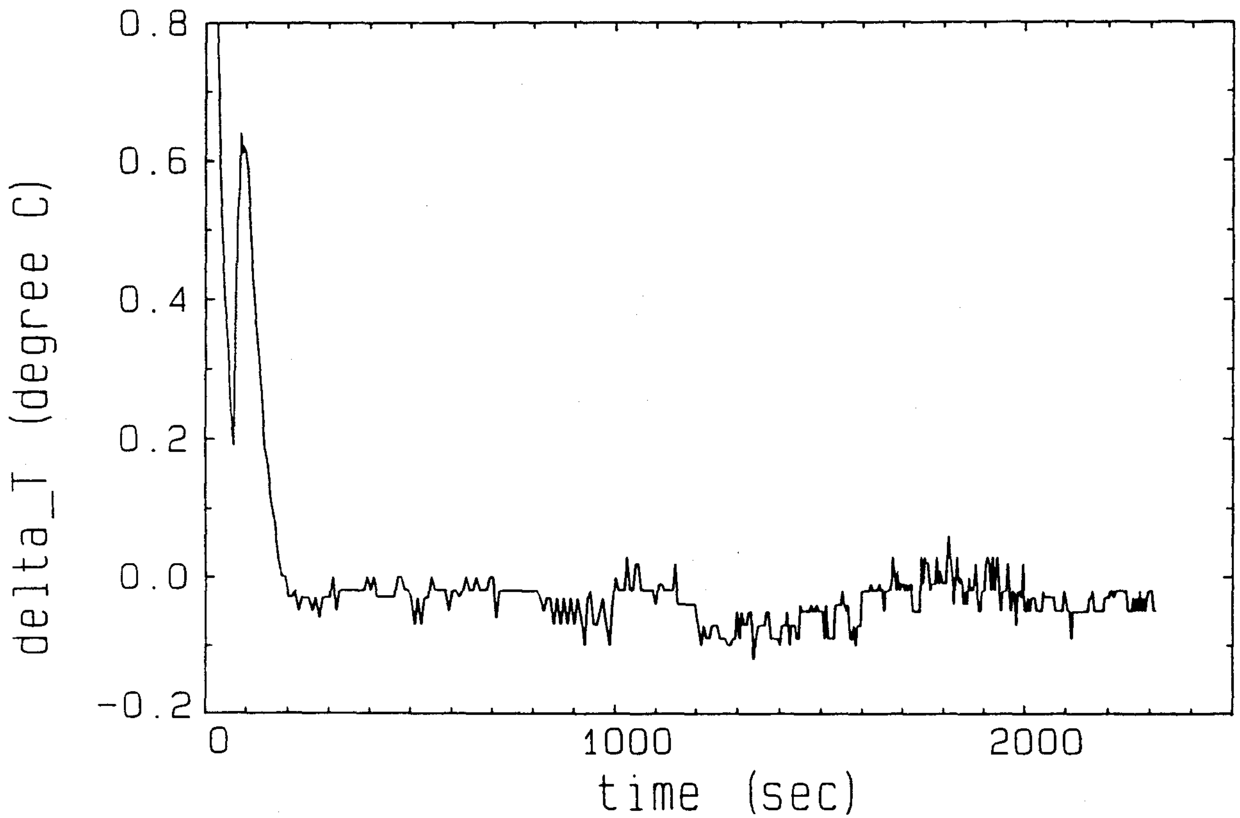
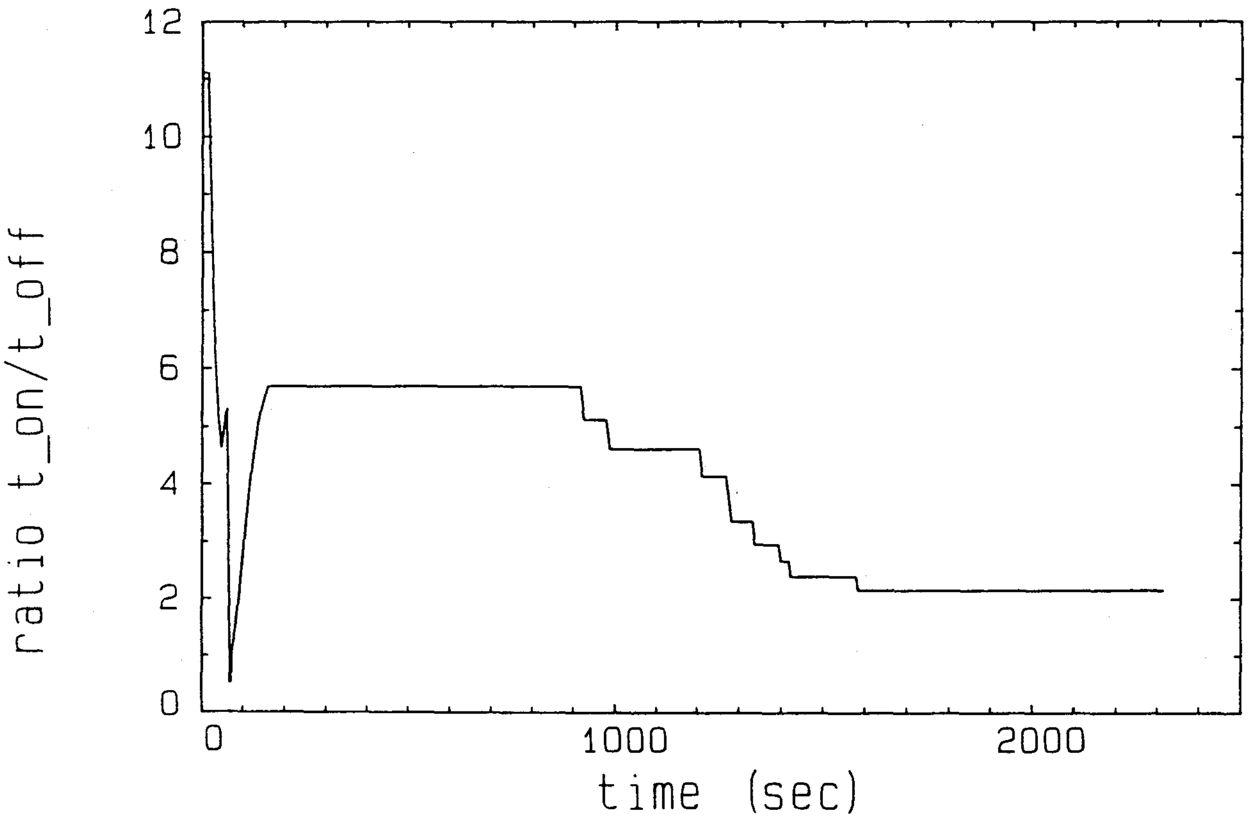


Figure 4-8 Leg with tumor at 42 degree C



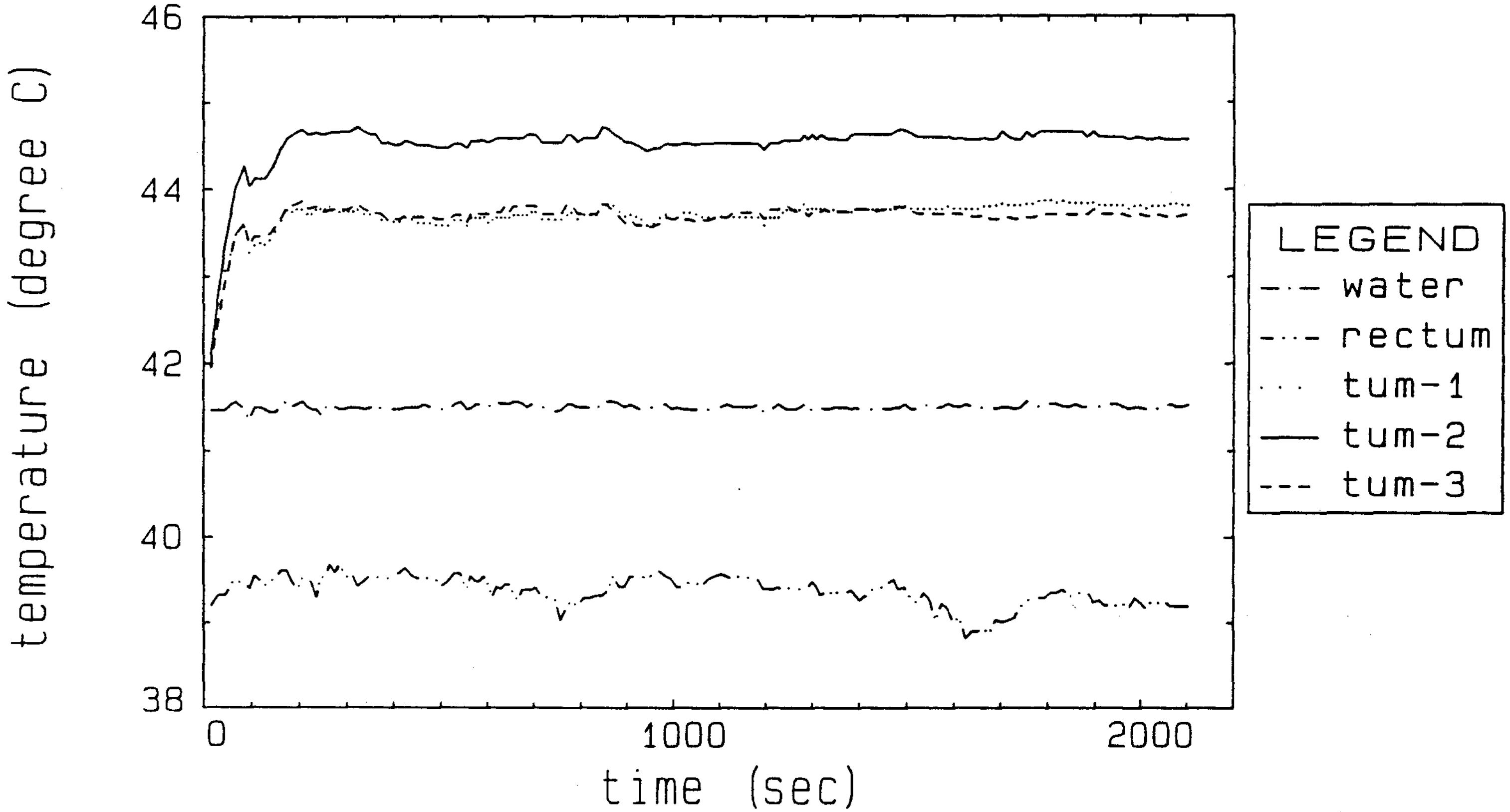
the heating, a fan helped cooling the body of the mouse. Thus the body temperature of the mouse was kept below the critical value of 40.5 $^{o}$C.

Separate graphs in figures 4-7 and 4-8 show, in analogy to experiment #2, the delta_T and the ratio versus time curves. The ratio dropped steadily from a value of 6 to a value of about 2 in the critical 15th to 27th min.. The temperatures measured in the tumor converged during the period the ratio dropped. The average power delivered by the RF field to the tumor consequently also was decreased. The cooling of the tissue around the probe Tum-1 probably remained constant, whereas cooling was reduced at probe Tum-2. After the ratio was stable again, the two probes measured the same temperature.

Experiment #5

In this final experiment a steady temperature of 44 $^{o}$C in the tumor, which was reached 3 min. after the start of the experiment, was maintained for 32 min, before the experiment was stopped (see figure 4-9). The RF output power was increased to 70 W and the water was kept at 41.5 $^{o}$C. The temperature in the tumor was controlled with three TCs. Two of the probes, Tum-1 and Tum-3, were inserted at the same depth of 3 mm below the skin and they measured the same temperature. The third TC, Tum-2 had been placed at a depth

Figure 4-9 Leg with tumor at 44 degree C



of 5 mm in the tumor and therefore the temperature measured was ca. 1 $^{o}C$ higher. Another probe measured the rectal temperature. Again, as in the experiments #3 and #4, a fan, placed beside the waterbath, helped to keep the core temperature below 39.6 $^{o}C$.

## V. Conclusion

The system developed in this project has been used successfully to induce HT in tumors growing in the thighs of nude mice at temperatures ranging from 42 $^{o}$C to 44 $^{o}$C. The temperatures could be kept constant within 0.2 $^{o}$C after the initial heating for at least 1 hour. Small temperature gradients, however, exist in the tumor when the skin was maintained by a waterbath at a lower temperature than that of the tumor.

### Future improvements

Several changes could be made to improve the performance of the system. Inasmuch as the software is concerned, the procedure ratio_on_off could be further improved. The last 10 sampled measurements could be included in the calculation of the next ratio, to further reduce big fluctuations in the ratio. However, this will only improve the performance, once an equilibrium has been reached. This equilibrium is not a static one. It can shift, when a parameter, such as the blood flow, changes, so that the ratio does not have a fixed value. Thus a parameter, such as the standard deviation of the last 10 measured temperatures, has to be evaluated to indicate whether a change has

Streffer, C. and D. van Beuningen. 1987. The biological Basis for Tumor Therapy by Hyperthermia and Radiation. Recent Results in Cancer Research 104, 25-70

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