The cryosurgical open-cone-spray method

proefschrift

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Petrus Johannes Maria Joannes Bessems

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Promotor: Prof.Dr. W.J.B.M. van de Staak

Co-promotor: Dr. W.R. Faber

Beoordelingscommissie: Prof.Dr. J.A. Flendrig
Dr. B.A. Jagtman
Prof.Dr. R.A. Janknegt
Prof.Dr. A.H. Keijser
Prof.Dr. W.P.M.A. Lamers

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Contents

General introduction

1.1 What is cryosurgery?

Cryosurgery or cryotherapy is a freezing process in which cryogenic substances are used to cause tissue necrosis.
Usually, for the treatment of skin lesions, the term "cryosurgery" is encountered in the literature rather than "cryotherapy". In this thesis, the term "cryosurgery" will be used.
Both benign and malignant skin conditions can be treated by cryosurgery. In this study the cryosurgical method will be restricted to malignant skin lesions, the basal cell carcinoma in particular. This therapy modality appears to be very effective in the treatment of the basal cell carcinoma (1). The results are equal to those of radiotherapy and excision surgery (1). The cosmetic results are also very satisfactory and similar to or even better than the cosmetic results of radiotherapy (1). The indications, contraindications and complications are well known (1).
When performing cryosurgery, the target area is first locally anesthesized. Apart from the anesthesia effect, "ballooning" of the tissue is provided, which is very useful in preventing damage to structures beneath the skin, such as nerves, and in preventing painful irritation of bony structures. Vasoconstriction of the microcirculation is provided when adrenaline is also used.
The bulk of the tumour can be removed by curettage (2) which also helps to delineate the field to be treated.
Liquid nitrogen is supplied by a spray unit to the neoplastic lesion which is surrounded by a cone (3). The spray is directed at the target area and this creates a gradually spreading freeze front.
Normally, two freeze cycles are preferred in the treatment of skin malignancies (1, 3, 4, 5, 23). After the first freeze cycle of a relatively short duration of twenty to thirty seconds (4, 5), spontaneous thawing of the lesion is allowed. The second freeze cycle is then applied and the second spontaneous thaw period completes the cryosurgical procedure.

1.2 Historical review

Cryosurgical techniques have been used since the second half of the nineteenth century.
Sir James Arnott in 1851 was the first to use the cryogenic temperatures of a cold saline solution to destroy tumours (6). More cryogenics, such as liquid oxygen (1877) (7), liquid nitrogen (1883) (7), liquid air (1899) (8) and carbon dioxide (1907) (9), were later developed.
Carbon dioxide was widely available, was of low cost, and could be stored for prolonged periods in contrast to liquid air. The disadvantage of carbon dioxide was its shallow depth of freezing (10). Liquid oxygen was only used for a short period of time because it was flammable and caused dangerous situations (10).
Liquid nitrogen was found to be the most potent cryogen (11, 12) and was initially applied by the swab method (13).
In 1899, White introduced this swab method using liquid air (8). The spray method with liquid air was described by Whitehouse in 1907 (14), but was then abandoned until the 1960's (10).
In 1961, the neurosurgeon, Cooper, was the first to describe a closed-system apparatus with cryoprobes utilizing liquid nitrogen
(15).
After this reintroduction, liquid nitrogen was also adopted by dermatologists in the treatment of neoplastic conditions of the skin.
Around 1965, Torre described a prototype spray unit which also utilized liquid nitrogen (16).
Zacarian and Adham introduced modified cryoprobes consisting of copper cylindrical discs in 1966 (17). Later, Zacarian, in co-operation with Bryne, also adopted the spray method (1967).
It was Torre again who, in 1977, combined the open spray technique with neopreen cones. The open-cone-spray method was a fact (3).
Both Zacarian and Torre have made a great effort to develop these modern dermatocryosurgical techniques.

1.3 Pathogenesis of the cryogenic lesion

The exact pathophysiology of the tissue destruction that follows a cold injury is unknown. Cryonecrosis probably depends on a complex interaction of various factors.
Two important mechanisms, cell injury and microcirculatory arrest, will be discussed below.

1.3.1 Cell injury

Extracellular and intracellular ice formation and crystallization of electrolytes not only cause mechanical damage to the cell, but also cause dehydration. This is followed by an abnormally high concentration of electrolytes within the cell (osmotic damage). Eventually, all electrolytes can be crystallized. A fast fall in temperature can also cause thermal shock and cell death (18). Finally, lipoprotein complexes denaturate (1). These are complex events, influenced by both the freezing and the thawing process (19, 20, 21, 22, 23, 24).

The freezing process will first be discussed.

Freezing of tissue does not occur until a temperature of -5°C is reached (20, 21) because of the high concentration of substances within the cell. This applies to any cooling velocity.

Between -5°C and -15°C, extracellular ice crystals are formed. The temperature in the cell is below 0°C, but the intracellular space remains unfrozen. The period between 0°C and the beginning of freezing (from -5°C on) is called "the supercooled state of the cell" (21, 22).

From -5°C on, the cooling velocity plays an important role.

A slow freezing process (10°C/min) increases cellular dehydration and, outside the cell, the water freezes (23). There is no intracellular ice formation. The dehydration causes toxic concentrations of electrolytes within the cell and this can cause cell death.

A high cooling velocity (100°C/min) causes an increasing extracellular ice formation. Here intracellular ice is also formed and this destroys intracellular structures, leading to cell death (23). In a high-speed freezing process, mainly small ice crystals are formed and these are rather unstable.

In general, a fast cooling rate produces a more effective fall in temperature than a slower rate (3). The lethal temperature isotherms will be relatively close to the periphery of the ice ball border when the cooling velocity is high.

Many electrolytes are present in the tissues and these all have different temperatures at which they crystallize (eutectic temperature) (1, 22, 24). Therefore transition from the liquid to the solid state occurs in stages.

Since sodium chloride is the major electrolyte in the tissue, the eutectic point of this electrolyte, being -21.2°C, is the most important (22). When this temperature is reached, the intracellular space of the cell is crystallized to a great extent. However, not all tissue cells will be irreversibly damaged at this temperature (25).

Apart from injury incurred during freezing, the thawing process can also cause additional damage to the cell. Again, velocity is the most important factor in the thawing process.
Fast thawing causes the ice crystals to melt and recrystallization does not occur (23). The additional damage to the cell is limited.
When thawing is slow, recrystallization of the intracellular ice crystals into larger crystals occurs (23). This clearly increases the injury to the cells.
The conclusion is that fast freezing (≥100°C/min) together with slow thawing (≤10°C/min) provides the best combination in causing cell death. This is still a dogma in cryosurgery.
Initially, a tissue temperature as low as -25°C was advised (3, 4, 19, 23), but now tissue temperatures between -40°C and -60°C are frequently encountered (1, 3).
Two freeze cycles are usually recommended in treating skin malignancies (1, 3, 4, 5, 23). The second freeze cycle should be performed after completion of the first thaw period, since this will elongate the period of recrystallization (1). Additional elongation of the thaw period can be provided by also using adrenaline in the anesthetic fluid (26).

1.3.2 Microcirculatory arrest

The effect of cold on the vasculature plays a significant role in the development of cryonecrosis (1, 27).
The microcirculation of the skin is very sensitive to cryogenic temperatures (19). This is particularly so for the venules (19), resulting in thrombosis, stasis and ischemia of the tissues cells.
The first response to local cold is vasoconstriction of the arterioles and venules (1).
Vasoconstriction already occurs when the tissue temperature falls below +15°C (18, 19, 28). In the temperature range of between +11°C and +3°C, 62% of the capillary circulation will cease (19). If cooling continues (+10°C), the vasoconstriction is periodically interrupted by vasodilatation (hunting response of Lewis) (28). However, if local cooling is severe, as in cryosurgery, vasoconstriction proceeds and the vasodilatation is usually ineffective (29). Between -3°C and -9°C, freezing of microcirculatory vessels and complete stasis of blood flow has been observed (19, 30).
Sub-zero temperatures and subsequent thawing cause direct injury to microvascular endothelium (19, 30) with swelling and even lysis of the

endothelial cells. The endothelial damage is probably the most critical event in cryogenic necrosis (30).
Thawing causes vasodilatation (19, 28, 29, 30). Vascular permeability occurs because of the damaged endothelial cells, leading to capillary leakage of fluid and plasma protein (19, 29). Both the vasodilatation and the increased intravascular viscosity result in a decreased blood flow and in sludging.
The damaged vessel wall endothelium activates the coagulation process and aggregation of thrombocytes and other blood cells occurs. Immediately after thawing, emboli pass through the microcirculatory system and adhere to the injured vascular wall to form thrombi (19, 30). Finally, a total hemostasis is observed, caused by the combination of previously described factors.

1.4 Cryosurgical techniques for the application of liquid nitrogen

Since the 1960's, several dermatocryosurgical methods have been developed to destroy neoplastic skin lesions. The two main techniques are the probe method and the spray method. These will be discussed and compared to each other.

1.4.1 The probe method

The (closed-end-) probe is a metal device of which the surface is cooled by the cryogen. This can be done either by previous immersion of the probe in the cryogen or by circulating the cryogen through it (1, 31).
The size of the probe is adapted to suit the lesion to be treated. The probe acts as a heat-sink.
The shape of the freeze front depends on the shape of the probe and the contact of the probe with the target surface. By increasing the pressure upon the probe, it is possible to increase the depth of freezing (1, 31).

1.4.2 The spray method

In the spray technique, the cryogen is directly applied to the target surface. In this way, the cryogen is used at its coldest possible temperature and is delivered by an open spray nozzle attached to the spray unit (1, 31).

The ratio between liquid and vapour in the liquid nitrogen spray depends on which spraying device is used (32) and influences the intensity of the spray. The aperture of the spraytip largely determines the fan-shape of the spray.
Various spray patterns have been described for the supply of the cryogen to the target site (33).
The distance between spraytip and target area and the manner of spraying (continuously or intermittently) are the other variables influencing the supply of the cryogen.
In the spray method, run-off of cryogen droplets from the target surface is possible, but can be avoided by a slight adaptation of the spray technique.

1.4.3 The probe method versus the spray method

Probably the most important disadvantage of the probe method is the slowly advancing freeze front it causes (31).
The probe is attached to the target surface during the freezing process and, for practical purposes, often has to be rewarmed to be released from the surface (1, 31). This causes a rapid thawing phase, again a disadvantage in creating cryonecrosis.
The spray can be moved freely, causes a rapid freeze front and it also allows a slow thawing phase.
The contact of the cryogen with the surface to be treated is good in spraying, whereas in using a probe, an irregular target surface prevents good contact with the smooth probe (31).
According to the literature, the probe technique is not suitable for lesions that invade the skin by more than 3 mm: this is in contrast to the spray method (31).
In conclusion, the spray method is a more potent modality than the probe technique in the treatment of skin malignancies (32).

1.4.4 Modification of the spray method: the cone-spray method

In the spray method cones can be used. A cone is a hollow device that encloses the surface to be treated. The cone can be either open or closed (31).
The area to be frozen is limited by the use of a cone (1, 3, 31). This limitation can be improved by slightly pressing the cone onto the skin.

The dispersion of the spray is also limited by the cone, giving a more potent spray and probably creating a greater depth of freezing.
Furthermore, there is less run-off of the cryogen (31). When run-off develops, the cryogen droplets are arrested by the wall of the cone and a slight adjustment of the spray technique will prevent the run-off.
There are, evidently, advantages in using cones in combination with the spray technique.
The prototype open cone is made of a pliable material (neoprene) (1, 3). Its diameter is determined by the size of the lesion to be treated, including an area of normal tissue.
The cone is directed to the skin using one hand and, using the other hand, liquid nitrogen is sprayed into the cone.
Closed cones can also be used: these can be attached to the spray unit. Usually these are unpliable devices, made of metal or plastic. The accumulated vapour of the cryogen within the closed cone does prevent a good view of the extension of the freeze front. This results in a lesser degree of control of the treatment procedure.
In conclusion, the spray method, in combination with open cones, is preferred in treating skin malignancies.

1.5 Aim of the study

The ways in which cryosurgery is performed in dermatological practice are quite divergent and depend largely on the experience of the practitioner.
The open-cone-spray technique (3) appears to be the best technique available for the cryosurgical treatment of skin malignancies.
However, there are several different variables in this technique which influence the supply of liquid nitrogen. These variables are the subject of this study and will be investigated on the basis of temperature measurements in a model, wherein freezing experiments are performed. They include the spray pattern (chapter 3), the spraytip diameter (chapter 4), the distance between spraytip and target area (chapter 5) and the spraying technique (continuous or intermittent) (chapter 7).
To provide an optimal cryogenic necrosis, it is important to create a rapid freezing phase (chapter 1.3.1).
On the other hand, freezing times should be limited to prevent extensive cryonecrosis of healthy dermal tissue (5).
Therefore a short freezing phase (maximum 30 seconds) should be applied with a high cooling velocity.

In studying the above-mentioned variables, an optimal supply of liquid nitrogen has to be found to provide a sufficient cryonecrosis. This is probably not equal to a maximum supply of liquid nitrogen.
An optimal delivery of liquid nitrogen also implies that as little discomfort as possible is caused to the patient to be treated by the cryosurgical procedure. This is particularly applicable to spattering and run-off of liquid nitrogen.
The final aim is to provide a guideline for a standardized supply of liquid nitrogen in the open-cone-spray technique, implying an extension of Torres method and the introduction of a cone made of unpliable polymethylmethacrylate.

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2

Materials

2.1 Introduction

In this chapter the materials used for all experiments are described.

2.2 Materials

In a pilot study, several models were investigated for the performance of the temperature measurements: dead pig skin, live pig skin, dead human skin and a 10% gelatin solution. These were studied by the experimental model as described in this chapter, using similar experimental conditions.
The gelatin solution, dead pig skin and dead human skin were found to give equal results in the temperature measurements. In these models the most important substance to be frozen is water. The 10% gelatin solution was previously used to investigate the thermal conductivity in heart muscle (1). The thermal conductivity of this gelatin solution was shown to be 12.2×10^{-4} cal/g^2/sec/°C (1).
This value approximates to that of various tissues such as skin and muscle ($7\text{-}14 \times 10^{-4}$ cal/g^2/sec/°C) (2, 3, 4) and also to that of water (14×10^{-4} cal/g^2/sec/°C) (2, 3).
The thermal diffusivity is calculated from the equation:

thermal diffusivity = thermal conductivity : (density x specific heat) (2).

The density and specific heat of a 10% gelatin solution are very similar to those of water and tissues. Therefore, only minor variations in thermal diffusivity are present between the 10% gelatin solution, dead pig skin and dead human skin.
In general, it was found that the thermal characteristics of the 10% gelatin solution are very similar to those of various tissues, including skin tissue.
Live pig skin was also studied, using the same experimental conditions.

Here the temperature measurements were somewhat different from the measurements found in the comparison of the other three models. The fall in temperature in live pig skin was generally slower, caused by the presence of an intact microcirculation. In live pig skin tissue, however, the ratio between the variables under investigation was similar to the ratio of these found in gelatin.
In addition, the gelatin model provided the most reproducible and workable medium for the registration of the freezing process by the thermocouples. Therefore this model is used for the performance of the temperature measurements in order to ascertain the relationship of the variables to each other.
The gelatin medium was produced as follows: gelatin powder was dissolved in water by heating; a 10% solution was made, poured in petri-dishes to coagulate and stored at an average room temperature of 20°C (the initial temperature in all measurements). Three or four areas of the gelatin layer in one petri-dish were used for the freezing experiments; each area was used just once.
For the delivery of liquid nitrogen (-195.6°C), normally used as cryogen (5, 6, 7, 8), the CRY-AC (Brymill Corporation, Vernon, Connecticut, USA, distributed by Alcon Pharmaceuticals Ltd.) was employed. This spray-unit (figure 2.1) has an operational pressure of 10 psi (pressure per

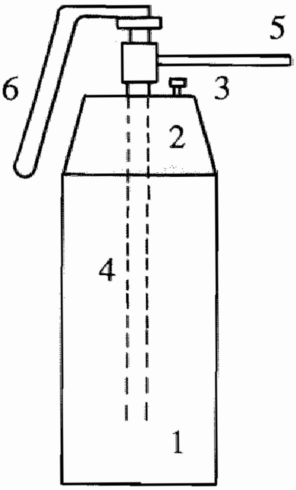

FIGURE 2.1. Spray-unit. 1 = Self-pressurizing liquid nitrogen reservoir; 2 = Screw cover; 3 = Pressure valve; 4 = Tube; 5 = Spray canula; 6= Operating handle.

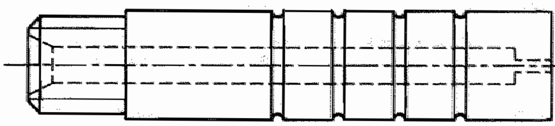

FIGURE 2.2. Spraytip. 1 = Aperture with a diameter of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm respectively; 2 = Indentation to attach the spraytip to the cone.

square inch) with a variation of 0.5 psi, caused by minor variations (5%) in the pressure relief valve. The unit consists of a self-pressurizing insulating liquid nitrogen reservoir, locked by a screw cover with a pressure valve. A narrow tube originating in the cover conveys the liquid nitrogen to a spray canula, positioned at rectangles to the spray unit. The liquid nitrogen is delivered by the operation of a handle.
Spraytips of various diameters can be connected to the spray canula. The spraytips (figure 2.2) are made of brass and have diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm (accuracy of 0.02 mm). External to the spraytips are four indentations to attach the spraytip to the cone in such a way that the distance between spraytip and target surface can be 5 mm, 10 mm, 15 mm or 20 mm.
For temperature registration, standard stainless steel hypodermic needles were used with a diameter of 0.8 mm (Brymill Corporation, Vernon, Connecticut, USA, distributed by Alcon Pharmaceuticals Ltd.). These have insulated copper-constantan thermocouple wires threaded through a canula and are fixed to the proximal edge of the needle bevel. The variation in temperature registration is ±1°C for all temperatures. The thermocouples were regularly calibrated.
The thermocouples were connected to a model 740 System Scanner thermometer (Keithley Instruments BV, Gorinchem, the Netherlands) with an accuracy of ±0.6°C between -200°C and -100°C and of ±0.5°C between -100°C and +400°C (figure 2.3).
The scanner thermometer was connected by an IEEE-488 interface board to a personal computer (Olivetti M-24) (figure 2.3).
A computer program was developed to control the scanner thermometer and to record the results measured.
Holders (figure 2.4) were constructed from polymethylmethacrylate. By using the excavated openings for insertion, exact positioning of the thermocouples in the area to be frozen was realized. Funnel-shaped cones with diameters of 11 mm, 16 mm and 21 mm were created

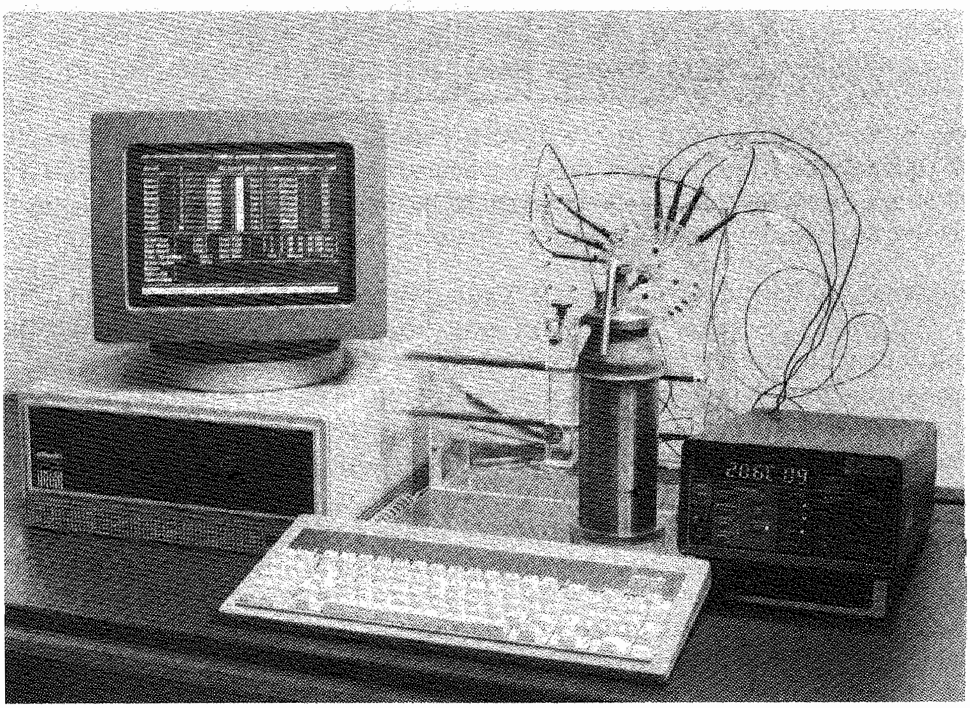

FIGURE 2.3. Experimental model.

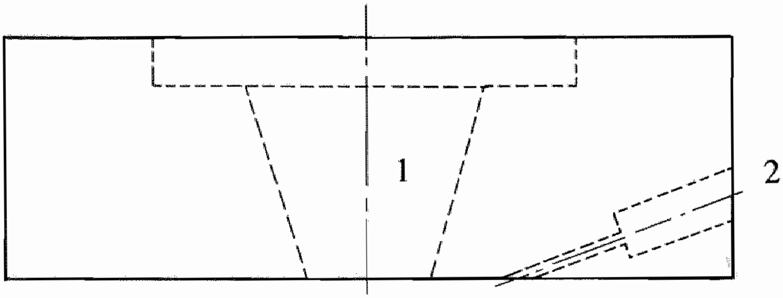

FIGURE 2.4. Holder. 1 = Funnel-shaped cone with diameters of 11 mm, 16 mm and 21 mm respectively; 2 = Opening for insertion of the thermocouple.

(accuracy ±0.5 mm) in the centre of the holders.
Finally, a rectangular construction consisting of a vertical and horizontal plate was made (figure 2.3).
The gelatin layer was placed on the vertical plate. The holder with the cone was directed smoothly against the gelatin layer and the thermocouples were inserted (figure 2.3).
The spray unit on the horizontal plate was coupled with its spraytip to the cone-holder by way of an opening in the centre of a small plate overlying the cone opening (figure 2.3).
All experiments were performed in an air-conditioned room, providing a constant temperature.

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3

Spray patterns

3.1 Introduction

The spray pattern is one of the variables that influence the supply of liquid nitrogen in the open-cone-spray technique.
This chapter presents the study of the temperature measurements in three spray patterns frequently encountered in cryosurgery (1): central, circular and "paintbrush" spraying.

3.2 Materials and methods

3.2.1 Materials

These are discussed in chapter 2.

3.2.2 Methods

The spray patterns are defined in this study as follows:
In the central spray pattern, the spraytip is fixed exactly in the centre of the cone.
Circular spraying is performed by slowly directing the spraytip in a circular motion against the inner wall of the cone.
The "paintbrush" pattern is started at the top of the cone carrying the spraytip horizontally from one side of the cone to the other, back and forth, slowly proceeding downwards and then repeating the pattern in reverse.
These three patterns are reproducible. In addition to circular and "paintbrush" spraying, other spray patterns can also be considered, but they all appear to be equal or intermediate forms of either circular or "paintbrush" spraying.

Freezing times of 60 seconds are frequently used in dermatological practice, mostly in association with circular or "paintbrush" spraying (2,3). In central spraying, freezing times can be as short as 30 seconds (4) or even shorter.
For our experiments, a freezing time of 120 seconds was chosen. The delivery of liquid nitrogen was achieved by continuous spraying.
In circular spraying 80 circular motions were performed in 120 seconds and in "paintbrush" spraying 50 "paintbrush" motions were performed in 120 seconds.
All three spray patterns will be studied for the cone diameters of 11 mm, 16 mm and 21 mm. These are the cones most commonly used in cryosurgery and have, therefore, also been adopted in this study.
The temperatures were recorded in the centre and at the edge of the cones, in both places at 1 mm, 3 mm and 5 mm from the surface of the target area (gelatin). These temperatures were recorded at intervals of 10 seconds.
For each cone diameter the three spray patterns will be discussed individually and then compared with each other.

3.2.3 Statistical methods

Thirty different experiments were performed for each spray pattern in the cone diameter of 16 mm and six different registrations in the cone diameters of 11 mm and 21 mm. From those experiments the mean values of the recorded temperatures with the standard deviations were calculated (5). The standard error of the difference between the mean values was also determined (5). The difference between the mean values was considered significant when twice the standard error was exceeded ($p \leq 0.05$) (5).
The Wilcoxon-test (5) was used to investigate whether a constant pattern existed in the temperature values recorded in various gelatin areas.

3.3 Results

3.3.1 Pilot study

In a pilot study it was found that after 120 seconds, no further relevant decline in temperature was observed in any of the three spray patterns. The spraytip diameter of 0.8 mm from a distance of 15 mm to the target surface seemed to be most suitable for all the cone diameters 11 mm, 16 mm and 21 mm.

The Wilcoxon-test (5) was applied to find out if any systematic trends existed in the measurements of various gelatin areas used for the same experiments. In our data, no systematic phenomena were found in any of the cones.

The spray patterns for each cone diameter will now be discussed separately.

3.3.2 Measurements in the cone diameter of 16 mm

Measurements taken in the centre of the 16 mm cone using the central spray pattern are shown in figure 3.1*, while those recorded at the edge of the cone are presented in figure 3.2.
Figure 3.3 and figure 3.4 show the temperature measurements in the centre and at the edge of the 16 mm cone respectively using circular spraying.
In figures 3.5 and 3.6, the temperature results are presented for the centre and the edge of this cone using the "paintbrush" spray technique.
In table 3.1, the temperatures taken in the centre and at the edge of the cone are analyzed over time. There occurs a statistically significant difference between the temperatures taken at intervals of 10 seconds for all three spray patterns.

3.3.3 Measurements in the cone diameter of 11 mm

Figure 3.7 and figure 3.8 give the temperature measurements in the centre and at the edge of the 11 mm cone respectively using the central spray pattern.
Figures 3.9 and 3.10 show the results of the temperatures measured in the centre and at the edge of this cone respectively using the circular spray technique.

* See addendum chapter 3 for figures 3.1-3.30

TABLE 3.1. P-values of temperature change over 120 seconds for various cone diameters, depths and spray patterns. The temperatures recorded at succeeding intervals of 10 seconds were statistically compared to each other (= time interval in seconds, wherein significancy occurs).*

	central spraying		circular spraying		paintbrush spraying	
depth	centre	edge	centre	edge	centre	edge
16 mm cone						
1 mm	p≤0.01 0-60*	p≤0.04 0-120	p≤0.02 0-40 50-90	p≤0.04 0-120	p≤0.03 0-50	p≤0.04 0-80
3 mm	p≤0.04 0-90	p≤0.005 0-120	p≤0.03 0-120	p≤0.01 0-120	p≤0.04 0-70	p≤0.05 0-100
5 mm	p≤0.01 0-120	p≤0.003 0-120	p≤0.003 20-120	p≤0.01 20-120	p≤0.04 20-90	p≤0.04 0-100
11 mm cone						
1 mm	p≤0.03 0-60	p≤0.04 0-70	p≤0.004 0-60	p≤0.01 0-70	p≤0.03 0-50	p≤0.02 0-50
3 mm	p≤0.02 0-60	p≤0.04 0-80	p≤0.002 0-70	p≤0.04 20-90	p≤0.03 0-60	p≤0.04 0-60
5 mm	p≤0.04 20-90	p≤0.03 30-110	p≤0.003 20-80	p≤0.04 110-120	p≤0.03 20-60	p≤0.04 30-80
21 mm cone						
1 mm	p≤0.008 0-40	p≤0.02 0-40	p≤0.04 0-50	p≤0.02 0-50	p≤0.002 0-20	p≤0.003 0-30
3 mm	p≤0.003 0-50	p≤0.03 20-50	p≤0.03 20-60	p≤0.04 20-70	p≤0.03 0-30	—
5 mm	p≤0.02 20-60	p≤0.04 30-120	—	—	—	—

Figures 3.11 and 3.12 present the measurement of temperatures in the centre and at the edge of this cone respectively using the "paintbrush" spay pattern.
The statistical analysis of the temperatures over time for all three spray patterns is shown in table 3.1.

TABLE 3.2. P-values of the comparison of the depths in the centre and at the edge for various cone diameters and spray patterns. The temperatures recorded over a period of 120 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

depth	central spraying centre	central spraying edge	circular spraying centre	circular spraying edge	paintbrush spraying centre	paintbrush spraying edge
16 mm cone						
1 mm versus 3 mm	p<0.001 0-120*	p<0.001 0-120	p<0.001 0-120	p<0.001 20-120	p<0.003 0-120	p≤0.003 0-120
1 mm versus 5 mm	p<0.001 0-120	p<0.001 0-120	p<0.001 0-120	p≤0.05 0-120	p<0.001 0-120	p≤0.001 0-120
3 mm versus 5 mm	p<0.001 0-120	p<0.001 20-120	p<0.001 20-120	p≤0.05 20-120	p<0.001 20-120	p≤0.002 20-120
11 mm cone						
1 mm versus 3 mm	p<0.001 0-120	p<0.001 20-120	p<0.001 0-120	p<0.001 20-120	p<0.001 0-120	p<0.001 20-120
1 mm versus 5 mm	p<0.001 0-120	p<0.001 20-120	p<0.001 0-120	p<0.001 20-120	p<0.001 0-120	p<0.001 20-120
3 mm versus 5 mm	p<0.001 0-120	p<0.001 20-120	p<0.001 20-120	p≤0.04 20-120	p<0.001 20-120	p≤0.008 20-120
21 mm cone						
1 mm versus 3 mm	p<0.001 0-120	p<0.001 20-120	p≤0.003 0-120	p<0.001 20-120	p≤0.03 0-40	p<0.001 20-120
1 mm versus 5 mm	p<0.001 0-120	p<0.001 20-120	p≤0.002 0-120	p≤0.03 0-120	p≤0.04 0-120	p<0.001 20-120
3 mm versus 5 mm	p<0.001 20-120	p≤0.02 30-120	p≤0.035 30-80	p≤0.005 30-120	p≤0.03 30	p≤0.005 40-120

3.3.4 Measurements in the cone diameter of 21 mm

Figure 3.13 and figure 3.14 display graphically the results of the temperatures measured in the centre and at the edge of the 21 mm cone respectively using the central spray pattern.
Figures 3.15 and 3.16 give the results of the temperatures measured in the centre and at the edge of this cone respectively using circular spraying.

TABLE 3.3. P-values of the comparison of the temperature measurements in the centre to those at the edge for various cones, depths and spray patterns. The temperatures recorded over a period of 120 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	central spraying	circular spraying	paintbrush spraying
depth	centre versus edge	centre versus edge	centre versus edge
16 mm cone			
1 mm	p<0.001 0-120*	p<0.001 0-120	p<0.001 0-120
3 mm	p<0.001 0-120	p<0.001 70-120	p≤0.002 20-120
5 mm	p≤0.002 30-120	p≤0.03 20-80 100-120	p≤0.01 70-120
11 mm cone			
1 mm	p<0.001 0-120	p<0.001 0-120	p<0.001 0-120
3 mm	p<0.001 20-120	p≤0.008 0-120	p≤0.009 20-120
5 mm	p<0.001 30-120	p≤0.002 30-120	p≤0.035 30-120
21 mm cone			
1 mm	p<0.001 0-120	p≤0.046 20-30 p≤0.035 100-120	p≤0.04 0-120
3 mm	p<0.001 20-120	p≤0.046 80-120	p≤0.03 20-120
5 mm	p<0.001 30-120	p≤0.03 40-120	p≤0.025 30-120

Figures 3.17 and 3.18 show the temperature measurements in the centre and at the edge respectively using "paintbrush" spraying.
The statistical analysis of the temperatures over time for all three spray patterns is shown in table 3.1. In circular and "paintbrush" spraying, there is no significancy of the temperatures over time at 5 mm-depth either in the centre or at the edge of the cone. This also applies to the

temperatures recorded at the edge at 3 mm-depth using the "paintbrush" spray pattern. This is caused by a slow fall in temperature at these depths.

Significant temperature differences exist for each of the recorded times in all of the following paired comparisons of depth, both in the centre and at the edge of the cone: 1 mm and 3 mm; 1 mm and 5 mm; 3 mm and 5 mm for all cones and spray techniques (table 3.2).
Between the results of temperatures measured in the centre and at the edge, significancy of the temperatures occurs for each of the recorded times for the various cones, depths and spray techniques (table 3.3).

3.3.5 Comparison of the spray patterns

The spray patterns for every cone diameter will now be compared to each other.

3.3.5.1 *Cone diameter of 16 mm*

In figure 3.19, the comparison of the three spray patterns in the centre of the 16 mm cone is presented and in figure 3.20 the comparison at the edge is shown, both at 3 mm-depth.
The fastest fall in temperature is achieved by central spraying, followed by "paintbrush" and circular spraying respectively.
The statistical analysis of this comparison is presented in table 3.4. There is an evident significancy between the spray patterns.
Figures 3.21 and 3.22 display graphically the intervals of minimum and maximum values of the measurements of temperature in the centre and at the edge of the cone respectively, both at 3 mm-depth. From the moment temperature decline begins, there is an overlap between circular and "paintbrush" spraying during the whole course of freezing. Also, although to a much lesser degree, an overlap exists between central and "paintbrush" spraying (from 50-60 seconds in the centre and from 70 seconds at the edge) and between central and circular spraying (from 80 seconds in the centre and from 110 seconds at the edge).

3.3.5.2 *Cone diameter of 11 mm*

Figures 3.23 and 3.24 provide the comparison of the spray techniques for the centre and the edge of the 11 mm cone respectively, both at 3 mm-depth. Once again, the decline of the temperature is fastest in the

TABLE 3.4. P-values of the comparison of the central, circular and "paintbrush" spray patterns for the various cones and depths. The temperatures recorded over a period of 120 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth		3 mm-depth		5 mm-depth	
spray pattern	centre	edge	centre	edge	centre	edge
16 mm cone						
central versus circular	p≤0.001 0-120*	p<0.001 0-120	p<0.001 0-120	p≤0.025 0-120	p≤0.003 0-120	p≤0.04 0-120
central versus paintbr.	p≤0.04 0-110	p<0.001 20-120	p<0.001 0-120	p<0.001 20-120	p<0.001 20-120	p<0.001 30-120
circular versus paintbr.	p<0.01 0-100	p≤0.03 0-110	p≤0.009 0-90	p≤0.03 0-120	p≤0.025 0-120	p≤0.05 0-120
11 mm cone						
central versus circular	p≤0.009 0-60	p<0.001 20-120	p≤0.05 0-100	p<0.001 20-120	p≤0.04 20-110	p≤0.01 30-120
central versus paintbr.	p≤0.04 0-100	p<0.001 20-120	p≤0.003 0-120	p<0.001 20-120	p≤0.03 0-120	p<0.001 30-120
circular versus paintbr.	p≤0.007 0-10	—	p≤0.03 0-20	—	p≤0.02 0-20	—
21 mm cone						
central versus circular	p<0.001 0-120	p<0.001 30-120	p<0.001 20-120	p<0.001 40-120	p<0.001 30-120	p<0.001 50-120
central versus paintbr.	p<0.001 0-120	p≤0.009 20-120	p<0.001 20-120	p<0.001 40-120	p<0.001 30-120	p≤0.03 40-120
circular versus paintbr.	—	p≤0.035 20-120	—	p≤0.04 30-120	—	p≤0.04 70-120

central spray pattern. Circular spraying is slightly faster than "paint-brush" spraying.

Table 3.4 presents the statistical analysis of this comparison. Between central and circular and between central and "paintbrush" spraying, clear significancy exists. When circular spraying is compared to "paint-brush" spraying, there is hardly any significancy in the temperatures measured in the centre and no significancy at the edge.

Figures 3.25 and 3.26 give the intervals of minimum and maximum temperature values at 3 mm-depth. From the moment temperature decline starts, there is an extensive overlap for the whole course of freezing between circular and "paintbrush" spraying, at the edge in particular. Overlap occurs between central spraying and the other two spray patterns after 60-70 seconds of freezing, whilst at the edge, there is hardly any overlap between central spraying and the other two techniques.

3.3.5.3 *Cone diameter of 21 mm*

The comparison of the three spray patterns in the centre of the 21 mm cone at 3 mm-depth is presented in figure 3.27. The fastest fall in temperature is achieved by central spraying, followed by "paintbrush" and circular spraying. At the edge at 3 mm-depth, as shown in figure 3.28, the central spray technique also causes the fastest decline in temperature followed by circular and "paintbrush" spraying respectively.

The statistical analysis of this comparison is presented in table 3.4. Between central and circular and between central and "paintbrush" spraying, an evident significancy exists. Between circular and "paintbrush" spraying, there is only significancy at the edge, but none in the centre.

Figures 3.29 and 3.30 present the intervals of minimum and maximum values at 3 mm-depth. From the moment temperature declines begins, no overlap can be seen between the central and circular spray pattern in the centre, nor at the edge of this cone. Between central and "paintbrush" spraying, there is a slight overlap in the centre and no overlap at the edge. There is a great overlap between circular and "paintbrush" spraying in the centre. At the edge, overlap is also present, although to a lesser degree.

3.4 Discussion

From this comparison it can be concluded that for all three cone diameters in general, and certainly with regard to a short
period of freezing (maximum 30 seconds) (4, 6), the central spray pattern provides the fastest extension of the freeze front.

In central spraying, the liquid nitrogen is only supplied to the centre of the target area and the freeze front gradually extends from this point. In the circular and "paintbrush" spray patterns, the liquid nitrogen makes contact at diverse points in the target area and this heat exchange

results in relatively greater evaporation of liquid nitrogen than in central spraying. This explains why the decline of the temperature is much slower in circular and "paintbrush" spraying.
Given the fact that central spraying results in the fastest decline of temperature and has the smallest standard deviation, there occurs very little overlap between the central spray pattern and the circular and "paintbrush" spray patterns. In the centre of the cones, central spraying gives a degree of overlapping with the other two patterns, whilst at the edge of the cones, there is no, or, at the most, negligible, overlap.
Circular and "paintbrush" spraying often show similar temperature variations. These two techniques therefore overlap each other to a great extent, both in the centre and at the edge of the cones.
The irregular supply of liquid nitrogen in the circular and especially in the "paintbrush" spray pattern results in an increased spread in minimum and maximum temperature values which increases the standard deviations of these techniques.
In conclusion, the central spray pattern provides the fastest fall in temperature, has the smallest spread in minimum and maximum temperature values and has almost no overlap with circular and "paintbrush" spraying.
Central spraying therefore provides the best opportunity to reach the fastest fall in temperature within a short period of freezing. To provide an exact central spray pattern, a modified cone, made of polymethylmethacrylate, is introduced with the spraytip fixed in the centre (figure 3.31).

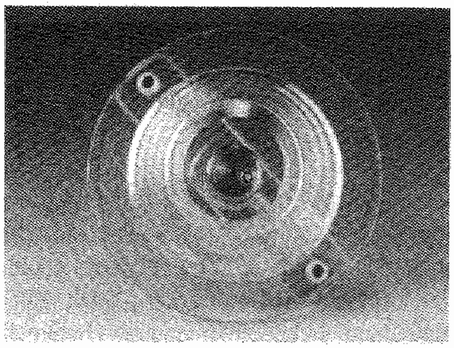

FIGURE 3.31. Polymethylmethacrylate cone with the spraytip fixed in the centre (bottom view).

3.5 References

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4

Spraytip diameters

4.1 Introduction

In chapter 3, it was concluded that the central spray pattern causes the fastest fall in temperature within a short period of freezing.
In this chapter the spraytip diameter will be discussed. This variable also influences the supply of liquid nitrogen in the freezing phase.
Spraytips with diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm will be compared to each other. These spraytips are frequently used in cryosurgical techniques but, however, the literature does not provide any precise guidelines for the use of spraytips (1, 2, 3, 4, 5). The choice of a specific spraytip diameter is determined by the preference guided by the experience of the cryosurgeon.

4.2 Materials and methods

4.2.1 Materials

These are discussed in chapter 2.

4.2.2 Methods

In this study four spraytips are discussed. The diameters of the spraytips are 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm respectively. These spraytips supply the following amounts of liquid nitrogen: 72 ml/min, 96 ml/min, 120 ml/min and 204 ml/min respectively in combination with the CRY-AC spray-unit. Other spraytip diameters can also be considered but the diameters selected are frequently used in cryosurgery.
The central spray pattern is applied with the spraytip fixed in the centre of the cone. The liquid nitrogen is supplied by continuous spraying over 60 seconds and from a distance of 15 mm to the target surface.

The four spraytips are studied in the cone diameters of 11 mm, 16 mm and 21 mm. These cones are most commonly used in cryosurgery and have, therefore, also been adopted in this study.
The temperatures are recorded in the centre and at the edge of the cones, in both places at 1 mm, 3 mm and 5 mm from the surface of the target area.
Registration of the temperatures is performed over a period of 60 seconds at intervals of 5 seconds.
For each cone diameter, the four spraytips will be discussed individually and then compared with each other. An optimal delivery of liquid nitrogen is the main starting point in this discussion. It implies a compromise between an optimal fall in temperature and a minimal run-off of liquid nitrogen.

4.2.3 Statistical methods

Four different experiments were performed for each spraytip (0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm) in each cone diameter (11 mm, 16 mm and 21 mm). From these experiments the mean values of the recorded temperatures with the standard deviations were calculated (6).
The standard error of the difference between the mean values was determined (6). The difference between the mean values was considered significant when twice the standard error was exceeded ($p \leq 0.05$) (6).
The mean values of the four experiments performed for the 0.8 mm spraytip in this study were compared to those of thirty equivalent experiments in the study of the spray patterns by means of the Wilcoxon-test (6).
The Wilcoxon-test (6) was used to investigate whether a constant pattern existed in the temperature values recorded in various gelatin areas.

4.3 Results

The Wilcoxon-test (6) was used to compare the data obtained from the four different experiments in this study with those obtained from thirty different experiments previously performed in the study of the spray patterns (chapter 3). This was done in the 16 mm cone using the central spraying technique and the 0.8 mm spraytip from a distance of 15 mm. There was no statistical difference between the data recorded at intervals of 10 seconds in the centre and at the edge, at depths of 1 mm, 3

mm and 5 mm. It was concluded that four experiments were sufficient for the comparison of the various spraytip diameters in this study.
The Wilcoxon-test (6) was also applied to ascertain whether any systematic trends existed in the different gelatin areas used for the same experiments. In our data, no systematic phenomena were found in any of the cones.

The spraytip diameters for each cone diameter will now be discussed separately.

4.3.1 Measurements in the cone diameter of 16 mm

Measurement of temperatures taken in the centre of the 16 mm cone using the 0.6 mm spraytip are shown in figure 4.1*, while those recorded at the edge of the cone are presented in figure 4.2.
Figure 4.3 and figure 4.4 show the temperature measurements in the centre and at the edge of the 16 mm cone respectively, using the 0.8 mm spraytip.
In figures 4.5 and 4.6, the temperature results are presented for the centre and the edge of this cone respectively, using the 1.0 mm spraytip.
Figures 4.7 and 4.8 provide the temperature results measured using the 1.7 mm spraytip, both in the centre and at the edge of the cone.
In table 4.1, the temperatures measured in the centre and at the edge of the cone are analyzed over time (60 seconds).
There occurs a variable statistical significancy over time between the temperatures taken at intervals of 5 seconds. At the edge of the cone at 5 mm-depth, no significancy occurs over time using the 1.0 mm spraytip.

4.3.2 Measurements in the cone diameter of 11 mm

Figures 4.9 and 4.10 display graphically the temperatures measured over time for the centre and the edge of the 11 mm cone respectively, using the 0.6 mm spraytip.
Figures 4.11 and 4.12 provide the results of temperature measurements in the centre and at the edge of this cone respectively, using the 0.8 mm

* See addendum chapter 4 for figures 4.1-4.36

TABLE 4.1. P-values of temperature change over 60 seconds for various cone diameters, depths and spraytip diameters. The temperatures recorded at succeeding intervals of 5 seconds were statistically compared to each other (= time interval in seconds, wherein significancy occurs).*

spraytip diameter	1 mm-depth		3 mm-depth		5 mm-depth	
	centre	edge	centre	edge	centre	edge
16 mm cone						
0.6 mm	p≤0.02 0-40*	p≤0.02 40-60	p≤0.04 0-45	p≤0.03 40-60	p≤0.008 25-60	p≤0.02 50-60
0.8 mm	p≤0.025 0-25	p≤0.04 5-40	p≤0.045 10-45	p≤0.04 15-50	p≤0.003 20-60	p≤0.04 20-50
1.0 mm	p≤0.03 0-20	p≤0.03 10-20	p≤0.03 10-50	p≤0.03 15-35	p≤0.05 20-40	—
1.7 mm	p≤0.02 0-15	p≤0.05 10-45	p≤0.046 10-20	p≤0.02 15-60	p≤0.04 10-25	p≤ 0.04 20-30
11 mm cone						
0.6 mm	p≤0.009 0-30	p≤0.046 10-35	p≤0.046 10-50	p≤0.05 25-60	p≤0.04 25-40	—
0.8 mm	p≤0.008 0-20	p<0.001 5-60	p≤0.04 5-35	p≤0.04 10-60	p≤0.02 10-60	p≤0.046 20-60
1.0 mm	p≤0.05 0-20	p≤0.05 5-35	p≤0.025 5-30	p<0.001 10-60	p≤0.025 10-30	p<0.001 20-60
1.7 mm	p≤0.04 0-25	p≤0.04 5-25	p≤0.04 0-45	p≤0.04 10-35	p<0.001 10-60	—
21 mm cone						
0.6 mm	p≤0.03 5-10	p≤0.05 5-40	p≤0.04 10-20	p≤0.01 15-50	p≤0.04 50-60	p≤0.006 35-60
0.8 mm	p≤0.035 5-20	p≤0.035 5-60	p≤0.02 10-15, 20-25	p≤0.035 20-60	p≤0.004 15-60	p≤0.04 30-60
1.0 mm	p≤0.02 0-30	p≤0.035 10-30	p≤0.035 10-50	p≤0.02 25-40	p≤0.03 15-20	p≤0.03 30-50
1.7 mm	p≤0.045 0-15	p≤0.035 5-40	p≤0.025 5-35	p≤0.02 15-50	p≤0.045 15-60	p≤0.03 25-50

spraytip, figures 4.13 and 4.14 the results using the 1.0 mm spraytip and figures 4.15 and 4.16 those using the 1.7 mm spraytip.
These graphs show a more or less similar pattern to the graphs of the 16 mm cone.
In table 4.1, the temperatures taken in the centre and at the edge are analyzed over time (60 seconds). At the edge, at 5 mm-depth, no significancy exists over time using the 0.6 mm and 1.7 mm spraytip.

4.3.3 Measurements in the cone diameter of 21 mm

In figures 4.17 and 4.18, the results of temperatures measured in the centre and at the edge of the 21 mm cone respectively are displayed graphically, using the 0.6 mm spraytip.
Figures 4.19 and 4.20 provide the results of temperatures measured in the centre and at the edge respectively, using the 0.8 mm spraytip.
Figures 4.21 and 4.22 show the temperature measurements in the centre and at the edge respectively, using the 1.0 mm spraytip and figures 4.23 and 4.24 present the results using the 1.7 mm spraytip.
In general, the fall in temperature in the 21 mm cone for all spraytip diameters is slower than in the 16 mm and 11 mm cones: this is so at the edge in particular.
Table 4.1 shows the statistical analysis of the temperatures over time.

Significant temperature differences exist for each of the recorded times in all of the following paired comparisons of depth, both in the centre and at the edge of the cone: 1 mm and 3 mm, 1 mm and 5 mm, 3 mm and 5 mm for all cones and spraytip diameters (table 4.2).
Between the results of temperatures recorded in the centre and at the edge, significancy of the temperatures occurs for each of the recorded times for the various cones, depths and spraytip diameters (table 4.3).

4.3.4 Comparison of the spraytip diameters

The spraytip diameters for every cone diameter will now be compared to each other.

4.3.4.1 *Cone diameter of 16 mm*

Figure 4.25 shows the comparison of the four spraytip diameters for the centre of the 16 mm cone and figure 4.26 for the edge, both at 3 mm-depth.

TABLE 4.2. P-values of the comparison of the depths in the centre and at the edge for various cone diameters and spraytip diameters. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm- versus 3 mm-depth		1 mm- versus 5 mm-depth		3 mm- versus 5 mm-depth	
spraytip diameter	centre	edge	centre	edge	centre	edge
16 mm cone						
0.6 mm	p≤0.009 5-60*	p≤0.025 20-60	p≤0.006 5-60	p≤0.03 20-60	p≤0.002 15-60	p≤0.009 50-60
0.8 mm	p<0.001 5-60	p≤0.001 15-60	p<0.001 5-60	p≤0.04 10-60	p≤0.007 15-60	p≤0.02 20-60
1.0 mm	p≤0.02 5-60	p≤0.025 15-60	p≤0.02 5-60	p≤0.01 15-60	p<0.001 15-60	p≤0.02 25-60
1.7 mm	p<0.001 5-60	p<0.001 15-60	p<0.001 5-60	p<0.001 15-60	p≤0.04 10-60	p<0.001 20-60
11 mm cone						
0.6 mm	p≤0.0035 5-60	p≤0.003 15-60	p<0.001 5-60	p<0.001 15-60	p<0.001 15-60	p≤0.03 20-60
0.8 mm	p<0.001 5-60	p≤0.04 5-60	p<0.001 5-60	p<0.001 5-60	p≤0.015 5-60	p≤0.04 5-60
1.0 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 5-60	p≤0.04 5-60	p<0.001 10-60	p<0.001 15-60
1.7 mm	p<0.001 5-60	p≤0.0025 10-60	p<0.001 5-60	p≤0.001 10-60	p<0.001 5-60	p<0.001 15-60
21 mm cone						
0.6 mm	p≤0.007 10-60	p<0.001 10-60	p≤0.005 10-60	p≤0.01 5-60	p≤0.025 15-60	p≤0.05 5-60
0.8 mm	p≤0.05 10-60	p<0.001 10-60	p≤0.0095 10-60	p<0.001 10-60	p≤0.009 15-60	p<0.001 25-60
1.0 mm	p≤0.01 5-60	p<0.001 15-60	p≤0.009 5-60	p≤0.035 10-60	p<0.001 15-60	p≤0.035 25-60
1.7 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 0-60	p<0.001 10-60	p<0.001 10-60	p<0.001 20-60

TABLE 4.3. P-values of the comparison of the temperature measurements in the centre to those at the edge for various cones, depths and spraytip diameters. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth	3 mm-depth	5 mm-depth
spraytip diameter	centre versus edge	centre versus edge	centre versus edge
16 mm cone			
0.6 mm	p≤0.009 5-60*	p≤0.004 15-60	p≤0.046 30-60
0.8 mm	p<0.001 5-60	p≤0.007 20-60	p≤0.04 45-60
1.0 mm	p≤0.03 5-60	p≤0.0075 15-60	p≤0.004 30-60
1.7 mm	p<0.001 5-60	p<0.001 15-60	p≤0.01 20-60
11 mm cone			
0.6 mm	p≤0.01 5-60	p<0.001 15-60	p≤0.015 25-60
0.8 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 15-60
1.0 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 15-60
1.7 mm	p<0.001 5-60	p≤0.046 5-60	p<0.001 15-60
21 mm cone			
0.6 mm	p≤0.004 10-60	p≤0.05 10-60	p<0.001 20-60
0.8 mm	p≤0.04 10-60	p≤0.005 15-60	p<0.001 20-60
1.0 mm	p≤0.005 5-60	p≤0.035 10-60	p<0.001 20-60
1.7 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 15-60

TABLE 4.4. P-values of the comparison of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm for the various cones and depths. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

spraytip diameter	1 mm-depth		3 mm-depth		5 mm-depth	
	centre	edge	centre	edge	centre	edge
16 mm cone						
0.6 mm versus 0.8 mm	$p \leq 0.03$ 5-60*	$p \leq 0.007$ 10-60	$p \leq 0.03$ 15-60	$p \leq 0.025$ 15-60	$p \leq 0.02$ 20-60	$p \leq 0.025$ 20-60
0.6 mm versus 1.0 mm	$p \leq 0.007$ 10-45	$p \leq 0.03$ 15-60	$p < 0.001$ 20-60	$p \leq 0.02$ 20-60	$p \leq 0.008$ 25-60	$p \leq 0.03$ 25-60
0.6 mm versus 1.7 mm	$p \leq 0.0025$ 5-60	$p < 0.001$ 15-60	$p \leq 0.002$ 15-60	$p < 0.001$ 20-60	$p \leq 0.015$ 20-60	$p \leq 0.015$ 25-60
0.8 mm versus 1.0 mm	—	—	—	$p \leq 0.04$ 15, 40-60	—	$p \leq 0.001$ 50
0.8 mm versus 1.7 mm	$p \leq 0.02$ 5-15	$p \leq 0.04$ 10 $p \leq 0.03$ 25-60	$p \leq 0.04$ 15-25	$p \leq 0.03$ 25-60	$p \leq 0.04$ 25-30	$p \leq 0.03$ 25-60
1.0 mm versus 1.7 mm	$p \leq 0.03$ 20-50	—	$p \leq 0.046$ 10-30	—	$p \leq 0.046$ 20	—
11 mm cone						
0.6 mm versus 0.8 mm	$p \leq 0.025$ 5-45	$p \leq 0.01$ 10-20 $p < 0.001$ 40-60	$p \leq 0.002$ 10-60	$p < 0.001$ 15-60	$p < 0.001$ 15-60	$p \leq 0.04$ 25-60
0.6 mm versus 1.0 mm	$p \leq 0.03$ 5-60	$p \leq 0.008$ 10-45	$p < 0.001$ 10-60	$p \leq 0.001$ 15-60	$p \leq 0.015$ 15-60	$p \leq 0.045$ 25-60
0.6 mm versus 1.7 mm	$p \leq 0.004$ 5-60	$p \leq 0.025$ 10-60	$p \leq 0.02$ 5-60	$p \leq 0.005$ 15-60	$p < 0.001$ 15-60	$p \leq 0.04$ 25-60
0.8 mm versus 1.0 mm	$p \leq 0.05$ 15-25 $p \leq 0.04$ 45	$p < 0.001$ 15-60	$p \leq 0.05$ 25-60	$p \leq 0.05$ 35-50	—	$p \leq 0.03$ 40-60
0.8 mm versus 1.7 mm	$p \leq 0.035$ 5-60	$p \leq 0.04$ 10-60	$p \leq 0.03$ 10-60	$p \leq 0.047$ 25-40	—	$p \leq 0.03$ 35-60
1.0 mm versus 1.7 mm	—	$p \leq 0.05$ 15-20	—	—	—	$p \leq 0.05$ 50-60

TABLE 4.4. (continued)

spraytip diameter	1 mm-depth		3 mm-depth		5 mm-depth	
	centre	edge	centre	edge	centre	edge
21 mm cone						
0.6 mm versus 0.8 mm	—	p<0.001 10-60	p≤0.03 15 p<0.001 25-60	p<0.001 10-60	p≤0.05 15-60	p<0.001 15-60
0.6 mm versus 1.0 mm	p≤0.007 10-60	p<0.001 10-60	p≤0.007 10-60	p≤0.009 25-60	p≤0.03 15-60	p<0.001 15-60
0.6 mm versus 1.7 mm	p<0.001 5-60	p<0.001 10-60	p<0.001 10-60	p<0.001 20-60	p≤0.004 15-60	p≤0.02 20-60
0.8 mm versus 1.0 mm	p≤0.0027 5-60	p≤0.04 55-60	—	p≤0.05 25-60	—	—
0.8 mm versus 1.7 mm	p<0.001 5-60	p≤0.04 15-60	p≤0.0075 10-60	p≤0.02 25-30	p≤0.025 20-60	—
1.0 mm versus 1.7 mm	p<0.001 5-60	p≤0.03 15-35	p≤0.0025 10-60	p≤0.04 25-45	p≤0.008 20-60	p≤0.04 35-50

The 0.6 mm spraytip provides the slowest fall in temperature. The other three spraytips are largely equal to each other. The statistical analysis of this comparison is presented in table 4.4.
There is a significant difference between the 0.6 mm spraytip and the other three spraytips, with the 0.6 mm spraytip giving the slowest fall in temperature.
Between the 0.8 mm and the 1.0 mm spraytips, there is no significancy in the temperatures measured in the centre at all depths. At the edge, there is no difference in the temperatures measured at 1 mm-depth and only moderate differences at 3 mm- and 5 mm-depth. When significancy exists, the 0.8 mm spraytip causes the faster fall in temperature.
Comparing the 0.8 mm spraytip with the 1.7 mm spraytip, there is a difference in the temperatures measured at all depths. The 1.7 mm spraytip causes the faster fall in temperature in the centre, but the 0.8 mm spraytip provides the faster fall in temperature at the edge.
Between the 1.0 mm and the 1.7 mm spraytips, significancy in the temperatures measured only exists in the centre at all depths, the

1.7 mm spraytip causing the faster fall in temperature. At the edge, no difference in the temperatures measured is present.
Figures 4.27 and 4.28 show the intervals of minimum and maximum values of the temperature measurements in the centre and at the edge of the cone respectively, at 3 mm-depth, for the various spraytip diameters.
From the moment temperature decline begins, there is no overlap between the 0.6 mm spraytip and the other three spraytips. The 0.8 mm, the 1.0 mm and the 1.7 mm spraytips overlap each other to a great extent.

4.3.4.2 *Cone diameter of 11 mm*
The comparison of the temperatures measured using the various spraytips in the centre of the 11 mm cone at 3 mm-depth is graphically presented in figure 4.29. Figure 4.30 shows the comparison in the temperatures measured at the edge at 3 mm-depth.
The 0.6 mm spraytip causes the slowest fall in temperature. The other three spraytips show a similar pattern in temperature fall.
Table 4.4 presents the statistical analysis of the comparison of the spraytips in the 11 mm cone.
Statistical significancy is obvious between the 0.6 mm spraytip and all the other spraytips, the 0.6 mm spraytip causing the slowest fall in temperature.
Between the 0.8 mm and the 1.0 mm spraytips and between the 0.8 mm and the 1.7 mm spraytips, there is a significant difference in the temperatures measured except for in the centre at 5 mm-depth. The 1.0 mm spraytip causes a faster fall in temperature than the 0.8 mm spraytip, although this difference is limited to the centre of the cone. The 1.7 mm spraytip causes a more rapid temperature decline than the 0.8 mm spraytip, both in the centre and at the edge of this cone.
In the centre of the cone no significant difference can be found between the 1.0 mm and the 1.7 mm spraytips. At the edge, there is only a moderate difference in the temperatures measured at 1 mm- and at 5 mm-depth and no significancy at 3 mm-depth. The 1.7 mm spraytip causes the fastest fall in temperature, when significancy exists.
In figures 4.31 and 4.32, the intervals of minimum and maximum temperature values of the spraytips are presented for the centre and the edge of the cone respectively, both at 3 mm-depth. The 0.6 mm spraytip shows no overlap with the other spraytips. In the centre, the 0.8 mm, the 1.0 mm and the 1.7 mm spraytips overlap each other to a great extent. At the edge, the interval of the 0.8 mm spraytip is similar to that

of the 1.0 mm spraytip and overlap is also clearly present with the 1.7 mm spraytip.

4.3.4.3 *Cone diameter of 21 mm*

Figures 4.33 and 4.34 show the comparison of the four spraytip diameters concerning the temperatures measured in the centre and at the edge of the 21 mm cone respectively, both at 3 mm-depth.
Once again, the 0.6 mm spraytip causes the slowest fall in temperature. The other three spraytips cause a similar fall in temperature.
Table 4.4 provides the statistical analysis of the comparison of the spraytips.
Significancy exists between the 0.6 mm spraytip and the other spraytips, except for the comparison with the 0.8 mm spraytip in the centre of the cone at 1 mm-depth. If significancy is present, the 0.6 mm spraytip shows the slowest fall in temperature.
Between the 0.8 mm and the 1.0 mm spraytips, a difference is present in the temperatures measured in the centre of the cone at 1 mm-depth only. The 1.0 mm spraytip shows the faster fall in temperature. At the edge, at 1 mm-depth, a very moderate significancy exists, the 1.0 mm spraytip providing the faster fall in temperature. At the edge, at 3 mm-depth, a difference does exist with the 0.8 mm spraytip causing the faster fall in temperature. At the edge, at 5 mm-depth, no significancy occurs.
In the comparison between the 0.8 mm and the 1.7 mm spraytips, a significant difference in the temperatures measured is present in the centre of the cone. At the edge, significancy exists at 1 mm-depth. At 3 mm-depth, hardly any significancy and at 5 mm-depth, no significancy at all is present. When significancy exists, the 1.7 mm spraytip shows the faster decline in temperature.
Finally, the 1.0 mm spraytip differs significantly from the 1.7 mm spraytip in the temperatures measured, both in the centre and at the edge, the 1.7 mm spraytip causing the faster fall in temperature.
Figures 4.35 and 4.36 present the intervals between minimum and maximum values of the temperatures measured for the spraytips for the centre and the edge of this cone respectively, both at 3 mm-depth. The 0.6 mm spraytip shows hardly any overlap with the other three spraytips in the centre of the cone. There is only a minor overlap with the 0.8 mm spraytip. At the edge of the cone, no overlap exists between the 0.6 mm spraytip and the other three spraytips. In the centre, there is a large overlap between the 0.8 mm and the 1.0 mm spraytip and just a minor overlap between these two spraytips and the 1.7 mm spraytip. At the

edge, the 0.8 mm, the 1.0 mm and the 1.7 mm spraytips overlap each other to a great extent.

4.4 Discussion

From this comparison it can be concluded that for all three cone diameters, the 0.6 mm spraytip shows the slowest fall in temperature, both in the centre and at the edge of the cones.
As far as the other three spraytip diameters are concerned, it is shown that in the centre of all the cones, the 1.7 mm spraytip gives a somewhat faster fall in temperature than the 0.8 mm and the 1.0 mm spraytips. For temperatures measured at the edge, this also applies to the results in the 11 mm cone. At the edge of the 21 mm cone, however, the temperature fall using the 0.8 mm spraytip equals that of the 1.7 mm spraytip. In the 16 mm cone, the 0.8 mm spraytip provides an even faster fall in temperature than the 1.7 mm spraytip.
The intervals of minimum and maximum values of the temperatures measured for the 0.6 mm spraytip overlap the intervals of the temperatures measured for the other three spraytip diameters marginally or not at all.
However a large overlap in the temperatures measured is present between the intervals of the 0.6 mm and the 0.8 mm spraytips in the centre of the 21 mm cone at 1 mm-depth.
It can be concluded that, in general, a large overlap exists between the intervals of the minimum and maximum values of the temperatures measured for the 0.8 mm, the 1.0 mm and the 1.7 mm spraytips, both in the centre and at the edge of the cones.
In the centre of the cones, it is shown that the use of a larger spraytip diameter increases the speed of temperature fall. There is an evident difference in temperature fall between the 0.6 mm and the 0.8 mm spraytips, but a marginal difference between the 0.8 mm and 1.7 mm spraytips.
At the edge of the 16 mm and the 21 mm cones, the 0.8 mm and the 1.7 mm spraytips show very similar results in the temperatures measured.
Every spraytip diameter produces a particular fan-shaped spray of liquid nitrogen. One can assume, therefore, that the spray is more intense in its centre than at its edge. By increasing the spraytip diameter, the amount of liquid nitrogen increases. In practice this results in run-off and spattering of liquid nitrogen. This process interacts with the liquid nitrogen being supplied to the target surface and diminishes

the intensity of the spray, at the outermost parts of the fan-shaped spray in particular.

In a small cone diameter (11 mm), the total amount of liquid nitrogen being supplied saturates the total target surface within the cone. When the cone diameter is increased (16 mm, 21 mm), the fan-shape of the spray will be more diffuse and thus the effectiviness of the spray will be even more vulnerable. The 1.0 mm and the 1.7 mm spraytips supply a greater amount of liquid nitrogen than does the 0.8 mm spraytip. This results in an overwhelming spattering and run-off of liquid nitrogen which diminishes the intensity of liquid nitrogen supplied by the 1.0 mm and the 1.7 mm spraytips.

In conclusion, the 0.8 mm spraytip can be used in all three polymethylmethacrylate cones for the following reasons:

a. The 0.8 mm spraytip provides a faster fall in temperature than the 0.6 mm spraytip.
b. The fall in temperature of the 0.8 mm spraytip is very similar to that of the 1.0 mm and the 1.7 mm spraytips, or even better as shown in the temperatures measured at the edge of the cones.
c. The interval of minimum and maximum temperature values of the 0.8 mm spraytip overlaps with the intervals of the 1.0 mm and the 1.7 mm spraytips.
d. In using the 0.8 mm spraytip, run-off of liquid nitrogen is far less than in using the 1.0 mm and the 1.7 mm spraytips. This is of great practical importance.

The 0.8 mm spraytip, therefore, is the best choice of spraytip to attach to the centre of the 11 mm, 16 mm and 21 mm cones.

4.5 References

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5

Spray Distances

5.1 Introduction

In this chapter the distance between the spraytip and the target surface will be discussed. This variable also influences the supply of liquid nitrogen in the freezing phase.
The spray distances of 5 mm, 10 mm, 15 mm and 20 mm will be compared to each other. The literature does not provide a clear guideline with regard to the spray distance to be adopted. When advice is given at all, the distances range from 5 mm to 25 mm (1, 2, 3, 4, 5). In effect, the choice of a specific distance is determined by the preference guided by the experience of the cryosurgeon.

5.2 Materials and methods

5.2.1 Materials

These are discussed in chapter 2.

5.2.2 Methods

In this study the spray distances of 5 mm, 10 mm, 15 mm and 20 mm are discussed. These distances correspond approximately with those reported in the literature.
The central spray pattern is applied with the spraytip diameter of 0.8 mm fixed in the centre of the cone. The supply of liquid nitrogen is provided by continuous spraying over 60 seconds.
The four distances are studied in the cone diameters of 11 mm, 16 mm and 21 mm. These cones are most commonly used in cryosurgery and have therefore also been adopted in this study.

The temperatures are recorded in the centre and at the edge of the cones, in both places at 1 mm, 3 mm and 5 mm from the surface of the target area.
Registration of the temperatures is recorded over a period of 60 seconds at intervals of 5 seconds.
For each cone diameter, the four spray distances will be discussed individually and then compared to each other. An optimal delivery of liquid nitrogen is the main starting point in this discussion.

5.2.3 Statistical methods

Four different experiments were performed for each spray distance (5 mm, 10 mm, 15 mm and 20 mm) in each cone diameter (11 mm, 16 mm and 21 mm). From these experiments the mean values of the recorded temperatures with the standard deviations were calculated (6). The standard error of the difference between the mean values was determined (6). The difference between the mean values was considered significant when twice the standard error was exceeded ($p \leq 0.05$) (6).
The mean values of the four experiments performed for the 15 mm spray distance in this study were compared to those of thirty equivalent experiments in the study of the spray patterns (chapter 3) using the Wilcoxon-test (6).
The Wilcoxon-test (6) was also used to investigate whether a constant pattern existed in the temperature values recorded in various gelatin areas.

5.3 Results

The Wilcoxon-test (6) compared the data obtained from the four different experiments in this study with those obtained from thirty different experiments previously performed in the study of the spray patterns. This was carried out in the cone diameter of 16 mm using the spraytip of 0.8 mm in the central spraying technique from a distance of 15 mm. There was no statistical difference between the data recorded at intervals of 10 seconds in the centre and at the edge both at 1 mm-, 3 mm- and 5 mm-depth. It was therefore concluded that four experiments were sufficient for the comparison of the various spray distances in this study.

The Wilcoxon-test (6) was applied to find out if any systematic trends existed in the different gelatin areas used for the same experiments. In our data no systematic phenomena were found in any of the cones.

The spray distances for each cone diameter will now be discussed separately.

5.3.1 Measurements in the cone diameter of 16 mm

Figure 5.1* and figure 5.2 show the graphs of the temperature measurements over time (60 seconds) for the centre and the edge of the 16 mm cone respectively, for the 5 mm spray distance.
Figures 5.3 and 5.4 provide the results of the temperature measurements for the centre and the edge of this cone respectively, for the 10 mm spray distance; figures 5.5 and 5.6 those, for the 15 mm spray distance and figures 5.7 and 5.8 those, for the 20 mm spray distance.
In table 5.1, the temperatures recorded in the centre and at the edge of the cone are analyzed over time (60 seconds).

5.3.2 Measurements in the cone diameter of 11 mm

Figure 5.9 and 5.10 display graphically the temperature measurements over time for the centre and the edge of the 11 mm cone respectively, for the 5 mm spray distance.
Figures 5.11 and 5.12 provide the results for the centre and the edge of this cone respectively, for the 10 mm distance; figures 5.13 and 5.14 provide the results for the 15 mm distance and figures 5.15 and 5.16 provide the results for the 20 mm distance.
In general, for the 5 mm and the 10 mm spray distances, the fall in temperature in the 11 mm cone is faster than in the 16 mm cone, whilst the fall in temperature in the 11 mm cone is identical or slightly slower than in the 16 mm cone for the 15 mm and the 20 mm distances.
In table 5.1, the temperatures recorded in the centre and at the edge are analyzed over time (60 seconds).

* See addendum chapter 5 for figures 5.1-5.36

TABLE 5.1. P-values of temperature change over 60 seconds for various cone diameters, depths and spray distances. The temperatures recorded at succeeding intervals of 5 seconds were statistically compared to each other (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth		3 mm-depth		5 mm-depth	
spray distance	centre	edge	centre	edge	centre	edge
16 mm cone						
5 mm	p≤0.04 0-40*	p≤0.015 10-35	p≤0.02 10-50	p≤0.045 20-60	p≤0.046 15-50	p≤0.01 30-60
10 mm	p≤0.03 0-60	p≤0.045 5-20	p≤0.04 5-60	p≤0.05 20-30	p≤0.04 15-45 55-60	p≤0.045 20-40
15 mm	p≤0.025 0-25	p≤0.04 5-40	p≤0.045 10-45	p≤0.04 15-50	p≤ 0.003 20-60	p≤0.04 20-50
20 mm	p≤0.035 0-60	p≤0.05 10-60	p≤ 0.03 15-60	p≤0.025 20-60	p≤ 0.042 20-60	p≤0.02 25-60
11 mm cone						
5 mm	p≤0.047 0-25	p≤0.045 10-45	p≤0.015 5-30	p≤0.035 15-55	p≤0.047 15-40	p≤0.015 30-60
10 mm	p≤0.015 0-35	p<0.009 5-60	p≤0.02 5-45	p≤0.045 10-60	p<0.001 10-60	p≤0.008 20-60
15 mm	p≤0.01 0-20	p<0.001 5-60	p≤0.04 5-35 p≤0.016 40-50	p≤0.04 10-60	p≤0.02 10-60	p≤0.046 20-60
20 mm	p≤0.008 0-20	p<0.001 5-60	p≤0.04 5-35	p≤0.04 10-60	p≤0.02 10-60	p≤0.035 20-60
21 mm cone						
5 mm	p≤0.035 5-30	—	p≤0.04 5-35	—	p≤0.04 15-45	—
10 mm	—	p≤0.03 10-25	p ≤0.04 5-15	—	p≤0.025 10-30 p≤0.007 35-60	—
15 mm	p≤0.035 5-20	p≤0.035 5-60	p≤0.02 10-25 p≤0.015 20-25	p≤0.035 20-60	p≤0.004 15-60	p≤0.04 30-60
20 mm	p≤0.035 5-20	p≤0.035 5-60	p≤0.02 10-15 p≤0.015 20-25	p≤0.03 20-60	p≤0.004 15-60	p≤0.04 30-60

5.3.3 Measurements in the cone diameter of 21 mm

In figures 5.17 and 5.18, the results of the temperatures measured in the centre and at the edge of the 21 mm cone respectively are displayed graphically, for the 5 mm spray distance.
Figures 5.19 and 5.20 provide the results of the temperatures measured in the centre and at the edge respectively, for the 10 mm spray distance.
Figures 5.21 and 5.22 provide the results of the temperature measurements in the centre and at the edge respectively, for the 15 mm distance, and figures 5.23 and 5.24 present the results, of the temperatures measured for the 20 mm distance.
In the cone diameter of 21 mm, the fall in temperature is slower than in the other two cones for all spray distances, at the edge in particular.
Table 5.1 shows the statistical analysis of the temperatures over time (60 seconds). Almost no fall in temperature and almost no significancy can be found at the edge of this cone for the spray distances of 5 mm and 10 mm.

Significant temperature differences exist for each of the recorded times in all of the following paired comparisons of depth, both in the centre and at the edge of the cone: 1 mm and 3 mm; 1 mm and 5 mm; 3 mm and 5 mm for all cones and spray distances (table 5.2).
Between the results in the centre and at the edge, significancy of the temperatures occurs for each of the recorded times for the various cones, depths and spray distances (table 5.3).

5.3.4 Comparison of the spraytip diameters

The spray distances for every cone diameter will now be compared to each other.

5.3.4.1 *Cone diameter of 16 mm*

Figure 5.25 shows the comparison of the four spray distances for the centre of the 16 mm cone and figure 5.26 for the edge, both at 3 mm-depth.
Spraying from the 5 mm distance causes the slowest fall in temperature. The distances of 10 mm and 20 mm give a very similar decline in temperature, whilst the 15 mm spray distance shows the fastest fall in temperature.
The statistical analysis of this comparison is presented in table 5.4.

TABLE 5.2 P-values of the comparison of the depths in the centre and at the edge for various cone diameters and spray distances. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm- versus 3 mm-depth		1 mm- versus 5 mm-depth		3 mm- versus 5 mm-depth	
spray distance	centre	edge	centre	edge	centre	edge
16 mm cone						
5 mm	p<0.001 5–60*	p<0.001 15–60	p<0.001 5–60	p<0.001 15–60	p≤0.009 10–60	p≤0.006 25–60
10 mm	p<0.001 5-60	p≤0.035 10-60	p<0.001 5-60	p≤0.009 10-60	p≤0.01 10-60	p≤0.02 20-60
15 mm	p<0.001 5-60	p<0.001 15-60	p<0.001 5-60	p≤0.04 10-60	p≤0.007 15-60	p≤0.02 20-60
20 mm	p≤0.004 5-60	p≤0.035 15-60	p≤0.0022 5-60	p≤0.03 15-60	p≤0.006 15-60	p≤0.01 25-60
11 mm cone						
5 mm	p<0.001 5-60	p≤0.0025 15-60	p<0.001 5-60	p<0.001 15-60	p<0.001 10-60	p≤0.03 15-60
10 mm	p<0.001 5-60	p<0.001 15-60	p<0.001 5-60	p≤0.002 5-60	p<0.001 10-60	p≤0.03 10-60
15 mm	p<0.001 5-60	p≤0.04 5-60	p<0.001 5-60	p<0.001 5-60	p<0.001 10-60	p≤0.04 5-60
20 mm	p≤0.0027 5-60	p<0.001 10-60	p≤0.002 5-60	p<0.001 5-60	p≤0.02 5-60	p≤0.04 5-60
21 mm cone						
5 mm	p<0.001 10-60	p≤0.045 15-60	p<0.001 10-60	p≤0.01 15-60	p≤0.009 10-60	p≤0.03 40-60
10 mm	p≤0.045 10-60	p<0.04 10-60	p≤0.003 10-60	p<0.015 10-60	p≤0.006 10-60	p≤0.04 25-50
15 mm	p≤0.05 10-60	p<0.001 10-60	p≤0.0095 10-60	p<0.001 10-60	p≤0.009 15-60	p<0.001 25-60
20 mm	p≤0.05 10-60	p<0.001 10-60	p≤0.006 10-60	p<0.001 10-60	p≤0.02 15-60	p<0.001 25-60

TABLE 5.3. P-values of the comparison of the temperature measurements in the centre to those at the edge for various cones, depths and spray distances. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth	3 mm-depth	5 mm-depth
spray distance	centre versus edge	centre versus edge	centre versus edge
16 mm cone			
5 mm	$p<0.001$ 5-60*	$p<0.001$ 15-60	$p\leq0.04$ 20-60
10 mm	$p<0.001$ 5-60	$p<0.001$ 15-60	$p<0.001$ 20-60
15 mm	$p<0.001$ 5-60	$p\leq0.007$ 20-60	$p\leq0.04$ 45-60
20 mm	$p\leq0.008$ 5-60	$p\leq0.03$ 15-60	$p\leq0.04$ 25-60
11 mm cone			
5 mm	$p<0.001$ 5-60	$p<0.001$ 10-60	$p<0.001$ 20-60
10 mm	$p<0.001$ 5-60	$p<0.001$ 10-60	$p\leq0.04$ 10-60
15 mm	$p<0.001$ 5-60	$p<0.001$ 10-60	$p<0.001$ 15-60
20 mm	$p\leq0.003$ 5-60	$p<0.001$ 10-60	$p\leq0.04$ 10-60
21 mm cone			
5 mm	$p<0.001$ 10-60	$p\leq0.0095$ 10-60	$p<0.001$ 20-60
10 mm	$p<0.004$ 10-60	$p\leq0.003$ 10-60	$p<0.001$ 15-60
15 mm	$p\leq0.04$ 10-60	$p\leq0.005$ 15-60	$p<0.001$ 20-60
20 mm	$p\leq0.04$ 10-60	$p\leq0.005$ 15-60	$p<0.001$ 20-60

TABLE 5.4. P-values of the comparison of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm for the various cones and depths. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth		3 mm-depth		5 mm-depth	
spray distance	centre	edge	centre	edge	centre	edge
16 mm cone						
5 mm versus 10 mm	p≤0.01 10-60*	p≤0.03 25-60	p<0.001 20-60	p≤0.02 30-60	p≤0.03 25-60	p≤0.01 35-60
5 mm versus 15 mm	p≤0.004 10-60	p≤0.003 15-60	p<0.001 15-60	p<0.001 20-60	p<0.001 25-60	p<0.001 25-60
5 mm versus 20 mm	p≤0.006 15-60	p≤0.003 20-60	p<0.001 25-60	p≤0.01 25-60	p≤0.01 30-60	p<0.001 30-60
10 mm versus 15 mm	—	p≤0.003 10	p≤0.02 35-60	p≤0.002 20-60	—	p≤0.006 25-60
10 mm versus 20 mm	p≤0.02 5 p≤0.05 45-60	—	—	—	—	—
15 mm versus 20 mm	p≤0.04 5-60	—	p≤0.009 35-60	p≤0.03 20-60	p≤0.04 60	p≤0.015 25-60
11 mm cone						
5 mm versus 10 mm	p≤0.0015 10-60	p≤0.0027 15-60	p≤0.02 10-60	p≤0.035 15-60	p≤ 0.015 15-60	p≤ 0.03 25-60
5 mm versus 15 mm	p<0.001 10-60	p≤0.01 10-60	p≤0.04 10-60	p≤0.002 15-60	p≤0.02 15-60	p≤0.04 25-60
5 mm versus 20 mm	p<0.001 10-60	p<0.001 15-60	p<0.001 15-60	p≤0.035 15-60	p≤0.005 20-60	p≤0.025 30-60
10 mm versus 15 mm	—	—	—	—	—	—
10 mm versus 20 mm	p≤0.042 20-35	—	—	—	—	—
15 mm versus 20 mm	—	—	—	—	—	—

TABLE 5.4. (continued)

	1 mm-depth		3 mm-depth		5 mm-depth	
spray distance	centre	edge	centre	edge	centre	edge
21 mm cone						
5mm versus 10 mm	p≤0.03 20-60	p≤0.03 30-35	p≤0.009 25-60	—	p≤0.042 25-60	—
5 mm versus 15 mm	p≤0.01 25-60	p≤0.009 15-60	p≤0.006 30-60	p≤0.03 25-60	p<0.001 25-60	p≤0.035 35-60
5 mm versus 20 mm	p≤0.045 10-15 p≤0.009 30-60	p≤0.009 15-60	p≤0.003 35-60	p≤0.002 30-60	p≤0.001 35-60	p≤0.002 40-60
10 mm versus 15 mm	—	p≤0.02 10-60	p≤0.035 15 p≤0.047 50-60	p<0.001 25-60	p<0.001 35-60	p≤0.05 30-60
10 mm versus 20 mm	p≤0.03 15-20	p≤0.015 10-60	—	p<0.001 20-60	p≤0.012 50-60	p≤0.047 15-60
15 mm versus 20 mm	—	p<0.001 25-60	—	p≤0.006 30-60	p≤0.05 45-55	p≤0.006 40-60

There is a significant difference in the temperatures measured between the 5 mm spray distance and the other three spray distances. Spraying from 5 mm distance gives the slowest fall in temperature.
Between the 10 mm and the 15 mm spray distances, there is only significancy in the temperatures measured in the centre of the cone at 3 mm depth. At the edge of the cone, a difference in the temperatures measured is present at 3 mm- and 5 mm-depth and only a moderate difference at 1 mm-depth. When significancy exists, the 15 mm spray distance causes the faster fall in temperature.
Comparing the 10 mm with the 20 mm spray distance, there is no difference at all, except in the centre at 1 mm-depth, where a moderate difference is present. Here the 10 mm distance gives a slightly faster fall in temperature.
Between the 15 mm and the 20 mm spray distances, significancy in the temperatures measured exists in the centre at 1 mm- and 3 mm-depths, and at 5 mm-depth only at 60 seconds. At the edge, there is no

difference in the temperatures measured at 1 mm-depth in contrast with 3 mm- and 5 mm-depth.
Figures 5.27 and 5.28 show the intervals of minimum and maximum values of the temperature measurements for the various spray distances in the centre and at the edge of the cone respectively at 3 mm-depth. From the moment temperature decline begins, there is almost no overlap between the 5 mm spray distance and the other three spray distances, both in the centre and at the edge of the cone. The 10 mm, the 15 mm and the 20 mm spray distances overlap each other clearly. At 3 mm-depth, the 15 mm distance shows no overlap with the 10 mm and 20 mm distances in the centre of the cone and almost no overlap at the edge.

5.3.4.2 *Cone diameter of 11 mm*

The comparison of the temperatures measured for the spray distances in the centre of the 11 mm cone at 3 mm-depth is graphically presented in figure 5.29. Figure 5.30 shows the comparison of the temperatures measured at the edge at 3 mm-depth.
The 5 mm spray distance causes the slowest fall in temperature. The other three spray distances provide a very similar pattern in temperature fall.
Table 5.4 presents the statistical analysis of the comparison of the temperatures measured for the spray distances in the 11 mm cone.
Statistical significancy in the temperatures measured is obvious between the 5 mm spray distance and all the other distances, the 5 mm distance causing the slowest fall in temperature.
Almost no significant difference in the temperatures measured exists between the 10 mm, the 15 mm and the 20 mm spray distances. There is only a moderate difference in the temperatures measured between the 10 mm and the 20 mm spray distances in the centre of the cone at 1 mm-depth. The 20 mm spray distance provides the faster fall in temperature.
In figures 5.31 and 5.32, the intervals of minimum and maximum temperature values for the spray distances are presented for the centre and the edge of the cone respectively, both at 3 mm-depth. The 5 mm spray distance shows hardly any overlap with the intervals of the other spray distances when temperature decline begins. The 10 mm, the 15 mm and the 20 mm spray distances overlap each other to a great extent.

5.3.4.3 *Cone diameter of 21 mm*

Figures 5.33 and 5.34 show the comparison of the four spray distances for the temperatures measured in the centre and at the edge of the 21 mm cone respectively, both at 3 mm-depth.

The 5 mm spray distance causes the slowest fall in temperature in the centre and at the edge of the cone. At the edge, the temperature fall for the 10 mm distance almost equals that of the 5 mm distance, at 3 mm- and 5 mm-depth in particular. The 15 mm spray distance causes the fastest fall in temperature, followed by the 20 mm and the 10 mm distances respectively.

Table 5.4 provides the statistical analysis of the comparison of the spray distances.

Significancy exists between the 5 mm spray distance and the other spray distances, except for the comparison with the 10 mm spray distance at the edge of the cone at 3 mm- and 5 mm-depth. If significancy is present, the 5 mm spray distance shows the slowest fall in temperature.

Between the 10 mm and the 15 mm spray distances, significancy in the temperatures measured is present in the centre and at the edge of the cone, except for the centre at 1 mm-depth. The 15 mm spray distance shows the faster fall in temperature.

In the comparison between the 10 mm and the 20 mm spray distances, a significant difference in the temperatures measured is present in the centre and at the edge of the cone, except for the centre at 3 mm-depth. When significancy exists, the 20 mm spray distance shows the faster decline in temperature.

In the centre of the cone, a moderate significancy in the temperatures measured only exists between the 15 mm and the 20 mm spray distances at 5 mm-depth. At the edge a difference is present at all depths. When significancy occurs, the 15 mm distance causes the faster fall in temperature.

Figures 5.35 and 5.36 present the intervals between minimum and maximum temperature values of the spray distances for the centre and the edge of this cone respectively, both at 3 mm-depth. The 5 mm spray distance shows hardly any overlap with the other three spray distances in the centre of the cone when temperature decline begins. At the edge, there is overlap with the 10 mm spray distance.

In the centre, there is a large overlap between the 10 mm, the 15 mm and the 20 mm spray distances. At the edge, no overlap occurs between the intervals of the 5 mm and the 10 mm distances as compared to the intervals of the 15 mm and the 20 mm distances.

There is no overlap between the intervals of the 15 mm and the 20 mm spray distances.

5.4 Discussion

From this comparison it can be concluded that for all three cone diameters, the 5 mm spray distance shows the slowest fall in temperature, both in the centre and at the edge of the cones. At the edge of the 21 mm cone, the 10 mm spray distance provides very similar results to the 5 mm distance.

The 10 mm, 15 mm and 20 mm spray distances show very similar patterns in the 11 mm cone, both in the centre and at the edge. The 15 mm distance provides the fastest fall in temperature, both in the centre and at the edge of the 16 mm and the 21 mm cones. This is statistically significant as is shown in table 5.4.

The intervals óf minimum and maximum values of the 5 mm spray distance overlap the intervals of the other three spray distances marginally or not at all.

A large overlap, however, occurs between the intervals of the 5 mm and the 10 mm spray distances at the edge of the 21 mm cone at 3 mm- and 5 mm-depth. Here no significancy is present between these two spray distances.

Furthermore a large overlap exists between the intervals of the minimum and maximum values of the 10 mm, the 15 mm and the 20 mm distances, both in the centre and at the edge of the cones. At the edge of the 21 mm cone, however, there is no overlap between the intervals of the 15 mm and the 20 mm distances and a clearly significant difference exists.

It can be concluded that by increasing the spray distance to 15 mm, the speed of temperature fall is enhanced in all cone diameters.

Nevertheless, on exceeding the 15 mm distance, the fall in temperature generally does not further increase in the 11 mm cone, and even decreases in the 16 mm and the 21 mm cones. This is confirmed by the statistical analysis in table 5.4.

The spray distance influences the fan-shape of the spray of liquid nitrogen.

Spraying from a distance of only 5 mm results in run-off and spattering of liquid nitrogen. This process interacts with the liquid nitrogen being supplied to the target surface and diminishes the intensity of the spray. On increase of the spray distance, run-off and spattering will be less and the efficiency of the spray increases. Nevertheless, when exceeding

the 15 mm distance, the fan-shape of the spray widens and the intensity of the spray decreases.
In a small cone diameter (11 mm), the cone itself prevents the widening of the fan-shape of the spray. Therefore the 15 mm and the 20 mm spray distances give equal temperature results in the 11 mm cone. The fan-shape of the spray will be more diffuse with the increase in the cone diameter (16 mm, 21 mm). In these cones, the increase in spray distance widens even further the fan-shape of the spray and decreases its intensity. This explains why the fall in temperature decreases when spraying from a distance of 20 mm in the 16 mm and 21 mm cones.

In conclusion, spraying from a distance of 15 mm generally provides the fastest fall in temperature. Therefore this distance is the best choice for all three polymethylmethacrylate cones diameters.

5.5 References

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6

Cone diameters

6.1 Introduction

In this chapter the cone diameters will be compared with each other. It is important to know if the central spray technique causes a sufficient fall in temperature in all the cone diameters of 11 mm, 16 mm and 21 mm, at the edge of the cones in particular.

6.2 Methods

In this comparison, the temperature measurements recorded for the cone diameters of 11 mm, 16 mm and 21 mm are applied, using the spraytip diameters of 0.8 mm, 1.0 mm and 1.7 mm respectively. The data are obtained from the measurements reported in chapter 4.
The supply of liquid nitrogen is provided by continuous spraying with the spraytip fixed in the centre of the cone. The distance between spraytip and target area is 15 mm. The temperatures are recorded in the centre and at the edge of the cones, in both places at 1 mm, 3 mm and 5 mm from the gelatin surface. Registration of the temperatures is performed over a period of 60 seconds at intervals of 5 seconds.

6.3 Results

The cone diameters for every spraytip diameter will now be compared to each other. The data for comparison are taken from the centre and the edge of the cones, both at 3 mm-depth.

6.3.1 Spraytip diameter of 0.8 mm

Figure 6.1* and figure 6.2 show the comparison of the cone diameters of 11 mm, 16 mm and 21 mm for the centre and the edge of the cones respectively, both at 3 mm-depth. The 0.8 mm spraytip is applied in all cone diameters.
The fall in temperature is slowest in the 21 mm cone, at the edge in particular. Here a subzero temperature is reached after just 45 seconds of freezing. In the centre, the fall in temperature in the 11 mm cone is faster at first and then slower than in the 16 mm cone. At the edge, the 11 mm and 16 mm cones show a very similar pattern in temperature decline.
The statistical analysis of this comparison is presented in table 6.1. In the comparison of the 11 mm cone with the 16 mm cone, statistical significancy exists for all depths, both in the centre and at the edge of the cones. The 16 mm cone provides the faster fall in temperature. An evident differeñce also exists between the 11 mm cone and the 21 mm cone. Here the 11 mm cone causes the faster fall in temperature. Between the 16 mm cone and the 21 mm cone significancy is present, the 16 mm cone causing the faster fall in temperature.
Figures 6.3 and 6.4 show the intervals of minimum and maximum temperature values for the cone diameters, in the centre and the edge of the cones respectively, both at 3 mm-depth. In the centre, there is a large overlap between all cone diameters. At the edge, the 11 mm and 16 mm cones overlap each other, but there is no overlap between the 21 mm cone and the other two cones.

6.3.2 Spraytip diameter of 1.0 mm

The comparison of the cone diameters of 11 mm, 16 mm and 21 mm is presented in figures 6.5 and 6.6 for the centre and the edge of the cones respectively, both at 3 mm-depth. The 1.0 mm spraytip is applied in all cone diameters.
In the centre, the 11 mm cone shows the fastest fall in temperature. The other two cones show an identical pattern. At the edge, the fall in temperature is slowest in the 21 mm cone. A subzero temperature is

* See addendum chapter 6 for figures 6.1-6.12

TABLE 6.1. P-values of the comparison of the cone diameters of 11 mm, 16 mm and 21 mm for various spraytip diameters and depths. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth		3 mm-depth		5 mm-depth	
cone diameter	centre	edge	centre	edge	centre	edge
0.8 mm spraytip						
11 mm versus 16 mm	$p \leq 0.006$ 30-60*	$p < 0.001$ 25-60	$p \leq 0.02$ 10-20 $p \leq 0.04$ 40-60	$p \leq 0.025$ 15-20 $p \leq 0.01$ 45-60	$p \leq 0.003$ 15-45	$p \leq 0.002$ 25-60
11 mm versus 21 mm	$p \leq 0.0015$ 5-60	$p \leq 0.006$ 10-60	$p \leq 0.025$ 5-25	$p \leq 0.009$ 10-60	$p < 0.001$ 15-60	$p \leq 0.005$ 25-55
16 mm versus 21 mm	$p < 0.001$ 5-60	$p \leq 0.004$ 15-60	$p \leq 0.02$ 20	$p < 0.001$ 20-60	$p \leq 0.045$ 30-60	$p \leq 0.025$ 20-60
1.0 mm spraytip						
11 mm versus 16 mm	$p \leq 0.03$ 5-15	$p \leq 0.003$ 10-15	$p \leq 0.03$ 10-60	$p \leq 0.04$ 15-35	$p \leq 0.03$ 15-30	$p \leq 0.006$ 30-60
11 mm versus 21 mm	$p \leq 0.035$ 5-50	$p \leq 0.007$ 10-35	$p \leq 0.009$ 10-55	$p < 0.001$ 15-60	$p \leq 0.009$ 20-35	$p \leq 0.002$ 25-60
16 mm versus 21 mm	$p \leq 0.045$ 10-60	$p \leq 0.05$ 15-35	$p \leq 0.04$ 10	$p \leq 0.009$ 20-60	—	$p \leq 0.003$ 25-60
1.7 mm spraytip						
11 mm versus 16 mm	$p \leq 0.035$ 35-60	$p \leq 0.035$ 10-55	$p \leq 0.025$ 5-40	$p \leq 0.045$ 15-55	$p \leq 0.02$ 60	$p \leq 0.05$ 55-60
11 mm versus 21 mm	$p \leq 0.045$ 5 $p \leq 0.035$ 55-60	$p \leq 0.009$ 10-35	$p \leq 0.009$ 10-30 $p \leq 0.009$ 55-60	$p < 0.001$ 15-60	$p \leq 0.02$ 35-60	$p \leq 0.047$ 20-60
16 mm versus 21mm	—	$p \leq 0.045$ 40-60	$p \leq 0.04$ 50-60	$p < 0.001$ 20-60	$p \leq 0.04$ 45-60	$p \leq 0.035$ 20-60

reached after 50 seconds of freezing. The 11 mm cone causes a slightly faster fall in temperature than the 16 mm cone.
Table 6.1 provides the statistical analysis of this comparison.
In the comparison of the 11 mm cone with the 16 mm cone, a statistically significant difference exists for all depths, both in the centre and

temperature. Evidently, there is also a difference between the 11 mm cone and the 21 mm cone, the 11 mm cone causing the faster fall in temperature. Between the 16 mm cone and the 21 mm cone, significancy is present except for in the centre at 5 mm-depth, with the 16 mm cone causing the faster fall in temperature.
In figures 6.7 and 6.8, the intervals of minimum and maximum temperature values are presented for the centre and the edge of the cones respectively, both at 3 mm-depth.
In the centre, an overlap is present between the 16 mm and the 21 mm cones and almost no overlap between these two cones and the 11 mm cone. At the edge, a large overlap exists between the 11 mm and the 16 mm cones and no overlap between these two cone diameters and the 21 mm cone.

6.3.3 Spraytip diameter of 1.7 mm

Figures 6.9 and 6.10 present the comparison of the cone diameters of 11 mm, 16 mm and 21 mm for the centre and the edge of the cones respectively, both at 3 mm-depth. The 1.7 mm spraytip is applied in all cone diameters.
In the centre, the 11 mm cone shows the fastest fall in temperature, followed by the 21 mm and the 16 mm cones respectively. At the edge, the 11 mm cone also causes the fastest fall in temperature, followed by the 16 mm cone. The fall in temperature is slowest in the 21 mm cone, reaching a subzero temperature after 40 seconds of freezing.
Table 6.1 provides the statistical analysis of this comparison.
In the comparison of the 11 mm cone with the 16 mm cone, statistical significancy is present for all depths, both in the centre and at the edge of the cones. The 11 mm cone provides the faster fall in temperaure. A significant difference also exists between the 11 mm cone and the 21 mm cone. Here the 11 mm cone causes the faster fall in temperature. Between the 16 mm cone and the 21 mm cone, significancy is present, with the exception of in the centre at 1 mm-depth. When significancy occurs, the faster fall in temperature is caused by the 21 mm cone in the centre and by the 16 mm cone at the edge.
Figures 6.11 and 6.12 show the intervals of minimum and maximum values for the centre and the edge of the cones respectively, both at 3 mm-depth.
In the centre, a large overlap exists between the three cone diameters. At the edge, a moderate overlap occurs between the 11 mm and 16 mm

cones. These two cone diameters show no overlap with the 21 mm cone diameter.

6.4 Discussion

For the spraytip diameters of 0.8 mm, 1.0 mm and 1.7 mm, the fall in temperature is generally fastest in the 11 mm cone and slowest in the 21 mm cone.
The statistical analysis of this comparison shows, for all spraytip diameters, an evident difference between the 11 mm and the 16 mm cones respectively, and the 21 mm cone. The latter generally shows a statistically significantly slower fall in temperature, at the edge in particular.
In studying the intervals of minimum and maximum temperature values, it is shown, that in general, for all three spraytip diameters, the measurements of temperature for the three cone diameters overlap each other in their centre. At the edge of the cones, it is obvious that overlap exists between the 11 mm cone and the 16 mm cone, whilst no overlap at all is present between these two cone diameters and the 21 mm cone diameter.
From this comparison it can be concluded that the temperature fall is slowest at the edge of the 21 mm cone. At the edge of the 21 mm cone at 1 mm-depth, a subzero temperature is recorded after 25 seconds, using the 0.8 mm and 1.0 mm spraytips and after 20 seconds, using the 1.7 mm spraytip. At the edge, at 3 mm-depth, a subzero temperature is recorded after just 45 seconds of freezing using the 0.8 mm and 1.7 mm spraytips and after 50 seconds, using the 1.0 mm spraytip.
The results show that an increased spraytip diameter cannot improve the fall in temperature at the edge of the 21 mm cone, whilst the run-off of liquid nitrogen is increased when a wider spraytip is used. The data for this comparison were obtained from experiments using continuous spraying.
When applying a different spraying technique - for instance, an intermittent spray over 30 seconds of freezing - a subzero temperature will not be recorded at 1 mm-depth at the edge of the 21 mm cone, using the 0.8 mm and 1.0 mm spraytips. Only when using the 1.7 mm spraytip is a subzero temperature recorded at 1 mm-depth (unpublished data). With an increased cone diameter, the target area becomes widened and the semicircular iceball will flatten more and more. This finally results in an inadequate fall in temperature at the edge of the cone, particularly when using a short period of freezing.

It is concluded that the 21 mm cone provides an insufficient fall in temperature, at the edge in particular. From this study, the cone diameter of 16 mm appears to be the maximum diameter for cryosurgical practice. Other cone diameters between 16 mm and 21 mm could possibly also be applied, but this has not yet been investigated (1, 2, 3). It is of practical importance that the area to be frozen should be divided into several sectors when a cone with a diameter exceeding 16 mm is required.

6.5 References

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7

Spraying techniques: continuous versus intermittent

7.1 Introduction

The techniques used for spraying influence the amount of liquid nitrogen being supplied to the target area during freezing.
So far, the experiments have been performed using a continuous spray of liquid nitrogen. An intermittent spray is advised in cryosurgical literature (1, 2, 3, 4, 5), mainly to limit the run-off and spattering of liquid nitrogen.
In this chapter, four spraying techniques will be compared.

7.2 Materials and methods

7.2.1 Materials

These are discussed in chapter 2.

7.2.2 Methods

In this study, four spraying techniques are discussed: continuous spraying, intermittent spraying (once a second) and two different combinations of continuous and intermittent spraying. Run-off of liquid nitrogen occurs after 5-10 seconds of continuous spraying: therefore continuous spraying over 5 seconds and 10 seconds respectively are combined with intermittent spraying of once a second. Other combinations of continuous and intermittent spraying can be considered: for instance, continuous spraying over 3 or 4 seconds, with interruptions of 1-2 seconds. These techniques, however, produce too much run-off of liquid nitrogen, a disadvantage for clinical practice.

The central spray pattern is applied with the 0.8 mm spraytip fixed in the centre of the cone with a distance of 15 mm to the gelatin surface. The liquid nitrogen is supplied over a period of 30 seconds. In this study, the cone diameters of 11 mm and 16 mm are used.
The temperatures are recorded in the centre and at the edge of the cones, in both places at 1 mm, 3 mm and 5 mm from the surface of the target area.
Registration of the temperatures was performed over a period of 30 seconds at intervals of 5 seconds.
For each cone diameter, the four spraying techniques will be discussed individually and then compared with each other.

7.2.3 Statistical methods

Four different experiments were performed for each spraying technique in each cone diameter. From these experiments the mean values of the recorded temperatures with the standard deviations were calculated (6). The standard error of the difference between the mean values was determined (6). The difference between the mean values was considered significant when twice the standard error was exceeded ($p \leq 0.05$) (6).
The mean values of the four experiments applying the continuous spraying technique were compared to the mean values obtained from thirty different experiments in the study of the spray patterns in the 16 mm cone by the Wilcoxon-test (6). The Wilcoxon-test was also used to investigate whether a constant pattern existed in the temperature values recorded in various gelatin areas.

7.3 Results

It was found that four experiments were sufficient for the comparison of the various spraying techniques. In addition, no systematic phenomena were found in any of the cones.
The techniques used for spraying for each cone diameter (11 mm and 16 mm) will now be discussed separately.

TABLE 7.1. P-values of temperature change over 30 seconds for various cone diameters, depths and spraying techniques. The temperatures recorded at succeeding intervals of 5 seconds were statistically compared to each other (= time interval in seconds, wherein significancy occurs).*

	1 mm- depth		3 mm-depth		5 mm-depth	
spray techn.	centre	edge	centre	edge	centre	edge
16 mm cone						
continuous	$p \le 0.025$ 0-25*	$p \le 0.04$ 5-30	$p \le 0.04$ 10-30	$p \le 0.008$ 15-30	$p < 0.001$ 20-30	$p \le 0.015$ 20-30
interm. 1/sec	$p \le 0.009$ 0-15	$p \le 0.004$ 10-30	$p \le 0.035$ 5-25	$p \le 0.003$ 20-30	$p < 0.001$ 20-30	$p \le 0.023$ 25-30
cont.5 sec, interm. 1/sec	$p \le 0.05$ 0-25	$p \le 0.0025$ 5-30	$p < 0.001$ 5-30	$p \le 0.002$ 15-30	$p \le 0.02$ 10-30	$p \le 0.004$ 20-30
cont.10 sec, interm. 1/sec	$p \le 0.003$ 0-25	$p \le 0.009$ 5-30	$p < 0.001$ 5-30	$p < 0.001$ 10-30	$p < 0.001$ 10-30	$p < 0.001$ 15-30
11 mm cone						
continuous	$p \le 0.01$ 0-20	$p < 0.001$ 5-30	$p \le 0.04$ 5-30	$p < 0.001$ 10-30	$p < 0.001$ 10-30	$p \le 0.02$ 20-30
interm. 1/sec	$p \le 0.025$ 0-30	$p \le 0.008$ 5-30	$p < 0.001$ 5-30	$p \le 0.07$ 10-30	$p \le 0.025$ 10-30	$p \le 0.003$ 25-30
cont.5 sec, interm. 1/sec	$p \le 0.005$ 0-30	$p \le 0.01$ 5-30	$p \le 0.005$ 5-30	$p \le 0.03$ 10-30	$p \le 0.02$ 10-30	—
cont.10 sec, interm. 1/sec	$p \le 0.05$ 0-30	$p < 0.001$ 5-30	$p < 0.001$ 5-30	$p < 0.001$ 10-30	$p < 0.001$ 10-30	$p < 0.001$ 20-30

7.3.1 Measurements in the cone diameter of 16 mm

Figure 7.1* and figure 7.2 show the graphs of the temperature measurements over time (30 seconds) for the centre and the edge of the 16 mm cone respectively, applying continuous spraying, and figures 7.3 and 7.4, using intermittent spraying of once a second.
Figures 7.5 and 7.6 provide the results for the centre and the edge of this cone respectively, applying continuous spraying over 5 seconds combined with intermittent spraying of once a second. Figures 7.7 and

* See addendum chapter 7 for figures 7.1-7.24

7.8 provide the results applying, continuous spraying over 10 seconds combined with intermittent spraying of once a second.
In table 7.1, the temperatures from the centre and the edge of the cone are analyzed over time (30 seconds).

7.3.2 Measurements in the cone diameter of 11 mm

In figures 7.9 and 7.10, the results measured in the centre and at the edge of the 11 mm cone respectively are displayed graphically, using the continuous spraying technique.
Figures 7.11 and 7.12 provide the results for the centre and the edge respectively, practicing intermittent spraying of once a second.
Figures 7.13 and 7.14 show the temperature measurements for the centre and the edge respectively, applying continuous spraying over 5 seconds combined with intermittent spraying of once a second. Figures 7.15 and 7.16 present the results of using continuous spraying over 10 seconds combined with intermittent spraying of once a second. In general, the fall in temperature at the edge of the 11 mm cone is faster than at the edge of the 16 mm cone for all spraying techniques.
Table 7.1 shows the statistical analysis of the temperatures over time (30 seconds).

Significant temperature differences exist for each of the recorded times in all of the following paired comparisons of depth, both in the centre and at the edge of the cone: 1 mm and 3 mm, 1 mm and 5 mm, 3 mm and 5 mm for all cones (11 mm and 16 mm) and spraying techniques (table 7.2).
Between the results in the centre and at the edge, significancy of temperatures generally occurs for each of the recorded times for both cones and for the various depths and spraying techniques (table 7.3), except at 5 mm-depth for continuous spraying in the 16 mm cone.

7.3.3 Comparison of the spraying techniques

The spraying techniques for each cone diameter will now be compared with each other.

TABLE 7.2. P-values of the comparison of the depths in the centre and at the edge for various cone diameters and spraying techniques. The temperatures recorded over a period of 30 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm- versus 3 mm-depth		1 mm- versus 5 mm-depth		3 mm- versus 5 mm-depth	
spray techn.	centre	edge	centre	edge	centre	edge
16 mm cone						
continuous	p<0.001 5-30*	p≤0.001 15-30	p<0.001 5-30	p≤0.04 10-30	p≤0.007 15-30	p≤0.02 20-30
interm. 1/sec	p<0.001 5-30	p<0.001 15-30	p<0.001 5-30	p<0.001 15-30	p≤0.025 10-30	p<0.001 25-30
cont.5 sec, interm. 1/sec	p<0.001 5-30	p<0.001 10-30	p<0.001 5-30	p≤0.02 5-30	p<0.001 10-30	p<0.001 20-30
cont.10 sec, interm. 1/sec	p<0.001 5-30	p≤0.004 5-30	p<0.001 5-30	p<0.001 5-30	p≤0.05 5-30	p≤0.003 15-30
11 mm cone						
continuous	p<0.001 5-30	p≤0.04 5-30	p<0.001 5-30	p<0.001 5-30	p≤0.015 5-30	p≤0.04 5-30
interm. 1/sec	p<0.001 5-30	p≤0.015 10-30	p<0.001 5-30	p≤0.002 10-30	p<0.001 10-30	p<0.001 15-30
cont.5 sec, interm. 1/sec	p<0.001 5-30	p<0.001 10-30	p<0.001 5-30	p≤0.04 5-30	p<0.001 10-30	p≤0.035 5-30
cont.10 sec, interm. 1/sec	p<0.001 5-30	p≤0.043 5-30	p<0.001 5-30	p≤0.009 5-30	p≤0.01 5-30	p≤0.03 10-30

7.3.3.1 *Cone diameter of 16 mm*

Figure 7.17 shows the comparison of the four spraying techniques for the centre of the 16 mm cone and figure 7.18 for the edge, both at 3 mm-depth.

The continuous spray offers the fastest fall in temperature, at the edge in particular. The other three spraying techniques are, in the main, equal to each other. The temperature decline is, however, generally slowest in the intermittent spray.

Table 7.4 provides the statistical analysis of this comparison. The continuous spray differs significantly from the other three spraying techniques except in the centre of this cone at 5 mm-depth. When

TABLE 7.3. P-values of the comparison of the temperature measurements in the centre to those at the edge for various cones, depths and spraying techniques. The temperatures recorded over a period of 30 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth	3 mm-depth	5 mm-depth
spray techn.	centre versus edge	centre versus edge	centre versus edge
16 mm cone			
continuous	$p<0.001$ 5-30*	$p\leq0.007$ 20-30	—
intermit.. 1/sec	$p<0.001$ 5-30	$p\leq0.015$ 10-30	$p\leq0.04$ 20-30
cont.5 sec, intermit. 1/sec	$p<0.001$ 5-30	$p<0.001$ 10-30	$p\leq0.003$ 20-30
cont.10 sec, interm. 1/sec	$p<0.001$ 5-30	$p<0.001$ 10-30	$p\leq0.02$ 10-30
11 mm cone			
continuous	$p<0.001$ 5-30	$p<0.001$ 10-30	$p<0.001$ 15-30
intermit. 1/sec.	$p<0.001$ 5-30	$p<0.001$ 10-30	$p\leq0.002$ 15-30
cont.5 sec, intermit. 1/sec	$p<0.001$ 5-30	$p<0.001$ 10-30	$p\leq0.001$ 15-30
cont.10 sec, intermit. 1/sec	$p<0.001$ 5-30	$p<0.001$ 10-30	$p\leq0.03$ 10-30

significancy occurs, the continuous spray provides the fastest fall in temperature.

Significancy between the intermittent spray and the two combinations of continuous and intermittent spraying occurs only at the edge of this cone. When significancy is present, the combined spraying techniques cause the fastest fall in temperature.

No significant difference is present between the two combined spraying techniques.

In figures 7.19 and 7.20, the intervals of minimum and maximum temperature values of the spraying techniques are presented for the centre and the edge of the 16 mm cone respectively, both at 3 mm-depth. In the centre of the cone, all four spraying techniques overlap

TABLE 7.4. P-values of the comparison of the four spraying techniques for the various cones and depths. The temperatures recorded over a period of 30 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*
*A** = continuous spray.*
*B** = intermittent spray 1/second.*
*C** = continuous spray over 5 seconds, combined with intermittent spray 1/second.*
*D** = continuous spray over 10 seconds, combined with intermittent spray 1/second.*

	1 mm-depth		3 mm-depth		5 mm-depth	
spray techn.	centre	edge	centre	edge	centre	edge
16 mm cone						
A** versus B**	p≤0.008 5-10*	p≤0.03 10-30	p≤0.02 15-20	p≤0.035 15-30	—	p≤0.01 20-30
A versus C**	p≤0.03 10-30	p≤0.006 15-30	p≤0.03 10-25	p≤0.03 20-30	—	p≤0.025 25-30
A versus D**	p≤0.009 5-15	p<0.001 20-30	p≤0.005 10-30	p≤0.04 20-30	—	p≤0.03 25-30
B versus C	—	p≤0.015 10-30	—	p≤0.05 10-30	—	p≤0.05 10-30
B versus D	—	p≤0.03 10-20	—	p≤0.03 20-30	—	p≤0.03 25-30
C versus D	—	—	—	—	—	—
11 mm cone						
A** versus B**	p≤0.045 10-25	p<0.001 10-30	p≤0.05 5-10	p≤0.04 5-25	p≤0.003 15-30	p≤0.01 25-30
A versus C**	p≤0.04 10-30	p≤0.009 10-30	—	p≤0.045 10-30	p≤0.003 15-30	p≤0.025 15-20
A versus D**	p≤0.0025 10-30	p≤0.035 10-30	p≤0.05 5-30	p≤0.003 15-30	p≤0.009 10-30	p≤0.04 15-30
B versus C	p≤0.0015 5	p≤0.04 10	—	p≤0.047 25	—	—
B versus D	p≤0.002 5	p≤0.001 10-15	—	—	—	—
C versus D	p≤0.0015 5	p≤0.0025 15	—	p≤0.004 20-25	—	—

each other. At the edge, there is hardly any overlap between continuous spraying and the other three spraying techniques, except for a very slight overlap with the combination of continuous spraying over 10 seconds and intermittent spraying of once a second. The other three spraying techniques show overlap to a great extent.

7.3.3.2 *Cone diameter of 11 mm*

The comparison of the four spraying techniques in the 11 mm cone diameter is presented in figures 7.21 and 7.22 for the centre and the edge of the cone respectively, both at 3 mm-depth.

In the centre of this cone, the continuous spray causes the fastest and the intermittent spray of once a second the slowest fall in temperature. The other two spraying techniques show a very similar temperature decline. At the edge of the cone, the fastest fall in temperature is caused by continuous spraying, followed by intermittent spraying and the two combinations of continuous and intermittent spraying respectively.

The statistical analysis of this comparison is presented in table 7.4. There exists a significant difference between the continuous spray and the other three spraying techniques, except in the centre of this cone, at 3 mm-depth between continuous spraying and the combination of continuous spraying over 5 seconds and intermittent spraying once a second. When a significant difference is present, continuous spraying produces the fastest fall in temperature.

Hardly any significancy occurs between the other three spraying techniques. If significancy is present at all, this generally only refers to one time point. The combined spraying techniques cause the fastest fall in temperature in comparison with the intermittent spray. In comparison between the two combined spraying techniques, the combination of continuous spraying over 10 seconds with intermittent spraying of once a second provides the fastest fall in temperature.

Figures 7.23 and 7.24 show the intervals of minimum and maximum temperature values for the centre and the edge of the cone respectively, both at 3 mm-depth.

In the centre, overlap exists between all four spraying techniques, except between continuous spraying and the combination of continuous spraying over 10 seconds and intermittent spraying of once a second. At the edge, there is no overlap between the continuous spray and the other spraying techniques. The other three spraying techniques overlap each other.

7.4 Discussion

It is clear that continuous spraying produces the fastest fall in temperature in both cone diameters (11 mm and 16 mm).
The intermittent spray causes the slowest fall in temperature.
The two combination spraying techniques are very similar to each other. The temperature results of these two spraying techniques resemble the results of intermittent spraying more closely than those of continuous spraying.
In general, the statistical analysis of the comparison confirms the aforementioned findings.
The intervals of minimum and maximum temperature values show a clear overlap between all four spraying techniques in the centre of the cones. At the edge of the cones, hardly any overlap exists between continuous spraying and the other three spraying techniques, whilst these show an extensive overlap.
The differences in the fall in temperature between continuous spraying and the other three spraying techniques are most striking at the edge of the cone diameters of 11 mm and 16 mm. It is also shown that the other three spraying techniques show a similar temperature decline.
In clinical practice, use of a continuous spray is undesirable because of the production of an overwhelming run-off and spattering of liquid nitrogen. Controlling the supply of liquid nitrogen becomes very difficult in continuous spraying.
An intermittent spray is therefore generally advised in cryosurgery.
Clear advice, however, concerning the application of an intermittent spray cannot be found in the literature (1, 2, 3, 4, 5). In this study, various other ways of intermittent spraying were investigated, but all of them caused considerable run-off of the cryogen. The best technique to control the intermittent spray was spraying once a second. With this method, run-off of liquid nitrogen still occurs, but to a very moderate degree.
Nevertheless, intermittent spraying causes a slower fall in temperature, both in the centre and at the edge of the cone diameters of 11 mm and 16 mm.
Over a short period of freezing of 30 seconds, the intermittent spray produces subzero temperatures in the centre of the 16 mm and 11 mm cones, both at 1 mm-depth and 3 mm-depth (figures 7.3 and 7.11).
At the edge of the 16 mm and 11 mm cones at 1 mm-depth, a subzero temperature is reached after 25 seconds of freezing (figures 7.4 and 7.12). At the edge of the 16 mm cone at 3 mm-depth, no subzero temperature is recorded within 30 seconds of freezing (figure 7.4), whilst

at the edge of the 11 mm cone at 3 mm-depth, a subzero temperature is reached after just 30 seconds of freezing (figure 7.12).
In conclusion, an intermittent spray is necessary to control the supply of liquid nitrogen. It is debatable whether this manner of spraying provides a sufficient fall in temperature at the edge of the cones.

7.5 References

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8

Clinical aspects of the standardized cryosurgical open-cone-spray method.

8.1 Introduction

In this chapter the clinical application of the standardized open-cone-spray method is discussed.
First, the cryosurgical procedure is explained; the applied cone diameters are then discussed followed by an evaluation of the freezing effect. The results of this treatment for the basal cell carcinoma are then presented.

8.2 The clinical application of the standardized cryosurgical open-cone-spray method.

8.2.1 Standardized cryosurgical open-cone-spray method.

The tumour area to be treated is first anaesthesized and the bulk of the tumour is removed by curettage. This curettage removes the tumour mass beyond the skin surface, often causing a skin lesion with a depth of a few millimetres. The cone diameter can then be determined. Usually a 2-3 mm margin of "healthy" skin surrounding the tumour is incorporated in the cone diameter. In the literature there is no consensus about the margins to be taken (1, 2, 3, 4, 5). It is advised that these margins should be increased as the tumour diameter increases. Generally, a margin of 3-5 mm is used in clinical practice; this also applies to larger basal cell carcinomas. Tumours with diameters exceeding 10-12 mm are divided into several areas before freezing starts.
The 0.8 mm spraytip is utilized and attached to the centre of the cone selected. The distance between spraytip and target area is 15 mm. The spray unit with the attached cone is directed gently at the skin surface. An intermittent spraying of once a second is performed over a period, usually of 20 seconds. The lesion thaws spontaneously. Absence of a

palpable iceball in the skin is the first sign of clinical thaw. Furthermore, the lesion begins to ooze and a moderate bleeding sometimes occurs. The second freeze cycle is then performed by means of an intermittent spraying of once a second over 20 seconds and, again, thawing occurs spontaneously. This completes the cryosurgical procedure.

8.2.2 Cone diameters applied in the standardized open-cone-spray method.

8.2.2.1 *Introduction*

The 11 mm, 16 mm and 21 mm cone diameters are generally used in clinical practice. These are discussed in chapter 6 where it is shown that the fall in temperature at the edge of the 21 mm cone is insufficient. The 0.8 mm spraytip can be used in the 11 mm and 16 mm cones. By extrapolation, it can be assumed that the 0.8 mm spraytip can also be used in the cone diameters of between 11 mm and 16 mm.
Small basal cell carcinomas are also treated by cryosurgery.
Very small tumours with a diameter of 2-3 mm can be treated very easily by other therapy modalities, such as excision surgery and electro surgery. For example, when these tumours are located at the eyelid margins, cryosurgery offers an excellent alternative but then a small cone diameter has to be available. A study of the 6 mm cone diameter is presented below.

8.2.2.2 *Cone diameter of 6 mm*

The smaller cone diameter of 6 mm fits in the sequence of the previously studied diameters of 21 mm, 16 mm and 11 mm. It is important to know whether the 0.8 mm spraytip can also be used successfully in the 6 mm cone. Unfortunately, the 6 mm cone causes run-off of liquid nitrogen because of its small diameter, even when the spray technique is intermittent. Run-off occurs in using both the 0.8 mm and 0.6 mm spraytip diameters. These spraytips will be compared with each other for the 6 mm cone.

8.2.2.3 *Materials*

These are discussed in chapter 2. A new holder was constructed and a cone with a diameter of 6 mm was created in its centre.

TABLE 8.1. P-values of the comparison of the spraytip diameters of 0.6 mm and 0.8 mm for the 6 mm cone at various depths. The temperatures recorded over a period of 60 seconds were statistically compared to each other at identical time points (= time interval in seconds, wherein significancy occurs).*

	1 mm-depth		3 mm-depth		5 mm-depth	
spraytip diam.	centre	edge	centre	edge	centre	edge
6 mm cone						
0.6 mm versus 0.8 mm	p≤0.04 5-35*	p≤0.035 5-45	p≤0.035 5-40	p≤0.05 10-50	p≤0.045 10-60	p≤0.02 20-60

8.2.2.4 *Methods*

The 0.8 mm and 0.6 mm spraytips are discussed. The central spray pattern is applied with the spraytip fixed in the centre of the cone. The liquid nitrogen is supplied by continuous spraying over a period of 60 seconds from a distance of 15 mm to the gelatin surface.
Registration of the temperatures was performed over a period of 60 seconds at intervals of 5 seconds.
The statistical methods are similar to those previously described (chapter 4).
The two spraytips will now be compared to each other.

8.2.2.5 *Results: comparison of the 0.8 mm and 0.6 mm spraytip diameters*

Figure 8.1* shows the comparison of the two spraytips for the centre and figure 8.2 for the edge of the 6 mm cone, both at 3 mm-depth.
The 0.6 mm spraytip provides the slower fall in temperature.
The statistical analysis of this comparison is presented in table 8.1. There is a statistically significant difference between the 0.8 mm and 0.6 mm spraytips for all depths, both in the centre and at the edge of the cone. The 0.8 mm spraytip provides the faster fall in temperature.
Figures 8.3 and 8.4 show the intervals of minimum and maximum temperature values for the 0.8 mm and 0.6 mm spraytips, in the centre and at the edge of the 6 mm cone respectively, both at 3 mm depth. An

* See addendum chapter 8 for figures 8.1-8.4

overlap occurs between the 0.8 mm and 0.6 mm spraytips from approximately 40 seconds of freezing, both in the centre and at the edge of the 6 mm cone.

8.2.2.6 *Discussion*

From this comparison, it can be concluded that the 0.8 mm spraytip shows a statistically significant faster fall in temperature, both in the centre and at the edge of the 6 mm cone.

The intervals of minimum and maximum temperature values of the 0.8 mm spraytip show a moderate overlap with those of the 0.6 mm spraytip after 40 seconds of freezing.

It is concluded that the 0.8 mm spraytip provides the faster fall in temperature in the 6 mm cone.

Taking the results reported in chapter 4 also into account, it appears by extrapolation that the 0.8 mm spraytip can be used for all cone diameters in the range from 6 mm to 16 mm.

The 0.8 mm spraytip produces just a moderate run-off of liquid nitrogen in the cone diameters of 11 mm and 16 mm. In the 6 mm cone, run-off increases and the supply of liquid nitrogen sometimes needs to be adapted by slightly increasing the interval between the flow phases of the cryogen.

The inexperienced cryosurgeon is advised to use the 0.6 mm spraytip when the 6 mm cone diameter is required for the treatment of small basal cell carcinomas located at the eyelid margin. The supply of liquid nitrogen, using the 0.6 mm spraytip, produces less run-off of liquid nitrogen, whilst the fall in temperature is found to be sufficient. Later, when cryosurgical skills are improved, the 0.8 mm spraytip can be used in the 6 mm cone very easily.

8.2.3 Clinical evaluation of the consequences of the freezing procedure

After each freeze cycle, the diameter of the iceball is measured. These measurements correlate well with the diameters of the tissue necrosis measured one week after the cryosurgical treatment. This is shown in table 8.2. In the 16 mm cone, no significant differences exist between these measurements. In the 11 mm cone, a difference exists only between the diameters measured after the first and the second freeze cycles ($p=0.008$). Table 8.2 shows that the diameters of the freeze front, measured immediately after freezing, are greater than the cone diameters. The freeze front extends a few millimetres outside the cone

edge. At the edge of the freeze front, one can assume that subzero temperatures do not occur. Cryonecrosis, however, occurs at the edge of the freeze front and is probably predominantly caused by temperatures around 0°C which induce microcirculatory arrest.
One month after treatment, a scar with hypopigmentation is formed. The diameters of the hypopigmentated areas are presented in table 8.3. The diameter of the hypopigmentation, one month after treatment, is smaller than the diameter of the skin necrosis one week after treatment: this is statistically significant ($p<0.001$). No significancy exists between the hypopigmentation diameters, measured one month and three months after treatment, and between three months and nine months after therapy. There is a significant difference between the hypopigmentation diameters measured one month and nine months after treatment ($p=0.025$), the latter having the greater diameter.

TABLE 8.2. Diameters (mean±sd) of the iceball measured after the first and after the second freeze cycle and the diameter of the skin necrosis one week after cryosurgery using the 16 mm and 11 mm cones and the 0.8 mm spraytip.

	diameter of the iceball after the first freeze cycle n=30	diameter of the iceball after the second freeze cycle n=30	diameter of the necrosis one week after therapy n=30
16 mm cone	mean=17.9 mm sd= 1.7 mm	mean=18.6 mm sd= 1.4 mm	mean=17.8 mm sd= 2.7 mm
11 mm cone	mean=14.3 mm sd= 1.1 mm	mean=15.2 mm sd= 1.4 mm	mean=14.8 mm sd= 1.4 mm

TABLE 8.3. Diameters (mean±sd) of the hypopigmentation measured one, three and nine months after cryosurgical treatment using the 16 mm cone and the 0.8 mm spraytip.

	diameter of the hypopigmentation		
	after 1 month n=21	after 3 months n=16	after 9 months n=10
16 mm cone	mean=10.5 mm sd= 3.1 mm	mean=11.6 mm sd= 5.2 mm	mean=14.9 mm sd= 5.6 mm

8.3 Clinical results of the treatment of the basal cell carcinomas by the standardized cryosurgical open-cone-spray method

8.3.1 Results

One hundred and eighty eight primary basal cell carcinomas were treated between April, 1986, and April, 1988, using the standard procedure discussed in chapter 8.2.
At the time of writing, the follow-up period ranges from one to three years.
The tumour sites are presented in table 8.4 and the tumour sizes in table 8.5. Most tumour sizes are between 5 mm and 14 mm.
Table 8.6 presents the histopathological classification of the treated basal cell carcinomas. The solid basal cell carcinoma is the most frequently encountered histopathological pattern. The term "invasive" basal cell carcinoma indicates small, finger-like, "spiky" histologic extensions of groups of cancerous basal cells extending from the edges or base of the larger masses of the tumour.

TABLE 8.4. Site of the basal cell carcinomas treated by cryosurgery.

tumour site	numbers
temple	37
ear: helix	2
lobe	4
mastoid	2
forehead/eyebrows	22
eyelids	7
periocular	7
inner canthus	9
outer canthus	3
cheek	21
nose: tip	6
ala	16
bridge	8
nasolabial fold	13
upper lip	4
neck	9
trunk	18

TABLE 8.5. Classification of the treated basal cell carcinomas by size (n=188).

tumour size	numbers
< 5 mm	11
5 mm- 9 mm	93
10 mm-14 mm	57
15 mm-19 mm	19
≥20 mm	8

TABLE 8.6. Histopathological classification of the treated basal cell carcinomas (n=188).

histopathological classification	numbers
solid	152
"invasive"	10
adenoid	14
basosquamous	4
morphea-like	3
superficial	14
keratotic	1

Tables 8.7.a and 8.7.b show the freeze cycles applied to the treatment of the basal cell carcinomas in one field (table 8.7.a) and in several fields (table 8.7.b). Two freeze cycles of 20 seconds each are usually utilized. The freeze cycles of 15 seconds applied, were to treat the superficial basal cell carcinomas.
Table 8.8 presents the cure rate of the treated basal cell carcinomas: this is 97.9%.
In table 8.9, a few characteristics of the four recurrent basal cell carcinomas are shown.
Finally, the recurrence chance by year of follow-up is calculated in table 8.10. It is suggested that after two years of follow-up, the chance of detection of a recurrent basal cell carcinoma is absent or minimal.

8.3.2 Discussion

A cure rate of 97.9% is highly satisfactory and is very similar to the results described in the literature (table 8.11). Different cryosurgical techniques, however, have been used in these reports. The main dif-

TABLE 8.7.a. Freeze cycles used in the cryosurgical treatment in one field of 142 basal cell carcinomas (≤11mm).

freeze cycles	numbers
2x15 seconds	7
2x20 seconds	131
2x25 seconds	1
2x30 seconds	3

TABLE 8.7.b. Freeze cycles used in the cryosurgical treatment in several fields of 46 basal cell carcinomas (>11mm).

freeze cycles	numbers
2x15 seconds	5
2x20 seconds	36
2x25 seconds	2
2x30 seconds	3

TABLE 8.8. Cure rate of the basal cell carcinomas treated by cryosurgery.

numbers	recurred	recurrence rate	cure rate
188	4	2.1%	97.9%

TABLE 8.9. Recurrent basal cell carcinomas (BCC) after cryosurgery (n=4).

histpath. classific.	tumour size	tumour site	freeze cycles	recurrence, months post cryosurgery
1. solid BCC	7 mm	periocular	2x20 sec.	18
2. solid BCC	12 mm	temple	2x20 sec.	19
3. solid BCC	8 mm	eyebrow	2x20 sec.	4
4. solid BCC	15 mm	cheek	2x20 sec.	13

ferences between these different techniques and that here advocated are the duration of the freeze cycle; the number of freeze cycles; the use of cones and the combination of curettage with cryosurgery.
In all reports, presented in table 8.11, there is a wide variation in the duration of the freeze cycle, ranging from 20-30 seconds to several minutes (6, 7, 8, 9, 10, 11, 12, 13, 14, 15). Only in one report (13) is the freeze time standardized, in this case at 30 seconds, whilst in all the other reports, wide variations in freezing times exist.
Spray freezing is the preferred technique, although in one report (8) probe-contact-freezing is also used.
In most reports, two freeze cycles are applied, but cones are not used consistently.
Several authors, among others Faber (6), Graham (11), Spiller and Spiller (16), Abadir (17) and Torre (18), have practised a combination of curettage and cryosurgery. The combination of curettage and cryosurgery, applying two freeze cycles, gives a cure rate of aproximately

TABLE 8.10. Recurrence chance by year of follow-up.

follow-up period	first year	second year	third year
number of primary tumours	188	138	69
recurrent tumours	1	3	0
recurrence chance	0.5%	2.2%	0%

TABLE 8.11. Cure rates in cryosurgery.

authors	cure rate	references
Faber, Rampen	93.8%	6
Lubritz	94.0%	7
Sebastian, Scholz	94.4%	8
Breitbart	96.5%	9
Torre	97.0%	10
Graham, Clark	97.1%	11
Holt	97.3%	12
McLean *et al*	97.7%	13
Fraunfelder *et al*	98.0%	14
Zacarian	98.0%	15

98%. This correlates well with the results in this study. In this study, however, a standardized cryosurgical procedure is used in the treatment of all 188 basal cell carcinomas, applying cones and mainly short freeze cycles of 20 seconds.
In this study, there is a maximum follow-up period of three years. Some authors (6, 11, 18) have reported recurrences after three years of follow-up. They advise a follow-up period of five to ten years because of the slow growth of the basal cell carcinoma. Other reports (12, 15, 16), however, suggest that a follow-up period of two to three years could be sufficient. Nevertheless, the traditional five-year follow-up seems desirable.

8.4 References

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General Discussion

9.1 Introduction

The aim of the present study is to provide a guideline for a standardized procedure for the cryosurgical treatment of skin malignancies, the basal cell carcinoma in particular. This guideline recommends an intermittent central spray pattern (of once a second), using a 0.8 mm spraytip fixed in the centre of the cone at a distance of 15 mm to the target area. This technique can be used in cone diameters in the range of 6 mm to 16 mm. The combination of spraytip and cone (figure 9.1) is attached to the spray unit.
In this chapter, the standardized cryosurgical open-cone-spray method will be discussed with regard to clinical practice.

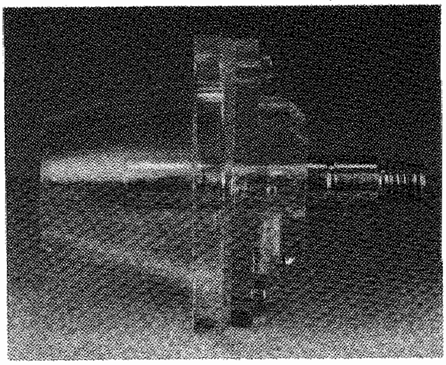

FIGURE 9.1. Cone with the spraytip fixed in the centre (side view).

9.2 The standardized open-cone-spray method and its clinical application

9.2.1 The variables studied which influence the supply of liquid nitrogen

In the open-cone-spray technique, as introduced by Torre (1), there are several different variables which influence the supply of liquid nitrogen. One of the main objectives of the present study is to standardize these variables to provide an optimal supply of liquid nitrogen in order to create a rapid freezing phase (chapter 1.3.1).
In chapters 3, 4 and 5, it is concluded that the central spray pattern, using the 0.8 mm spraytip at a distance of 15 mm to the target area, provides an optimal supply of liquid nitrogen. This technique produces a rapid freezing phase. A moderate run-off of liquid nitrogen is achieved when an intermittent spray of once a second is applied (chapter 7).
In chapter 6 and chapter 8.2.2, the clinical application of the cone diameters is discussed. From chapter 6 it is concluded that at the edge of the 21 mm cone, the fall in temperature is insufficient. For the present study this means that the 16 mm cone is the maximum cone diameter recommended for use in clinical practice. Tumours with diameters exceeding 10-12 mm should be divided into several areas before freezing. In addition, the 6 mm cone can be utilized for small tumours: for example, those located at the eyelid margin (chapter 8.2.2).
The present study shows that all cone diameters between 6 mm and 16 mm can be utilized in cryosurgical practice, using the 0.8 mm spraytip fixed in the centre of the cone at a distance of 15 mm to the target area.

9.2.2 The duration of the freeze cycle

In chapter 8 it is shown that two freeze cycles, each of 20 seconds' duration, applying an intermittent spray technique of once a second, give a high cure rate in the cryosurgical treatment of the basal cell carcinoma.
From the experiments reported in chapter 7, however, one can conclude that it is debatable whether an intermittent spray of once a second provides a sufficient fall in temperature at the edge of the cones.
There are several factors that influence the favourable clinical results of the standardized cryosurgical procedure, applying freeze cycles of mainly 20 seconds.

A margin of "healthy" skin tissue surrounding the tumour is incorporated within the cone. This means that the fall in temperature at the edge of the tumour is faster than at the edge of the cone because of the semicircular form of the iceball. In the present study, it is stressed that in the larger cone diameter of 21 mm, the semicircular iceball is flattened. An insufficient fall in temperature will not, therefore, occur only at the edge of the cone, but also at the edge of the tumour.

Temperatures to induce cryogenic necrosis are only necessary at a moderate depth when additional curettage of the tumours is performed. Cryosurgical literature advises temperatures between -20°C and -25°C (1, 2, 3, 4) and even between -40°C and -60°C (1, 5) to induce cryonecrosis. These temperatures are rarely achieved at the edge of the cones or at a depth of 3 mm to 5 mm when a short period of freezing (20 seconds) is applied.

It is possible, therefore, that cryonecrosis is predominantly caused by microcirculatory arrest (chapter 1.3.2) arising in the temperature range between +10°C and -10°C (3, 6). Unfortunately, the exact temperature that causes blood coagulation in the aforementioned range is not known.

The findings in chapter 8.2.3 also suggest that temperatures as low as -20°C are not necessary for the induction of cryonecrosis. It has been shown that the diameter of the cryonecrosis, measured one week after cryosurgery, correlates well with the diameter of the freeze front measured immediately after freezing. The edge of the freeze front is situated a few millimetres outside the cone edge where hardly any subzero temperatures will be registered. Nevertheless, cryonecrosis occurs.

It is concluded that the freezing period of 20 seconds is determined by a combination of a guideline for a standardized cryosurgical procedure and clinical experience.

9.2.3 Tumours for cryosurgical treatment

Histopathological classification and anatomical localisation of tumours are two important factors that determine the application of cryosurgery. In the present study, only the results of the cryosurgical treatment of the basal cell carcinoma are discussed.

Squamous cell carcinomas also respond very well to cryosurgery (7, 8, 9).

Bowen's tumours, keratoacanthomas and lentigo maligna are also treated successfully by cryosurgery (5, 7, 8, 9) and there have been a

few favourable reports on the cryosurgical treatment of lentigo maligna melanoma and superficial spreading melanoma (9).
Finally, a large number of other skin conditions, both premalignant and benign, can be the subject of cryosurgical treatment (3, 5, 9).

9.2.4 Cure rate for primary skin cancers using other treatment modalities than cryosurgery

In chapter 8, the cure rate of cryosurgical treatment is presented, ranging from 93.8% to 98%. In the literature (10), cure rates for primary skin cancers by other treatment modalities are reported as follows: in excision surgery, the cure rate ranges from 91% to 98.8%; in radiation therapy, from 92.1% to 98%; in electrosurgery, from 92.6% to 95.5% and in Mohs' chemosurgery, from 92.3% to 99.2%.
As is shown, most therapy modalities give more or less equal results. Mohs' surgery, however, usually produces the highest cure rates (11) and is generally used for difficult skin cancers.

9.3 Final remarks and recommendations

This standardized cryosurgical open-cone-spray method can serve as a clinical guide for the inexperienced cryosurgeon.
In the clinical application of this technique, however, there are still some variables that need to be clarified. Two freeze cycles, each of 20 seconds' duration, give very good results in the technique presented in this study.
The extension of the cryonecrosis could probably be established by experimental research on an animal model, then verified by clinical investigations in man. This should be performed for various freezing times.
The influence of low temperatures on the coagulation process of blood should be evaluated more exactly with regard to the effect of cryogenic temperatures on the microcirculation.
Finally, ultrasound equipment with a very high resolution is required to investigate the vertical extension of tumours in the skin (12, 13).
Determination of the depth of skin tumours, together with the induction of cryonecrosis predictable by freezing time, would offer the opportunity to provide very accurate depth-dose in cryosurgery.
A standardized cryosurgical technique is an important condition for further investigations into the induction of cryonecrosis.

9.4 References

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Summary

In this thesis a standardized procedure is proposed for the supply of liquid nitrogen in the cryosurgical open-cone-spray technique.
Chapter 1 provides a review of the cryosurgical literature.
A historical review of cryosurgical techniques is first presented.
Two important mechanisms, cell injury and microcirculatory arrest, are discussed in the pathogenesis of the cryogenic lesion.
The probe method and the spray method are the most frequently encountered cryosurgical techniques for the supply of liquid nitrogen to the target. It is concluded that the open-cone-spray method is the preferred technique in the cryosurgical treatment of skin malignancies.
Finally, in this chapter, the aim of this thesis is outlined: to provide a guideline for a standardized supply of liquid nitrogen in the open-cone-spray technique. Variables that influence this supply were studied on the basis of temperature measurements in a model in which the freezing experiments were performed. These variables concern the spray pattern, the diameter of the spraytip, the distance between spraytip and target, the cone diameters and the technique of spraying.
In chapter 2, the experimental model is described. Several models were investigated for the performance of the temperature measurements. The gelatin model was found to provide the most reproducible and workable medium for the registration of the freezing process by the thermocouples.
The equipment used in this study is described.
Chapters 3, 4, 5, 6 and 7 present the temperature measurements in order to ascertain the relationship of the variables to each other. The experiments were performed with three cone diameters (11 mm, 16 mm and 21 mm), with the exception of the experiments described in chapter 7 in which the 11 mm and 16 mm cones were used only.
In chapter 3, three spray patterns are compared with each other: central, circular and "paintbrush" spraying. It is concluded that central spraying provides the opportunity to reach the fastest fall in temperature within a short period of freezing.
A modified polymethylmethacrylate cone is introduced with the spraytip fixed in the centre.

Chapter 4 presents the study of four spraytips with the diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm. It is concluded that the 0.8 mm spraytip is the best choice for the supply of liquid nitrogen.
Chapter 5 describes the comparison of four spray distances (5 mm, 10 mm, 15 mm and 20 mm). Spraying from a distance of 15 mm generally provides the fastest fall in temperature.
In chapter 6, the cone diameters (11 mm, 16 mm and 21 mm) are compared with each other. The 21 mm cone is found to provide an insufficient fall in temperature, at the edge of this cone in particular. This cone diameter should probably not be used. It is of practical importance, therefore, that the area to be frozen should be divided into several areas when a cone with a diameter exceeding 16 mm is required.
In chapter 7, four spraying techniques are discussed: continuous spraying, intermittent spraying of once a second and two different combinations of continuous spraying and intermittent spraying of once a second. Continuous spraying is found to provide the fastest fall in temperature. This spray technique, however, appears to produce an overwhelming run-off and spattering of liquid nitrogen. It is concluded that an intermittent spray of once a second is necessary to control the supply of liquid nitrogen.
Chapter 8 offers a discussion of the clinical aspects of the standardized cryosurgical open-cone-spray method. A standard cryosurgical technique is described with regard to the conclusions of the chapters 3, 4, 5, 6 and 7 for application in clinical practice.
For small tumours, a 6 mm cone is introduced and discussed for the application of the 0.6 mm and the 0.8 mm spraytips. The 0.8 mm spraytip provides the faster fall in temperature in the 6 mm cone.
A clinical evaluation of the freezing effect on skin tissue shows a correlation between the diameter of the iceball measured immediately after the freeze cycle, and the diameter of the tissue necrosis measured one week after cryosurgery.
The clinical results of the treatment of 188 basal cell carcinomas by the standardized cryosurgical open-cone-spray method are presented, resulting in a cure rate of 97.9%.
In chapter 9, the findings of the previous chapters are integrated into a clinical guideline for the standardized procedure for the cryosurgical open-cone-spray method. The conclusions of the comparisons of the various variables influencing the supply of liquid nitrogen are summarized.
The duration of the freeze cycle is discussed. Several factors are indicated that influence the favourable clinical results of the standardized cryosurgical procedure, applying freeze cycles of mainly 20 seconds.

The first factor concerns the inclusion within the cone of a safety margin of "healthy" skin tissue surrounding the tumour. A second factor is curettage of the bulk of the tumour in addition to cryosurgery. Thirdly, temperatures around zero are suggested as a cause of microcirculatory arrest which is possibly the most important factor for the induction of cryonecrosis.

The tumours for cryosurgical treatment are indicated.

The results of cryosurgery are compared to the results of other therapy modalities for primary skin cancers.

Finally, techniques providing accurate depth-dose in cryosurgery are recommended.

A standardized cryosurgical technique, as presented in this thesis, is an important condition for further investigations in the field of inducing cryonecrosis in live skin tissue.

Samenvatting

In dit proefschrift wordt een standaard procedure voorgesteld ten behoeve van het toedienen van vloeibare stikstof in de cryochirurgische open-conus-spray methode.
Hoofdstuk 1 geeft een overzicht van de cryochirurgische literatuur.
Allereerst wordt een historisch overzicht gegeven.
Vervolgens wordt de pathogenese van het vriesletsel besproken, waarin directe celbeschadiging en stasis van de microcirculatie de twee belangrijkste mechanismen zijn.
De probe methode en de spray methode worden in de literatuur de belangrijkste methoden genoemd voor het toedienen van vloeibare stikstof. De conclusie van deze literatuurstudie is, dat de open-conus-spray methode de voorkeur geniet bij de cryochirurgische behandeling van huidmaligniteiten.
Tot besluit van hoofdstuk 1 wordt het doel van het onderzoek, beschreven in dit proefschrift, uiteengezet: Het geven van een richtlijn ten behoeve van een gestandaardiseerde toevoer van vloeibare stikstof in de open-conus-spray methode. De variabelen die deze toevoer beinvloeden, werden bestudeerd aan de hand van temperatuurmetingen in een model, waarop de vriesexperimenten werden uitgevoerd. Deze variabelen betreffen het spraypatroon, de diameter van de spraypen, de sprayafstand, de diameters van de conus en de spraytechniek.
Hoofdstuk 2 beschrijft de voor het onderzoek gebruikte proefopstelling. Verschillende modellen werden onderzocht voor het uitvoeren van de temperatuurmetingen. Het gelatinemodel bleek het meest reproduceerbare en bruikbare medium te leveren voor het registreren van de bevriezings processen gebruik makende van thermokoppels.
Het voor deze studie gebruikte instrumentarium wordt beschreven.
De hoofdstukken 3, 4, 5, 6 en 7 bevatten de temperatuurmetingen van de experimenten. Deze temperatuurmetingen geven per hoofdstuk aan, hoe de verschillende variabelen zich ten opzichte van elkaar verhouden.
De experimenten werden uitgevoerd in drie conus (11 mm, 16 mm en 21 mm) met uitzondering van de experimenten beschreven in hoofdstuk 7, die alleen in de 11 mm en 16 mm conus werden uitgevoerd.

In hoofdstuk 3 worden drie spraypatronen met elkaar vergeleken, te weten centraal, circulair en zig-zag sprayen. Het centrale spraypatroon voorziet in een optimaal temperatuurverval, uitgaande van een korte vriestijd.
Een gemodificeerde conus, vervaardigd uit polymethylmethacrylaat, wordt geintroduceerd. De spraypen wordt gefixeerd in het centrum van de conus.
Hoofdstuk 4 presenteert de studie van vier spraypennen. Deze hebben respectievelijk de volgende diameters: 0,6 mm, 0,8 mm, 1,0 mm en 1,7 mm. De conclusie is, dat de spraypen met een diameter van 0,8 mm de beste keuze is voor de toediening van de vloeibare stikstof.
Hoofdstuk 5 beschrijft de vergelijking van vier spray-afstanden (5 mm, 10 mm, 15 mm en 20 mm). De sprayafstand van 15 mm laat over het algemeen het snelste temperatuurverval zien.
In hoofdstuk 6 worden de conus diameters met elkaar vergeleken. Het blijkt, dat aan de rand van de conus met een diameter van 21 mm het temperatuurverval onvoldoende is. Deze conus dient bij voorkeur niet te worden gebruikt. Daarom is het van practisch belang, dat het te bevriezen huidareaal verdeeld wordt in verschillende kleinere regio's, indien een conus met een grotere diameter dan 16 mm gewenst is.
In hoofdstuk 7 worden vier spraytechnieken besproken: continue sprayen, intermitterend sprayen (eenmaal per seconde) en twee verschillende combinaties van continue en intermitterend sprayen (eenmaal per seconde). Continue sprayen geeft het snelste temperatuurverval, doch veroorzaakt overvloedige "run-off" en aanzienlijk terugspatten van vloeibare stikstof. Een intermitterende spraytechniek van eenmaal sprayen per seconde blijkt noodzakelijk te zijn voor een gecontroleerde toevoer van vloeibare stikstof.
Hoofdstuk 8 bespreekt de klinische aspecten van de gestandaardiseerde cryochirurgische open-conus-spray methode. Voor klinische toepassing wordt een standaard cryochirurgische techniek beschreven, rekening houdende met de conclusies van de hoofdstukken 3, 4, 5, 6 en 7.
Voor de behandeling van kleine tumoren wordt een conus met een diameter van 6 mm geintroduceerd. Deze conus wordt besproken voor de spraypennen van 0,6 mm en 0,8 mm. Geconcludeerd wordt, dat de spraypen van 0,8 mm het snelste temperatuurverval veroorzaakt.
Vervolgens wordt in dit hoofdstuk een klinische evaluatie gegeven van het vriesletsel. De diameter van de ijsbal direct na vriezen blijkt te correleren met de diameter van de weefselnecrose, gemeten een week na cryochirurgie.
Bovendien worden in dit hoofdstuk de klinische resultaten van de gestandaardiseerde cryochirurgische open-conus-spray methode gepre-

senteerd in de behandeling van het basaalcelcarcinoom. Het genezings-percentage bedraagt 97,9%.
Hoofdstuk 9 integreert de resultaten van de voorafgaande hoofdstukken in een klinische richtlijn voor een gestandaardiseerde procedure van de cryochirurgische open-conus-spray methode.
De conclusies van de vergelijkingen van de verschillende variabelen, die de toevoer van vloeibare stikstof beinvloeden, worden samengevat.
De duur van de vriesfase wordt besproken. Factoren worden genoemd, die een gunstige invloed hebben op het klinische resultaat van de gestandaardiseerde cryochirurgische methode, waarin gebruik wordt gemaakt van vriescycli met een duur van voornamelijk 20 seconden. De eerste factor betreft het insluiten binnen de conus van een zone van "gezond" weefsel rondom de tumor. Een tweede factor is curettage van de tumormassa, voorafgaande aan cryochirurgie. Ten derde veroorzaken temperaturen rondom het vriespunt stasis van de microcirculatie, hetgeen mogelijk de belangrijkste factor is voor het induceren van cryonecrose.
De tumoren, die voor cryochirurgische behandeling in aanmerking komen, worden aangegeven.
De resultaten van de cryochirurgische behandeling van primaire huidmaligniteiten worden vergeleken met andere vormen van therapie.
Tenslotte wordt gepleit voor de ontwikkeling van technieken, die voorzien in een nauwkeurige diepte-dosis in de cryochirurgische behandeling.
Een gestandaardiseerde cryochirurgische techniek, zoals gepresenteerd in dit proefschrift, is een belangrijke voorwaarde voor verder onderzoek op het gebied van het induceren van cryogene necrose in levend huidweefsel.

Dankwoord

Dit proefschrift werd bewerkt binnen de vakgroep Dermatologie van het Academisch Ziekenhuis Maastricht.

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Curriculum Vitae

1955 Geboren te Maastricht.

1974 Eindexamen Gymnasium-B, Henric van Veldeke college, Maastricht.

1974-1982 Studie geneeskunde, Rijksuniversiteit Utrecht.

1982-1984 Arts-assistent Dermatologie (niet in opleiding), Ziekenhuis St. Annadal, Maastricht.

1984-1987 Opleiding Dermatovenereologie, Ziekenhuis St. Annadal, Maastricht, vanaf 1-1-1986 Academisch Ziekenhuis Maastricht, opleider: Prof.Dr. W.J.B.M. van de Staak.

1987 Inschrijving specialistenregister.

1987 Staflid vakgroep Dermatologie, Academisch Ziekenhuis Maastricht, universitair docent Rijksuniversiteit Limburg.

1989 Dermatovenereoloog, Maaslandziekenhuis, Sittard

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Figures of chapter 3

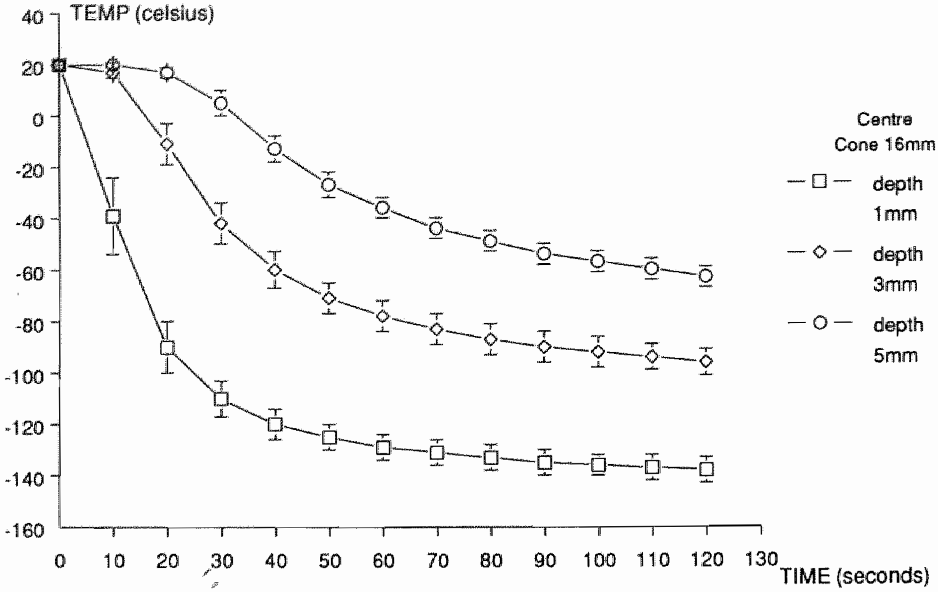


Figure 3.1. Temperature course (mean±sd, n=30) of the central spray pattern in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

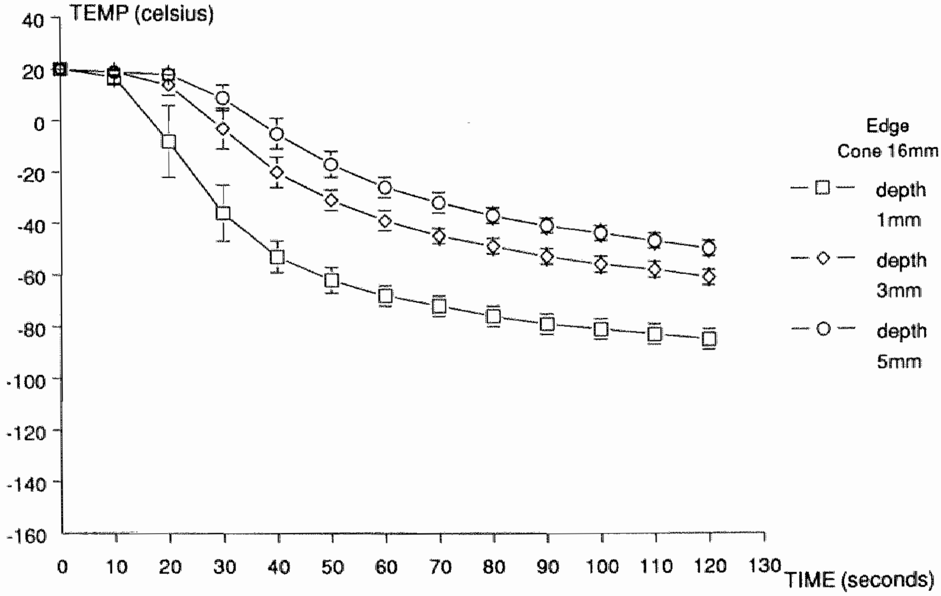


Figure 3.2. Temperature course (mean±sd, n=30) of the central spray pattern at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

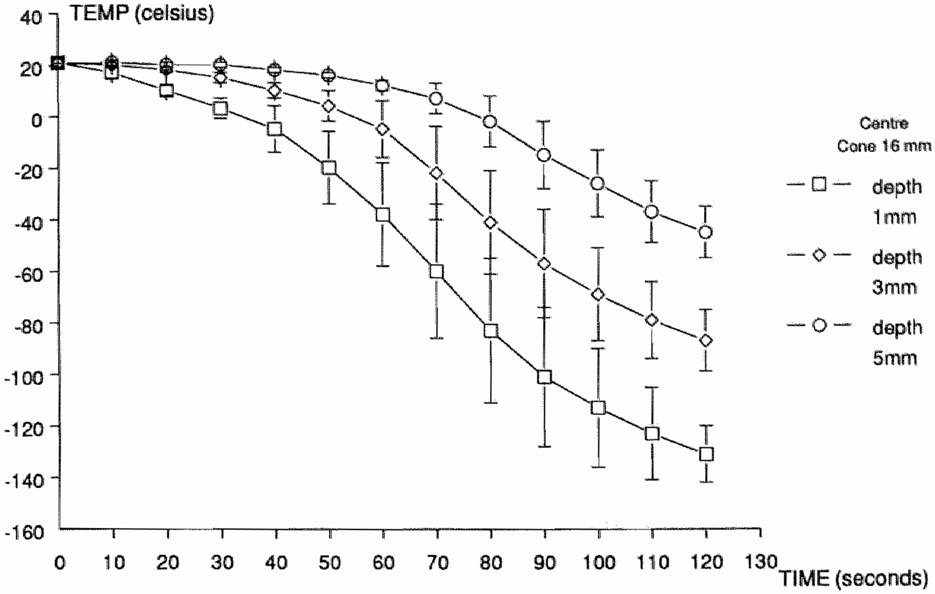


Figure 3.3. Temperature course (mean±sd, n=30) of the circular spray pattern in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

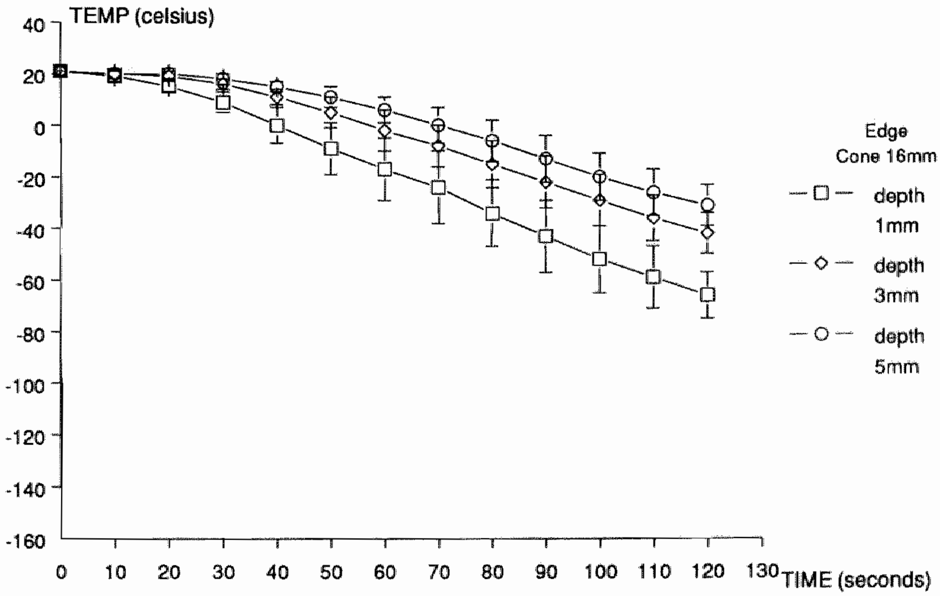


Figure 3.4. Temperature course (mean±sd, n=30) of the circular spray pattern at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

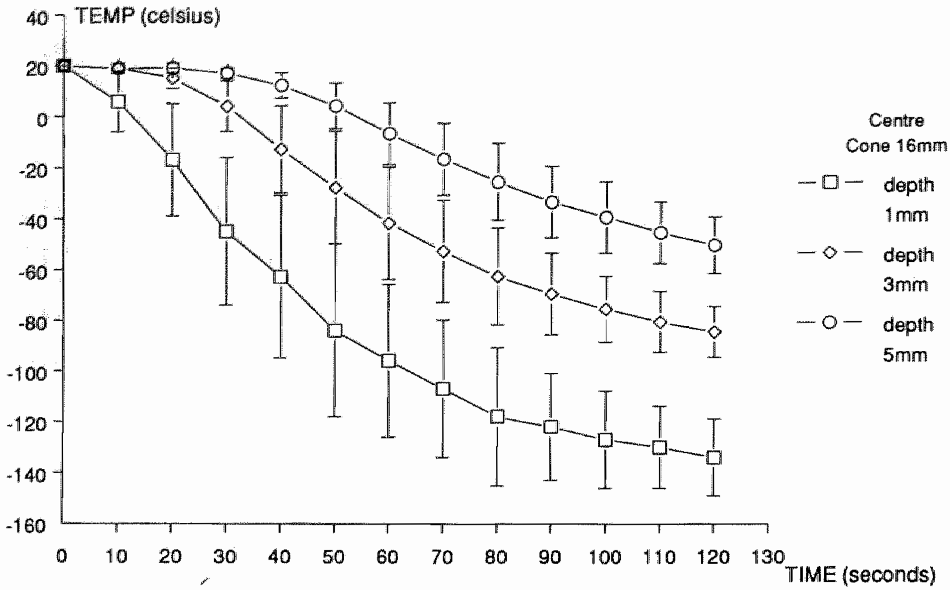


Figure 3.5. Temperature course (mean±sd, n=30) of the "paintbrush" spray pattern in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

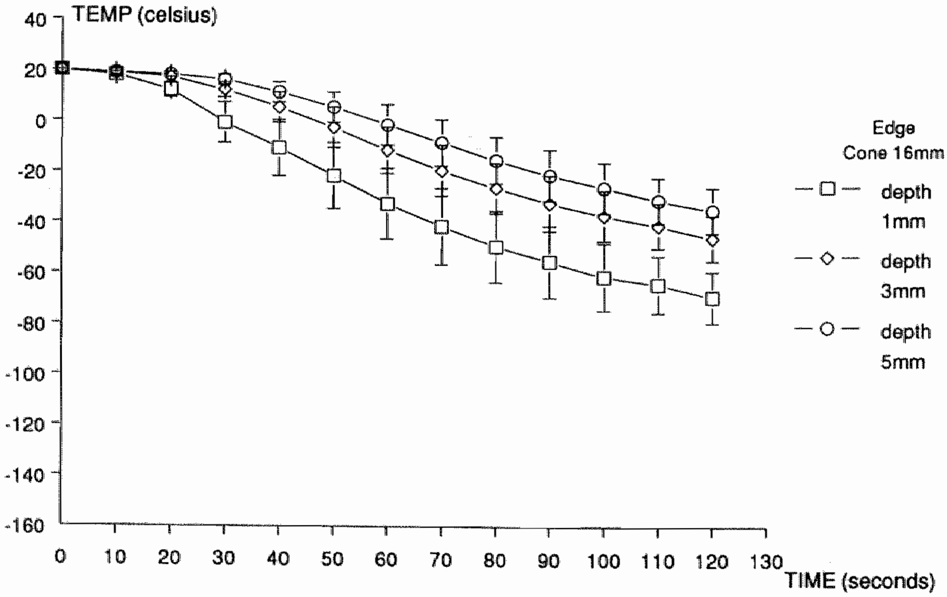


Figure 3.6. Temperature course (mean±sd, n=30) of the "paintbrush" spray pattern at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

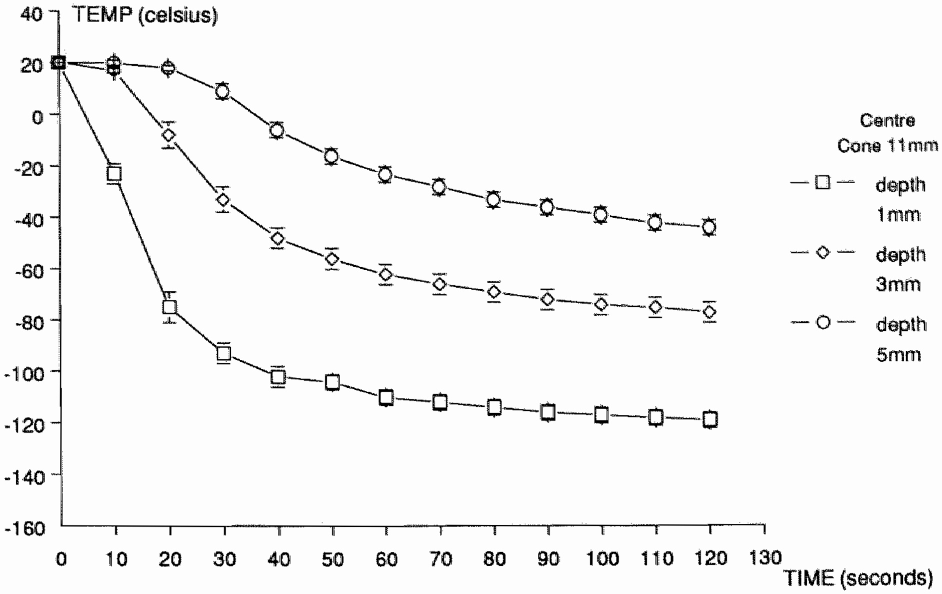


Figure 3.7. Temperature course (mean±sd, n=6) of the central spray pattern in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

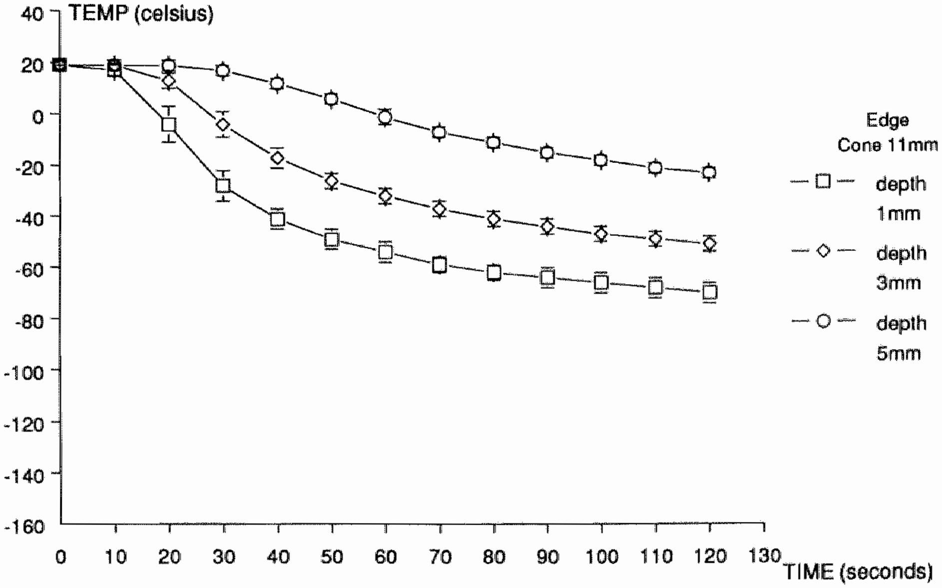


Figure 3.8. Temperature course (mean±sd, n=6) of the central spray pattern at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

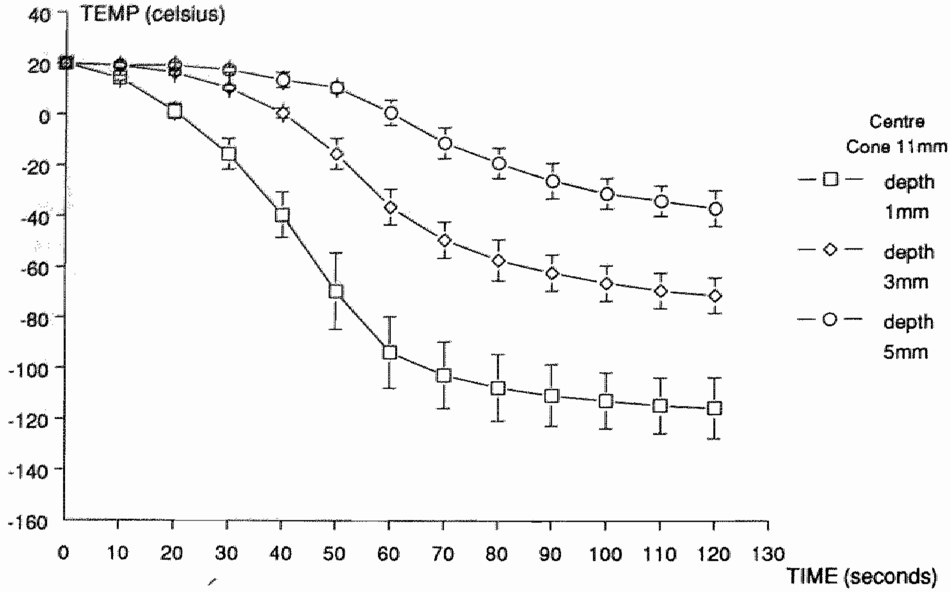


Figure 3.9. Temperature course (mean±sd, n=6) of the circular spray pattern in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

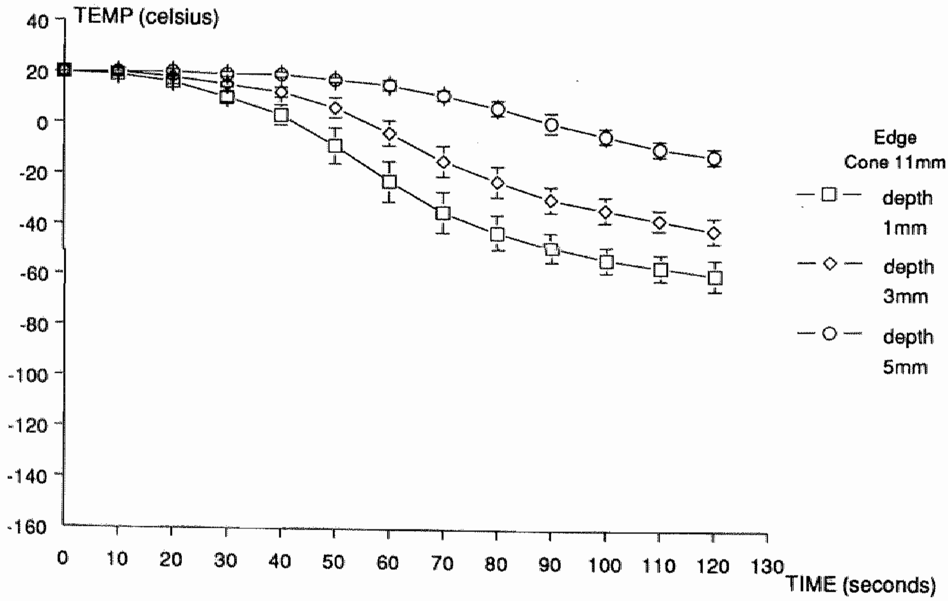


Figure 3.10. Temperature course (mean±sd, n=6) of the circular spray pattern at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

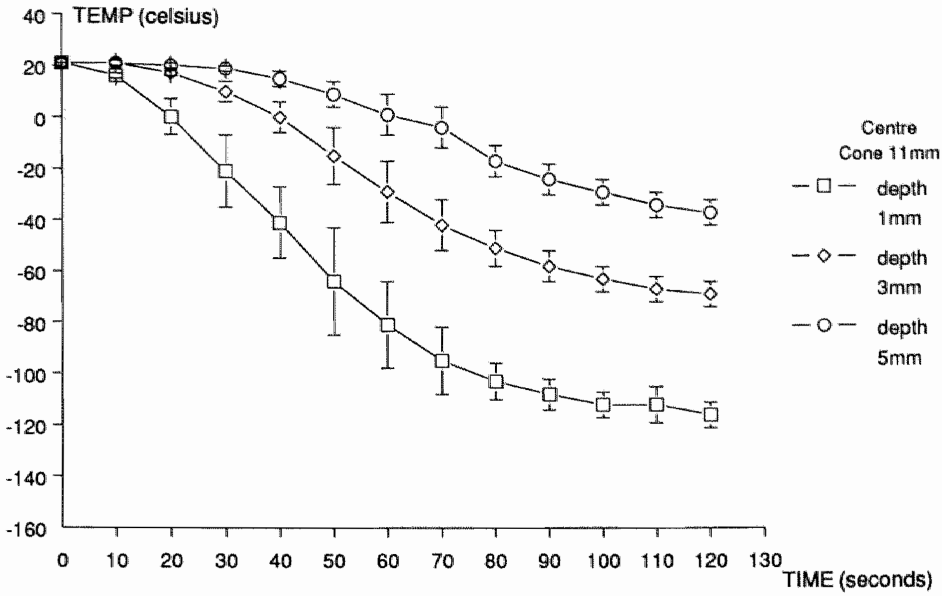


Figure 3.11. Temperature course (mean±sd, n=6) of the "paintbrush" spray pattern in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

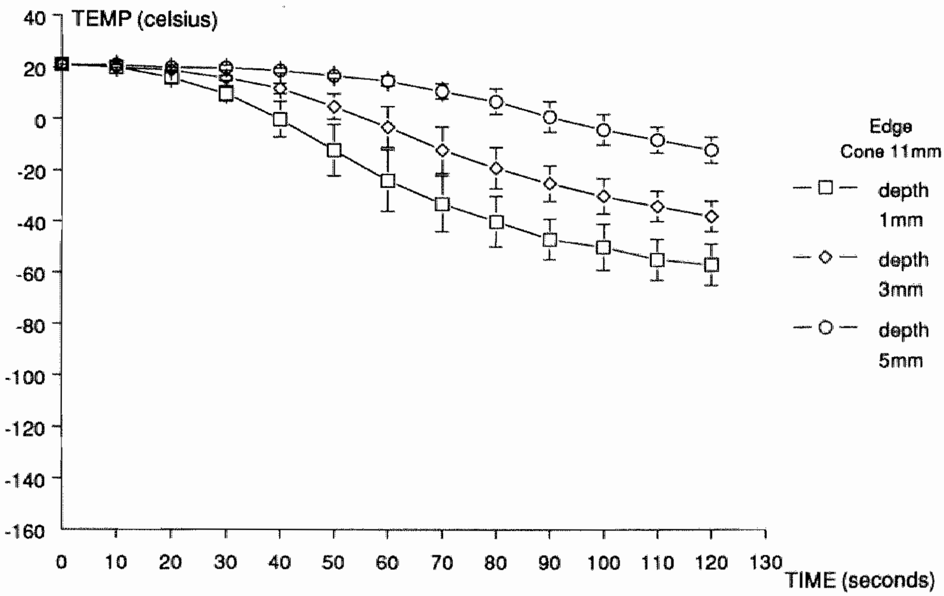


Figure 3.12. Temperature course (mean±sd, n=6) of the "paintbrush" spray pattern at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

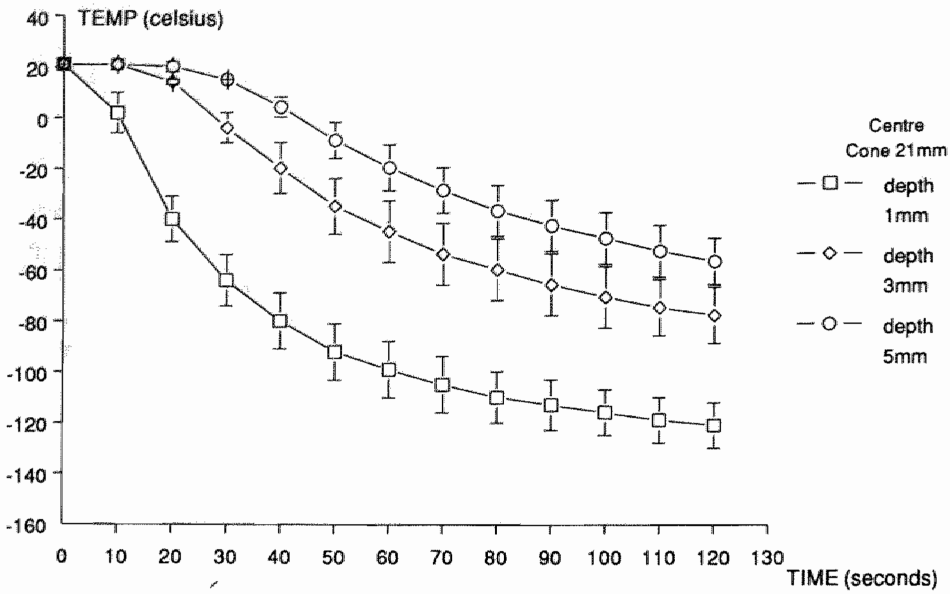


Figure 3.13. Temperature course (mean±sd, n=6) of the central spray pattern in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

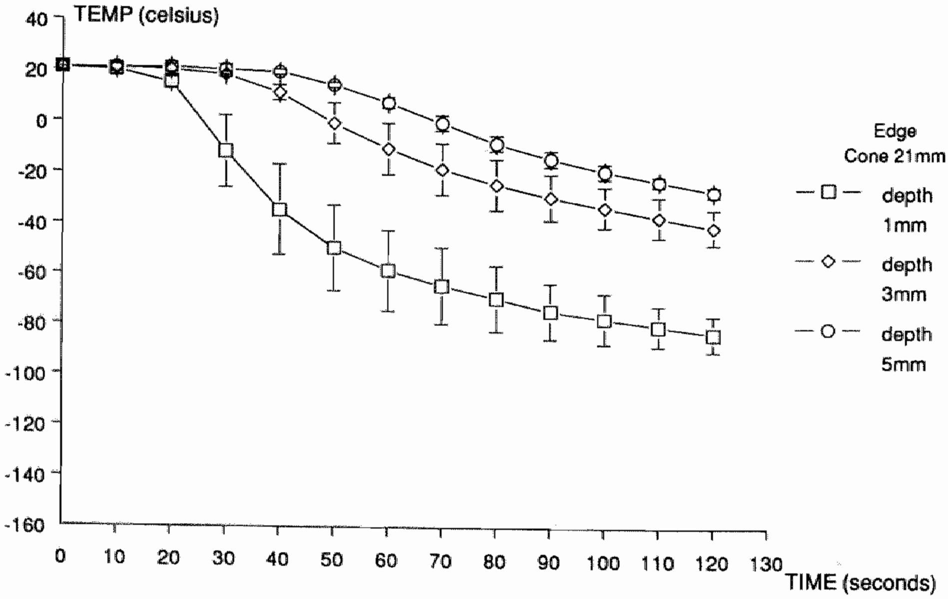


figure 3.14. Temperature course (mean±sd, n=6) of the central spray pattern at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

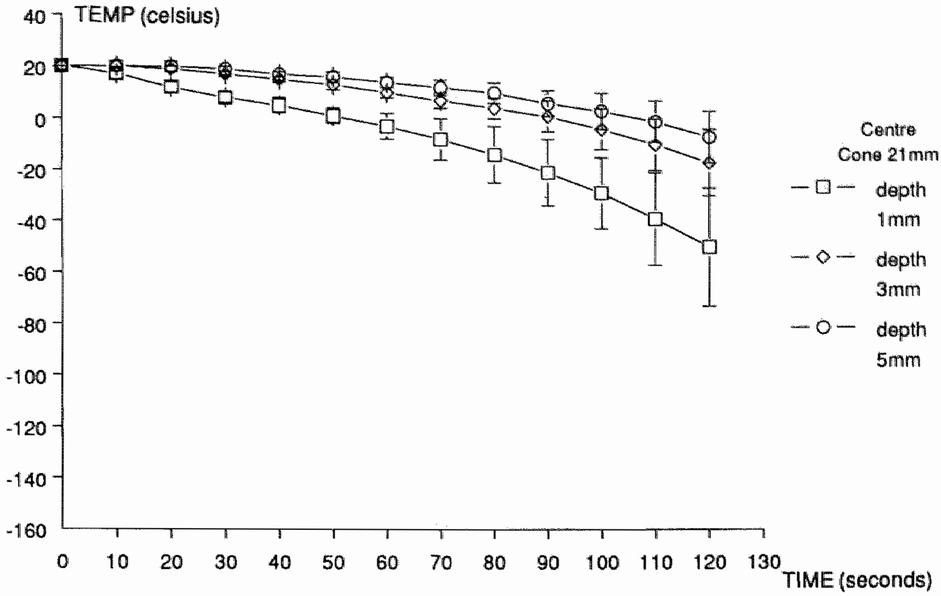


Figure 3.15. Temperature course (mean±sd, n=6) of the circular spray pattern in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

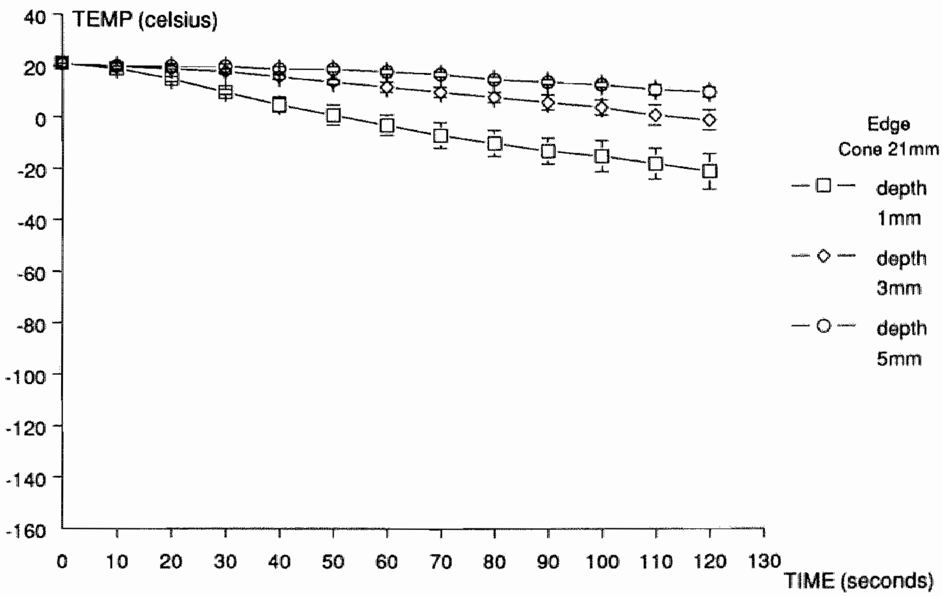


Figure 3.16. Temperature course (mean±sd, n=6) of the circular spray pattern at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

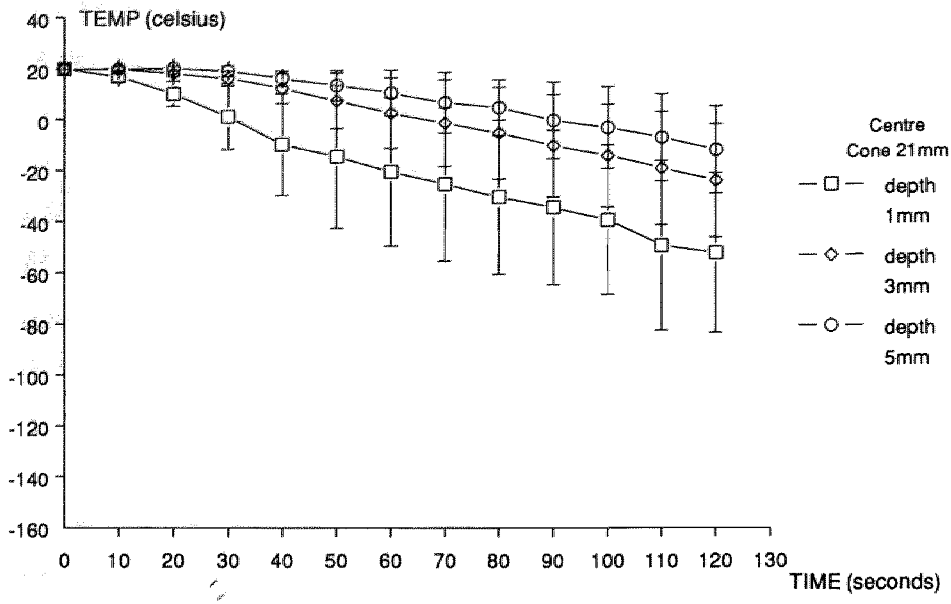


Figure 3.17. Temperature course (mean±sd, n=6) of the "paintbrush" spray pattern in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

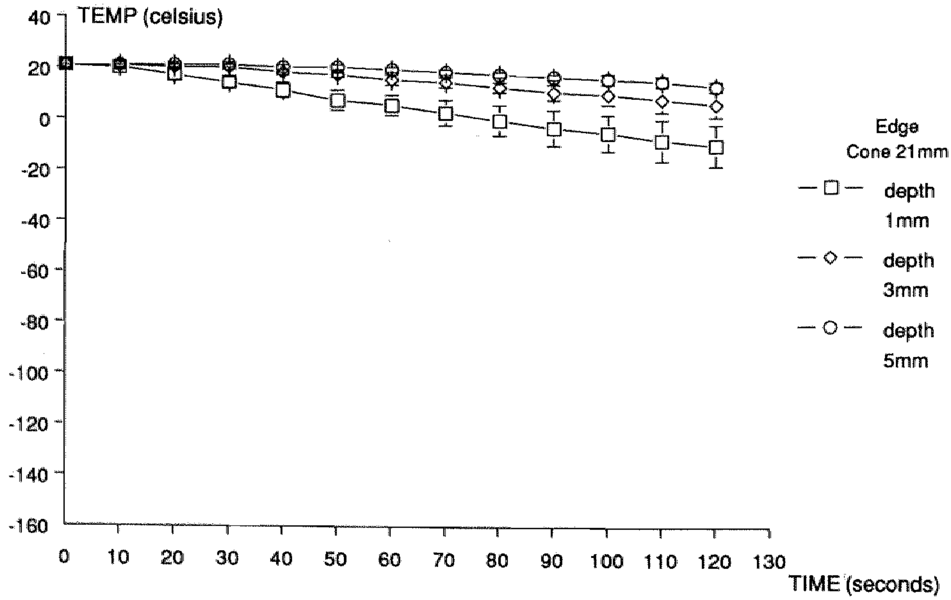


Figure 3.18. Temperature course (mean±sd, n=6) of the "paintbrush" spray pattern at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

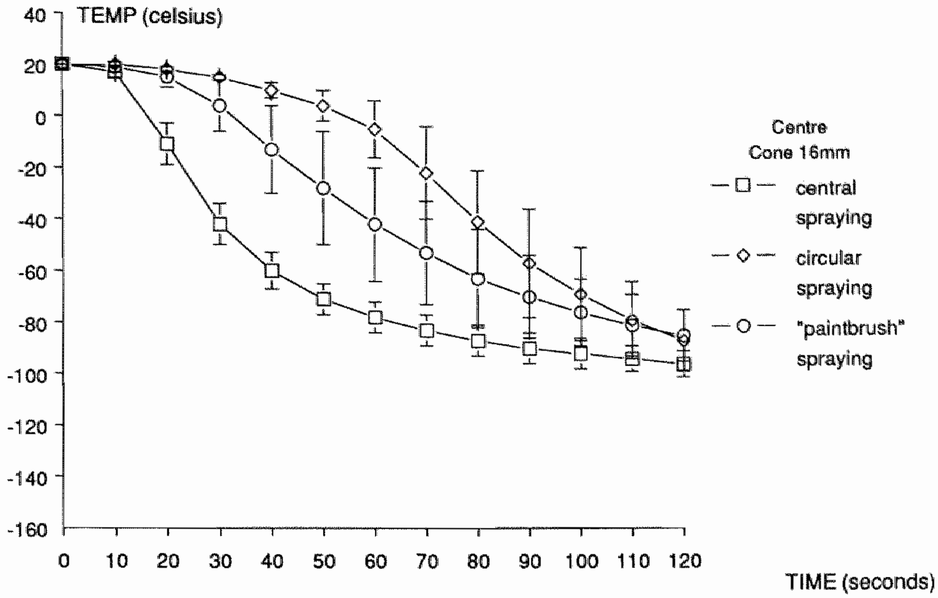


Figure 3.19. Comparison (mean±sd, n=30) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

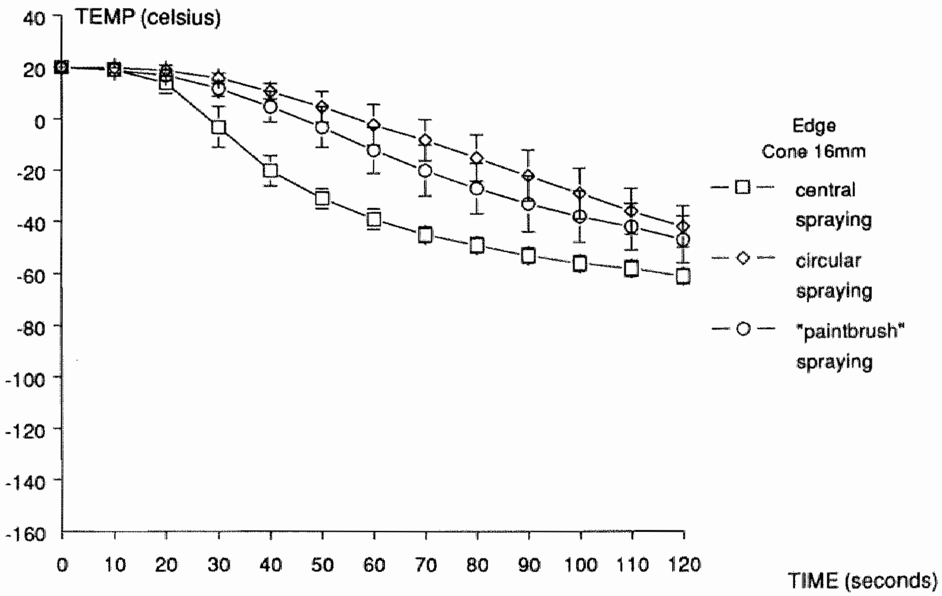


Figure 3.20. Comparison (mean±sd, n=30) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

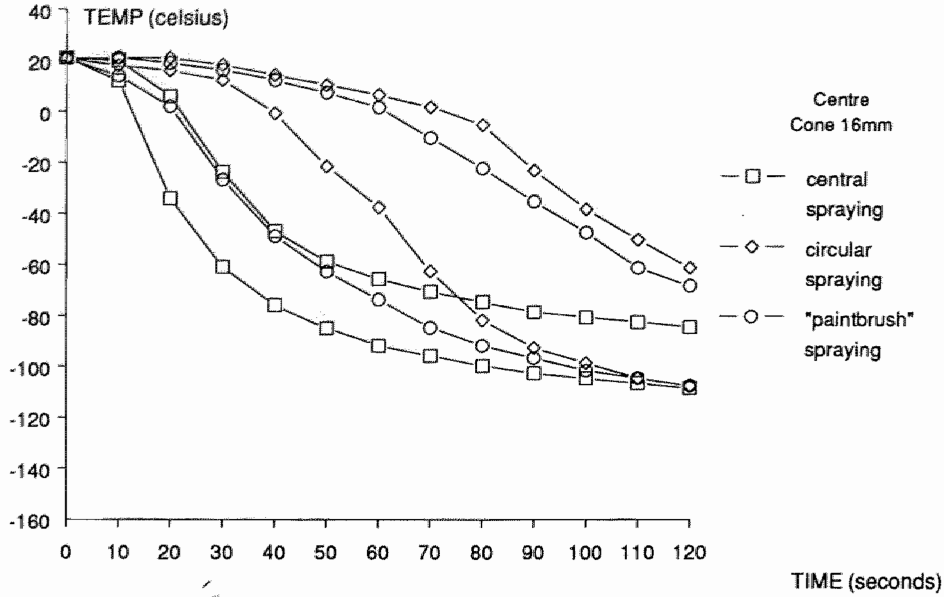


Figure 3.21. Comparison (intervals of minimum and maximum values, n=30) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

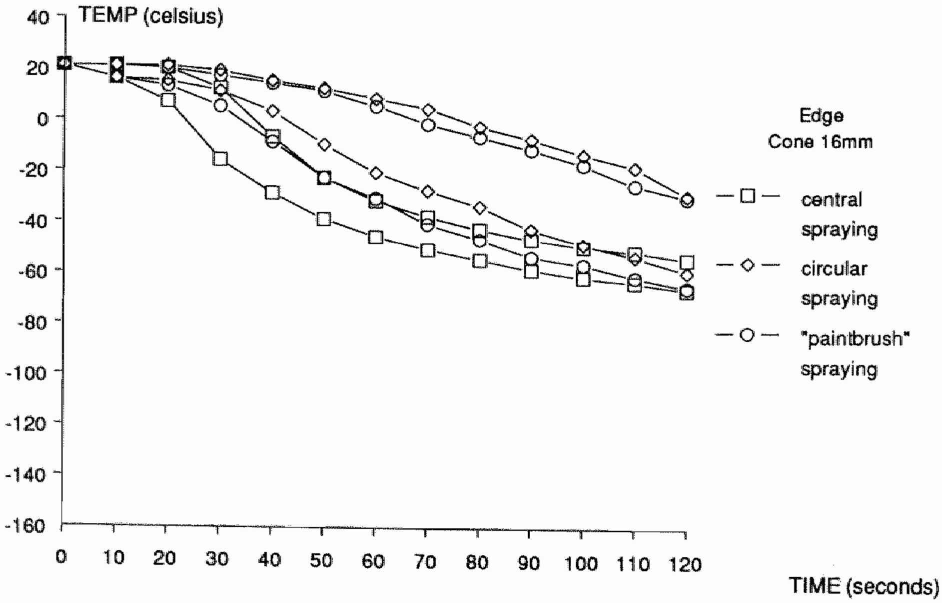


Figure 3.22. Comparison (intervals of minimum and maximum values, n=30) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

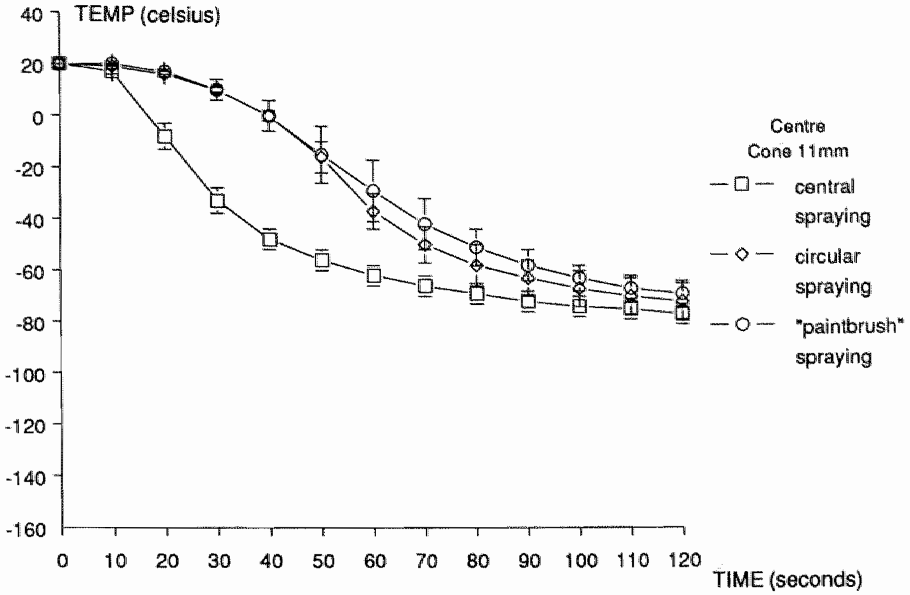


Figure 3.23. Comparison (mean±sd, n=6) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

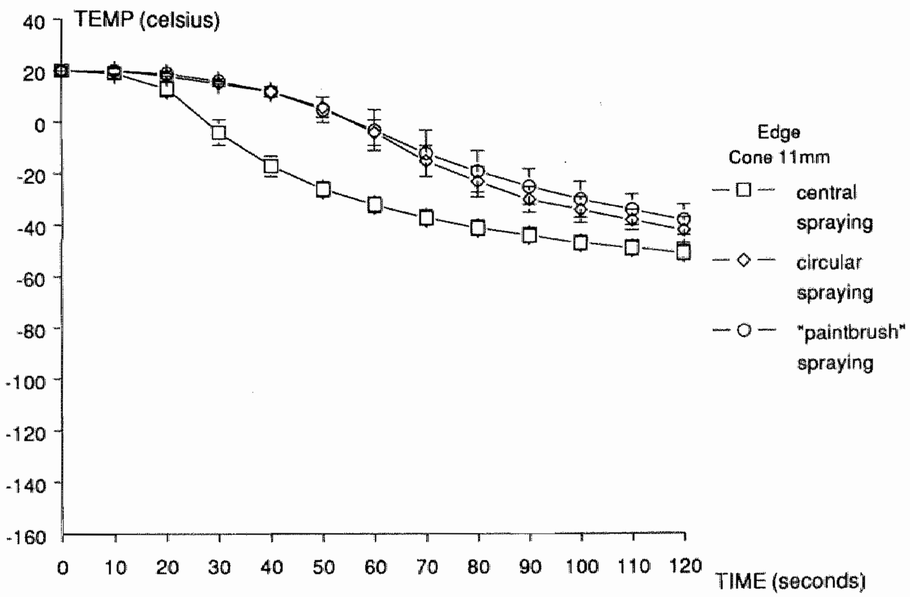


Figure 3.24. Comparison (mean±sd, n=6) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

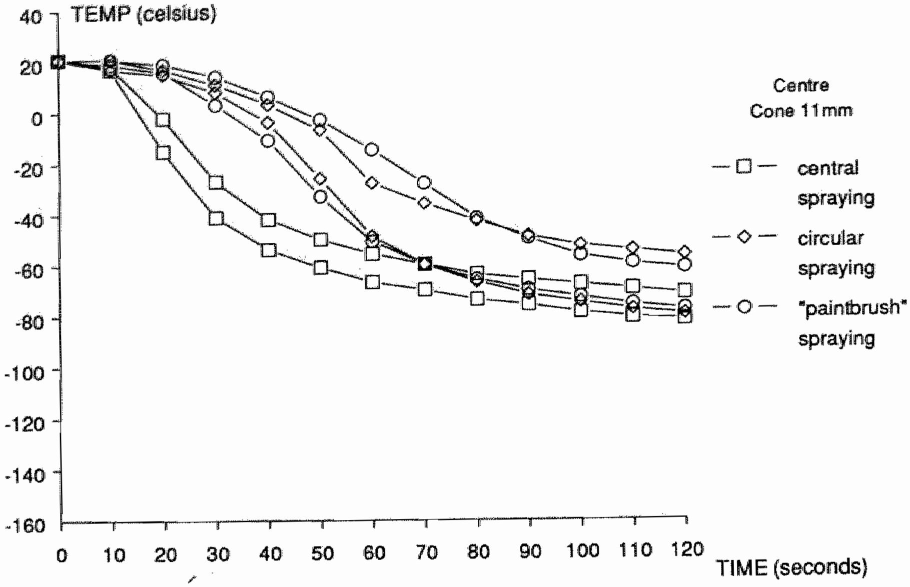


Figure 3.25. Comparison (intervals of minimum and maximum values, n=6) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

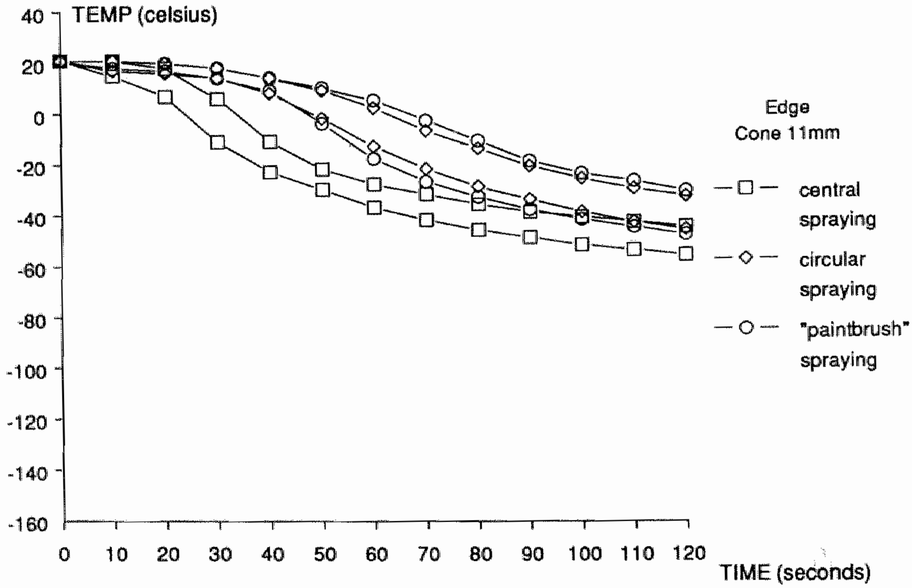


Figure 3.26. Comparison (intervals of minimum and maximum values, n=6) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

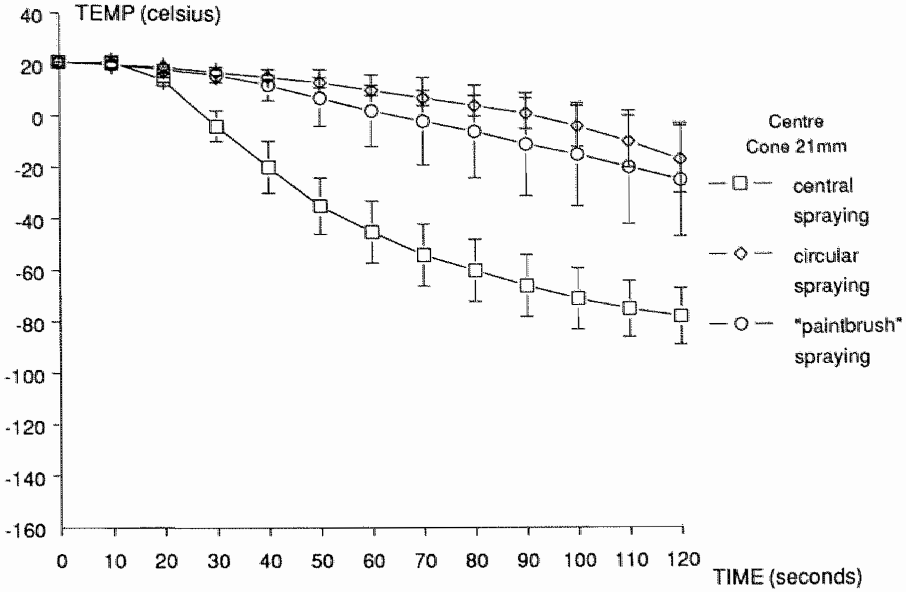


Figure 3.27. Comparison (mean±sd, n=6) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 21 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

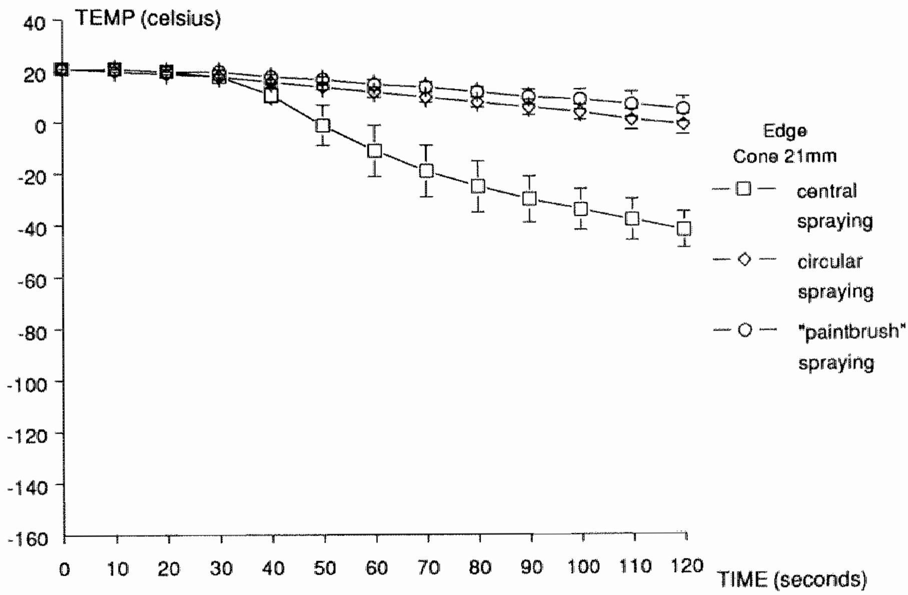


Figure 3.28. Comparison (mean±sd, n=6) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

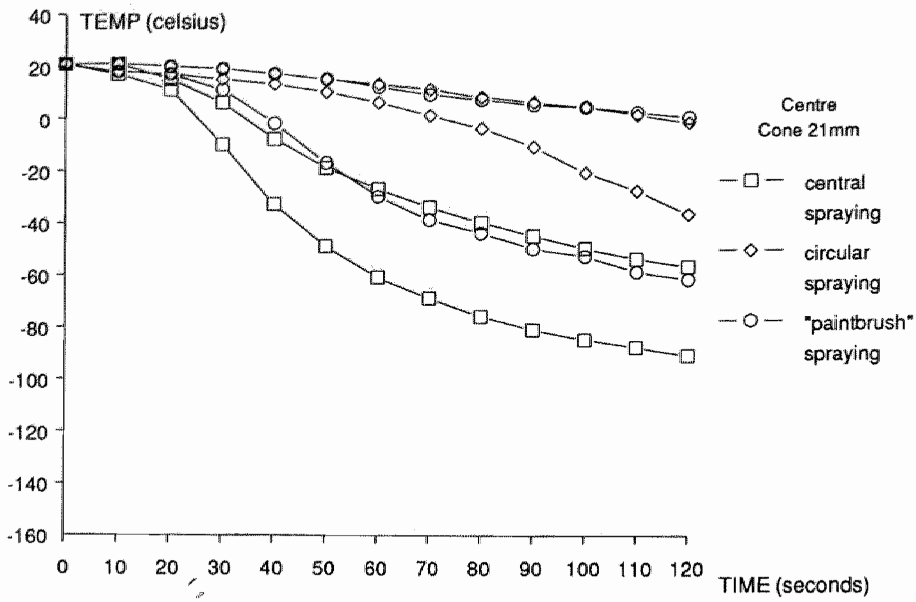


Figure 3.29. Comparison (intervals of minimum and maximum values, n=6) of the central, circular and "paintbrush" spray patterns in the centre of the cone diameter of 21 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

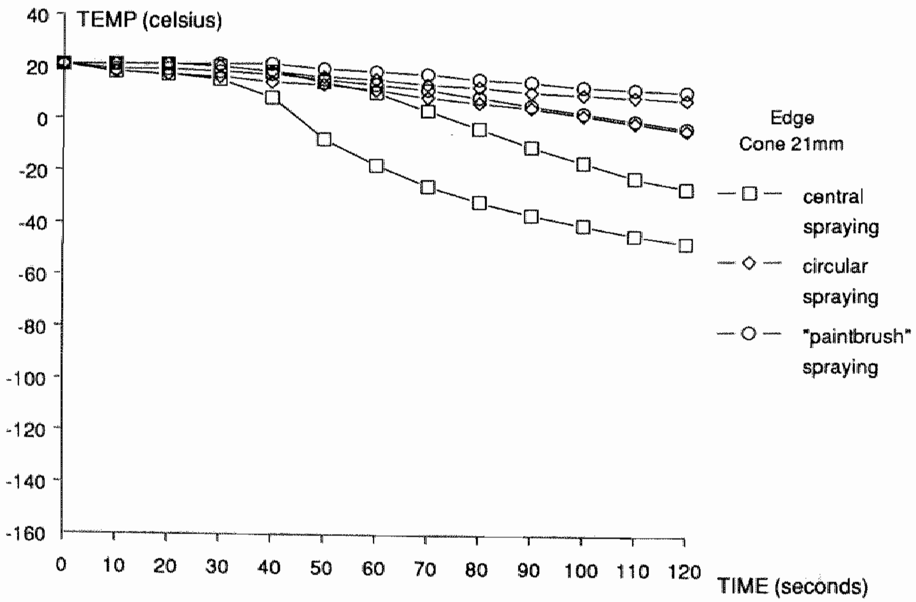


Figure 3.30. Comparison (intervals of minimum and maximum values, n=6) of the central, circular and "paintbrush" spray patterns at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

Figures of chapter 4

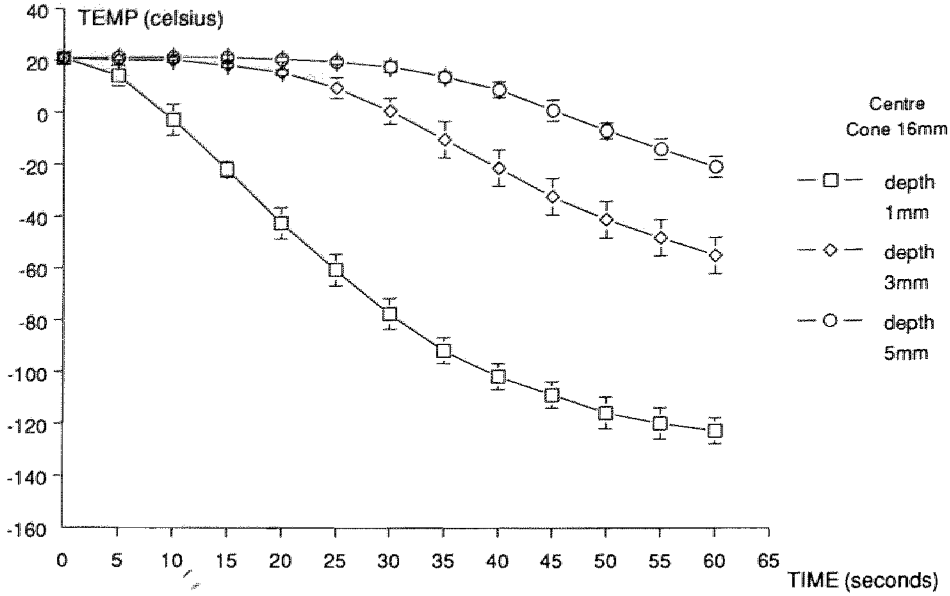


Figure 4.1. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

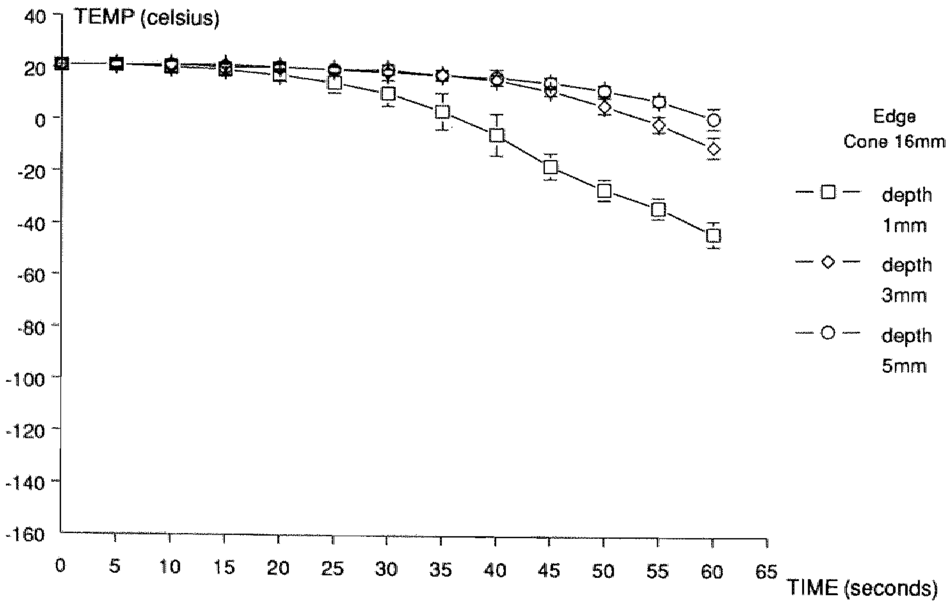


Figure 4.2. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

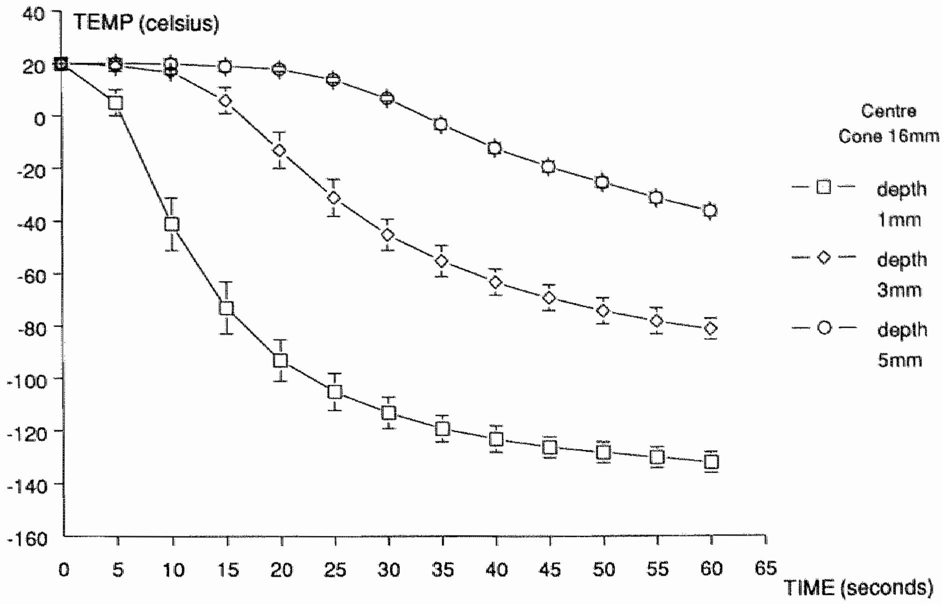


Figure 4.3. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

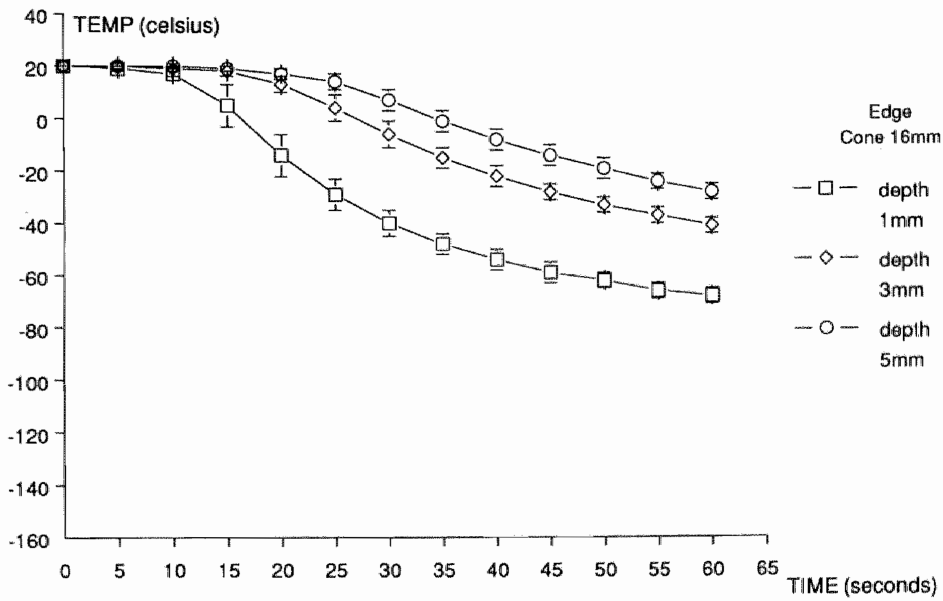


Figure 4.4. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

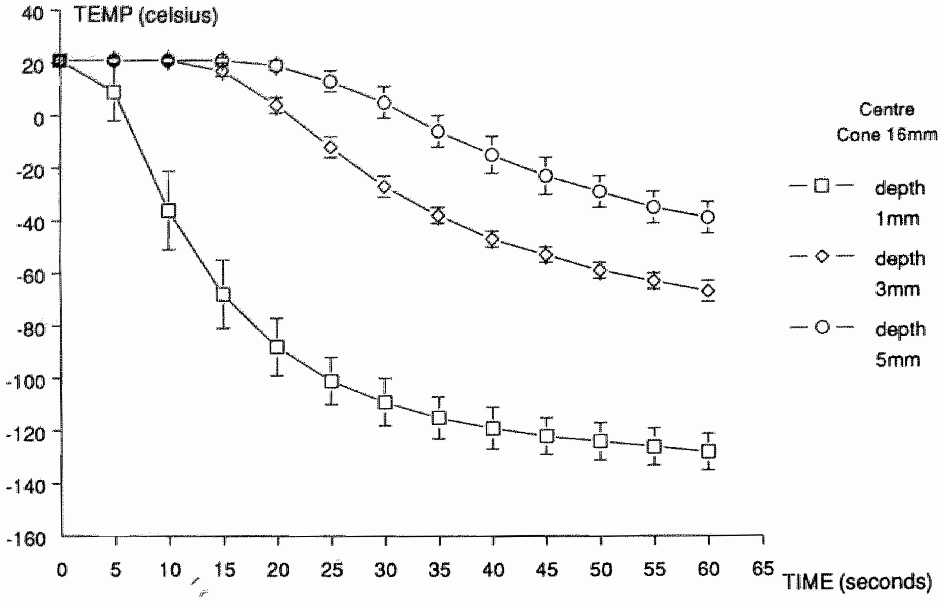


Figure 4.5. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

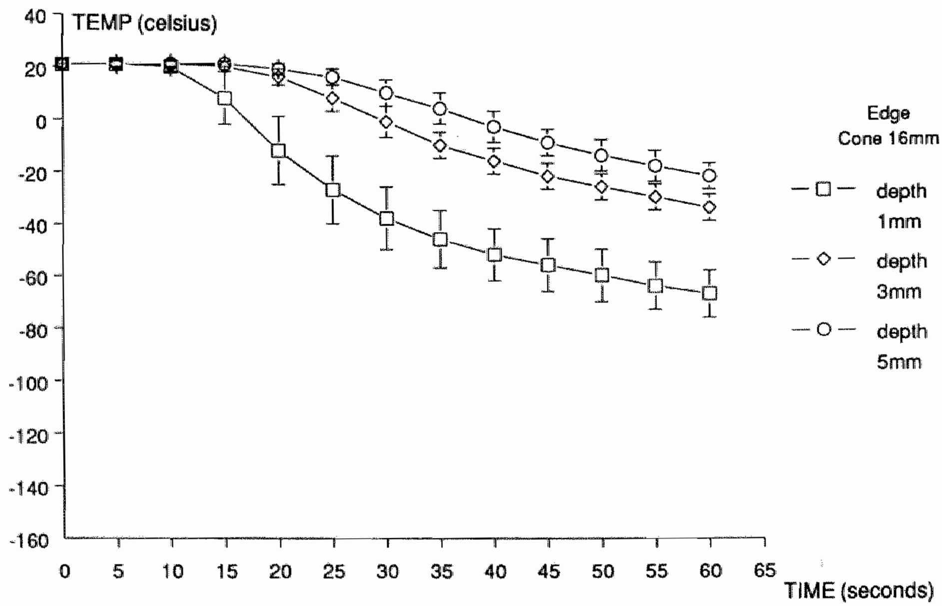


Figure 4.6. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

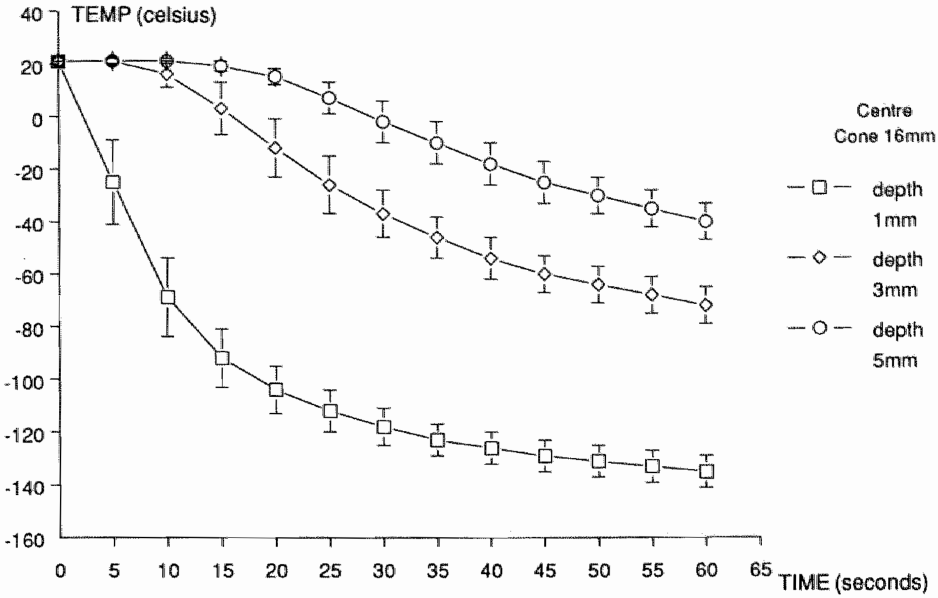


Figure 4.7. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

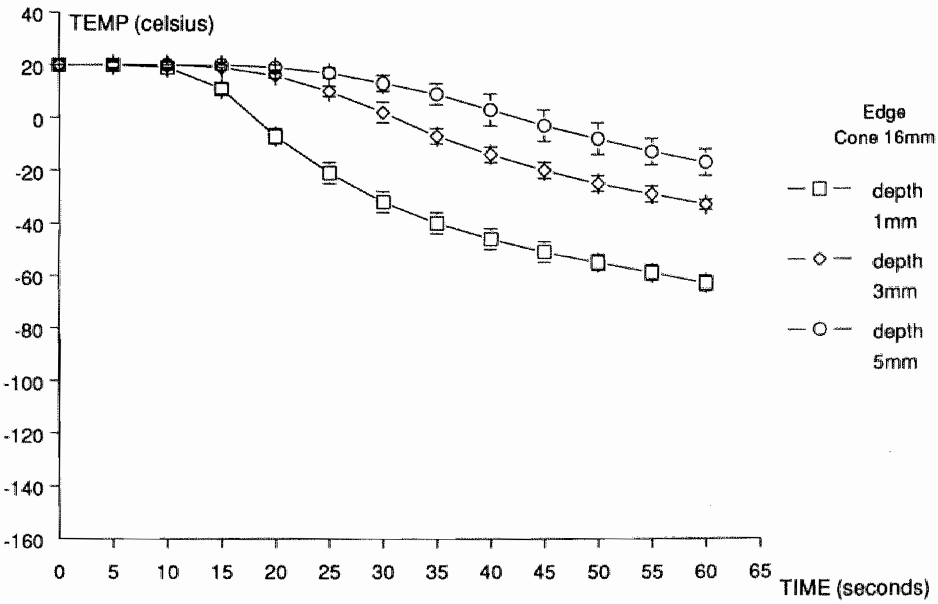


Figure 4.8. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central sparying from a distance of 15 mm).

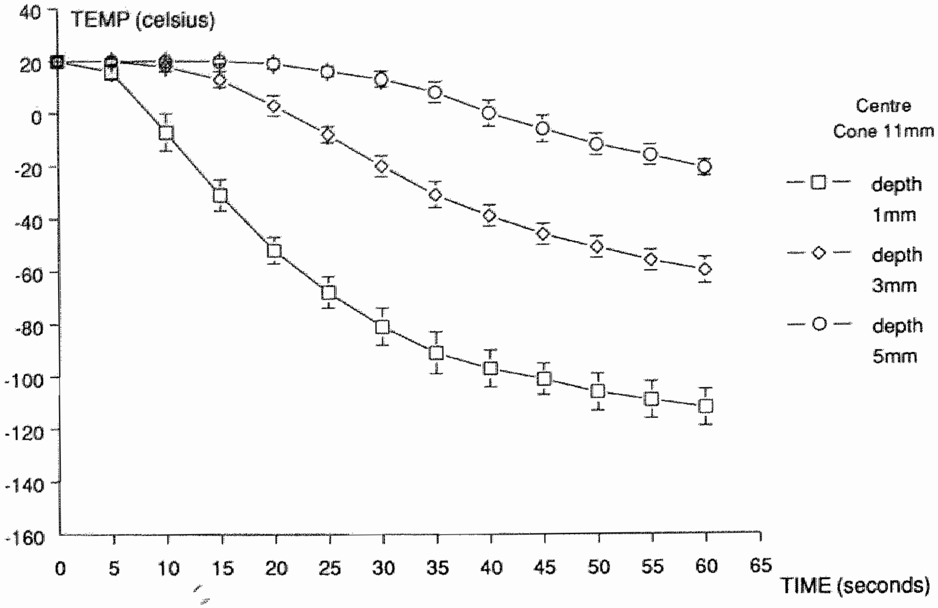


Figure 4.9. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

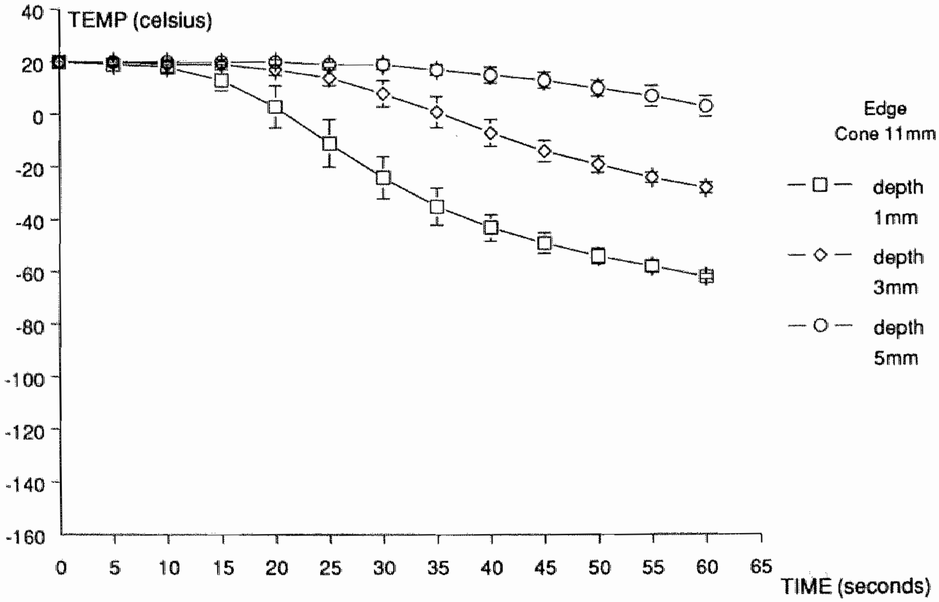


Figure 4.10. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

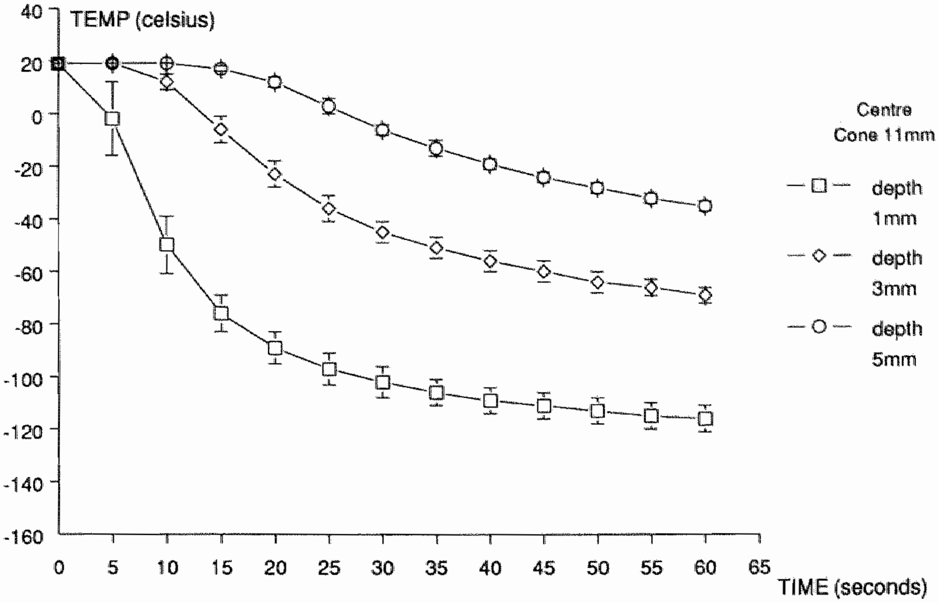


Figure 4.11. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

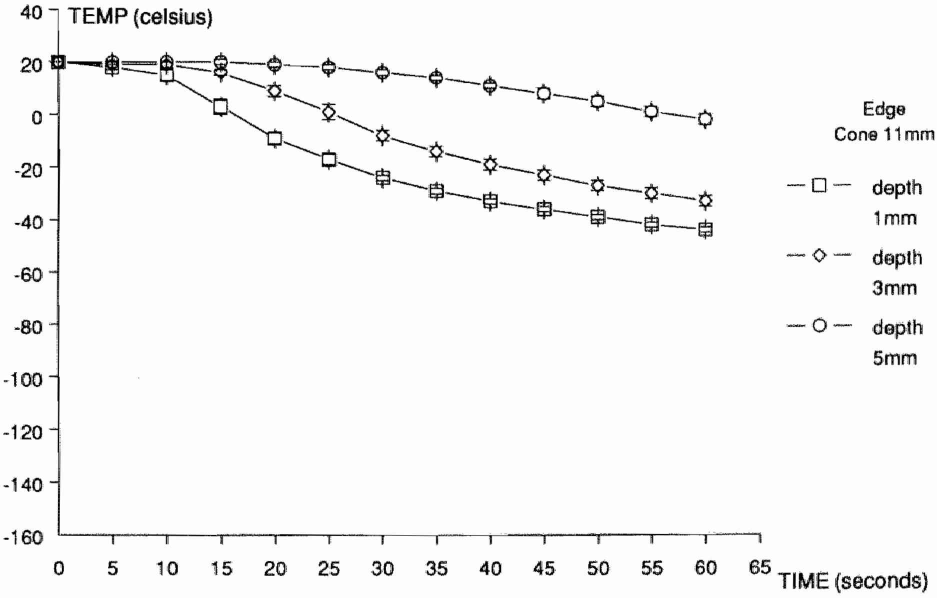


Figure 4.12. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

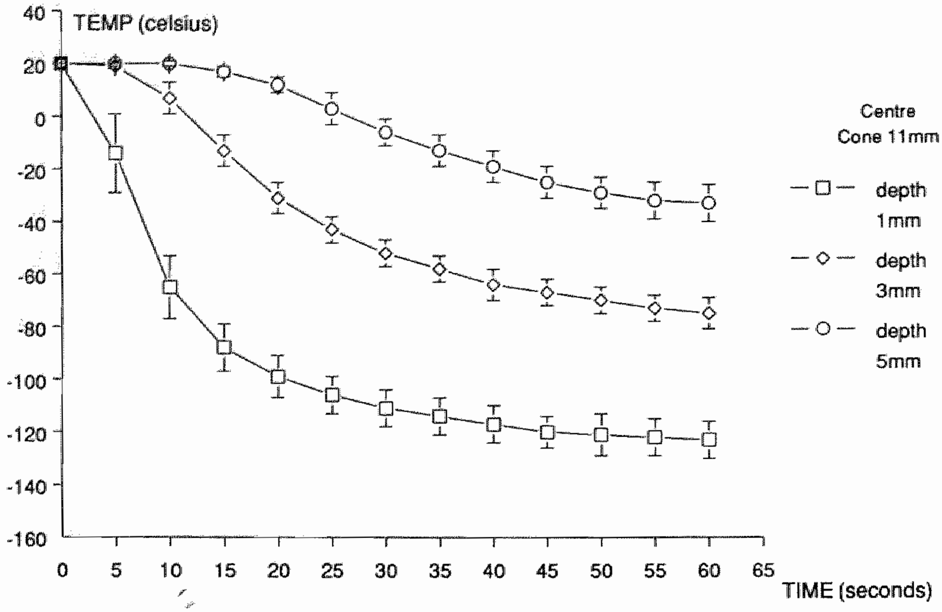


Figure 4.13. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

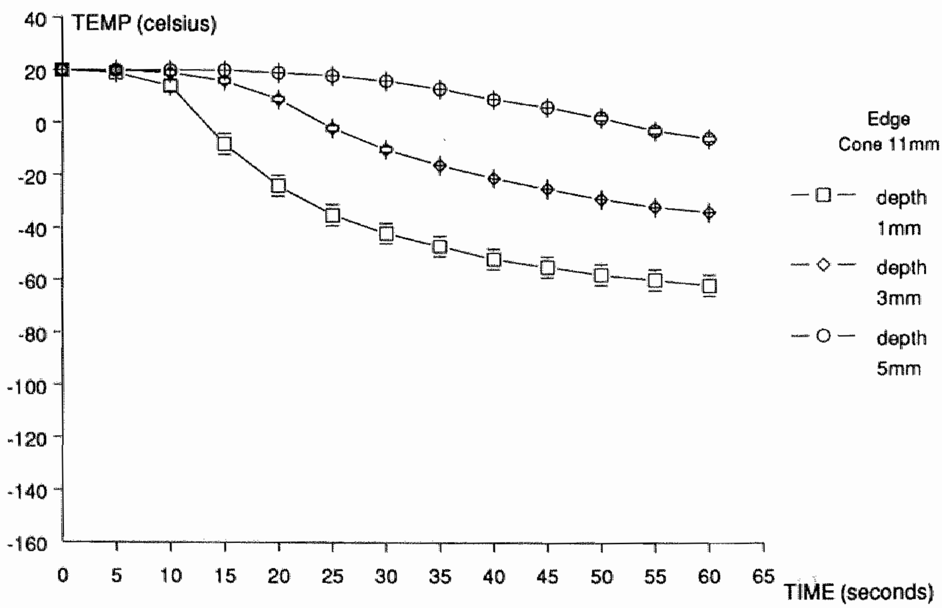


Figure 4.14. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

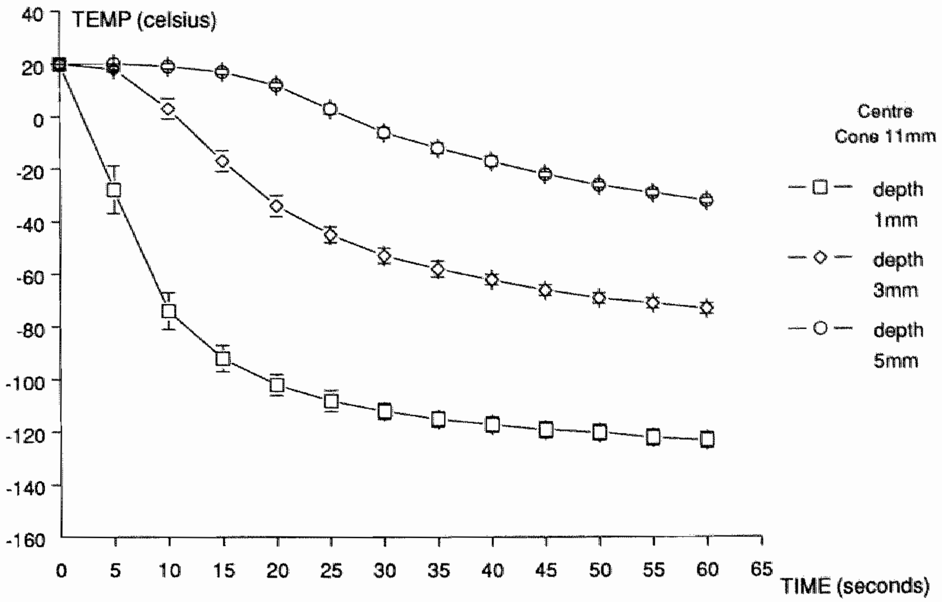


Figure 4.15. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

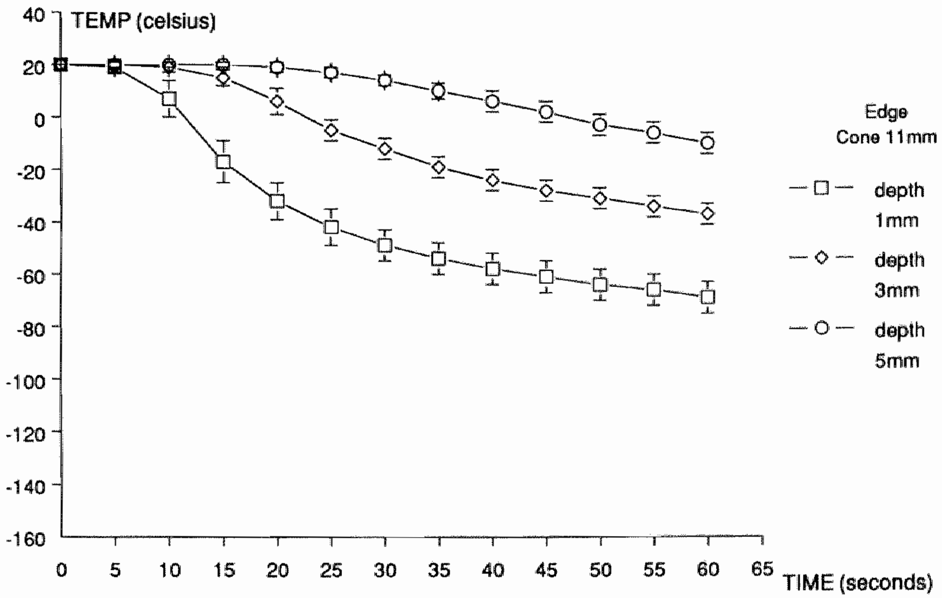


Figure 4.16. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central sparying from a distance of 15 mm).

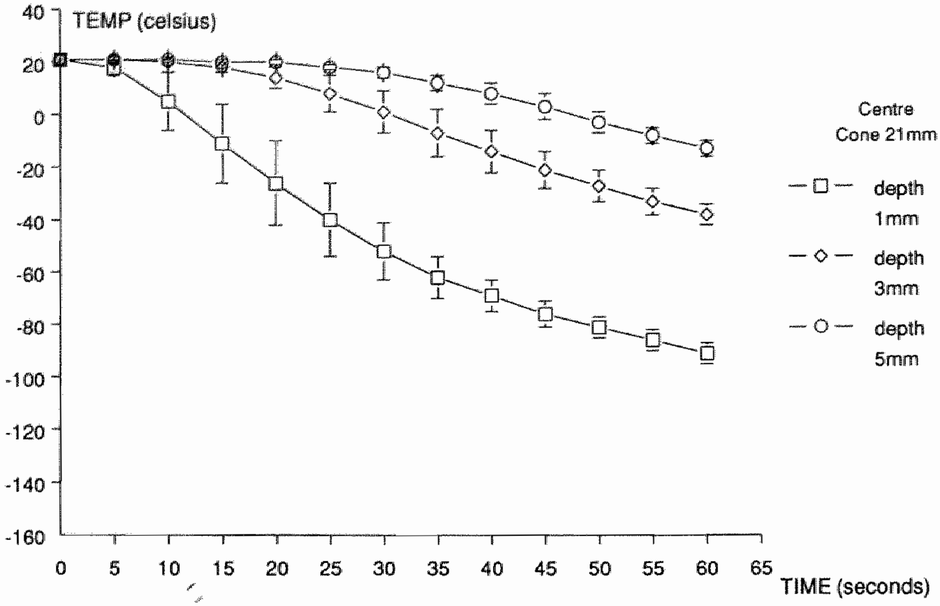


Figure 4.17. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

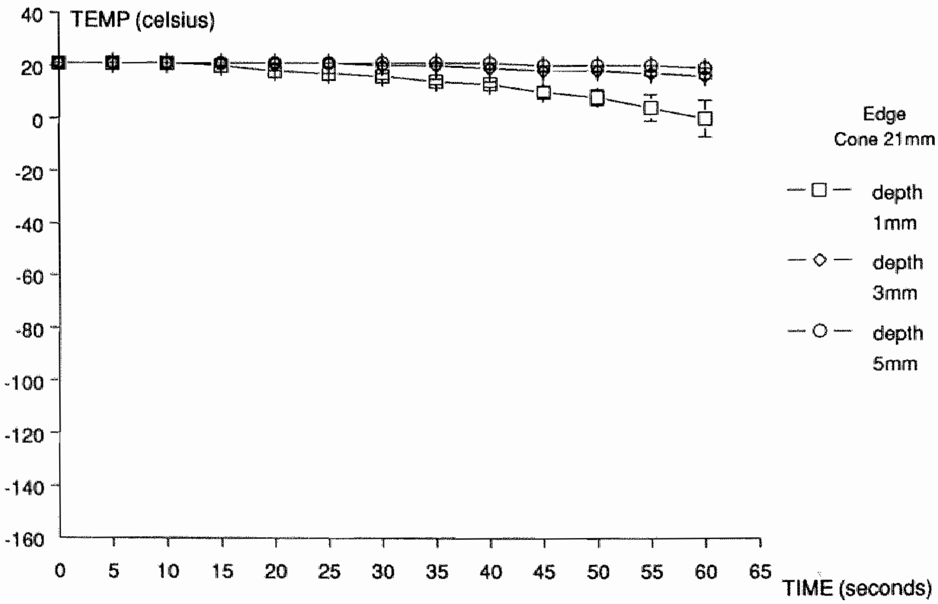


Figure 4.18. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.6 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

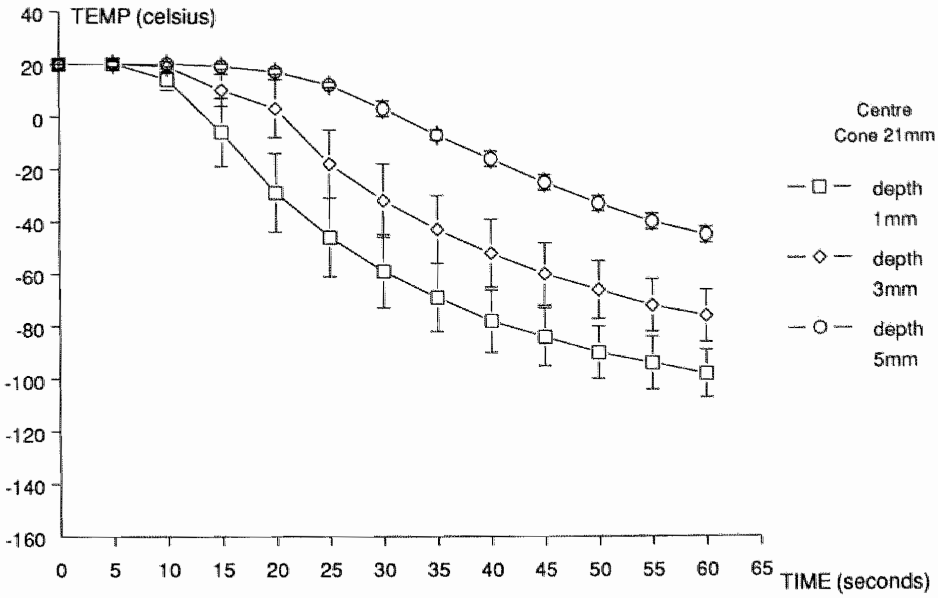


Figure 4.19. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

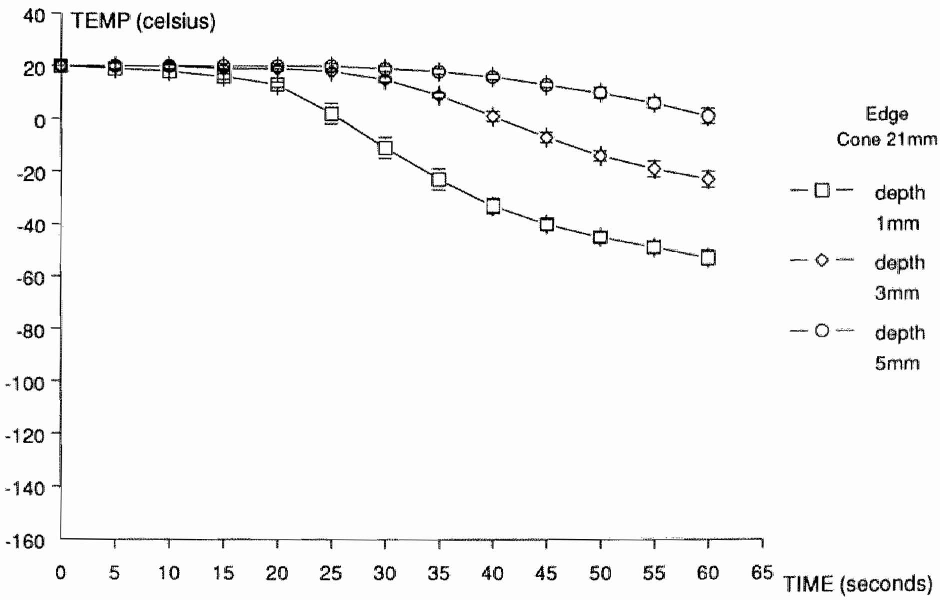


Figure 4.20. Temperature course (mean±sd, n=4) of the spraytip diameter of 0.8 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

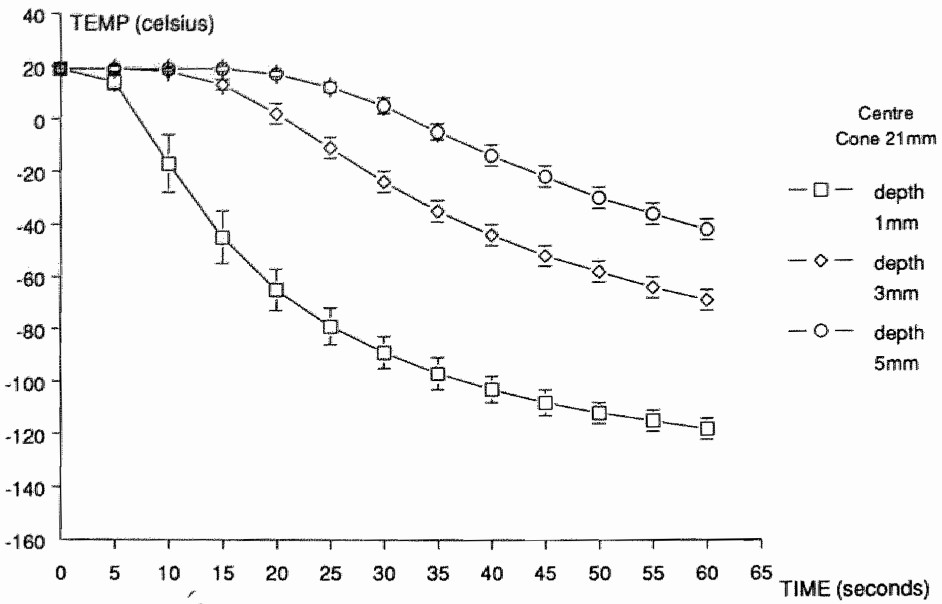


Figure 4.21. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

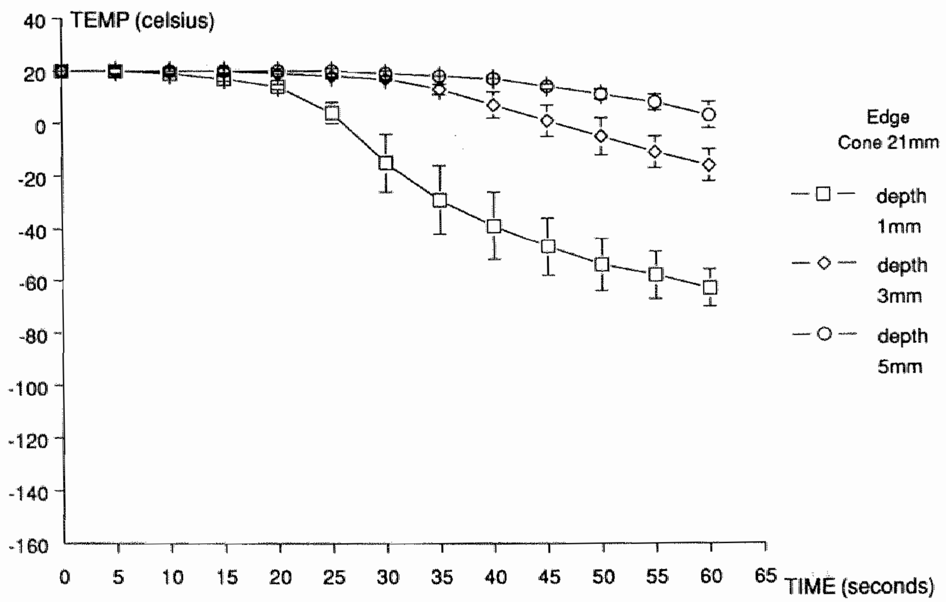


Figure 4.22. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.0 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

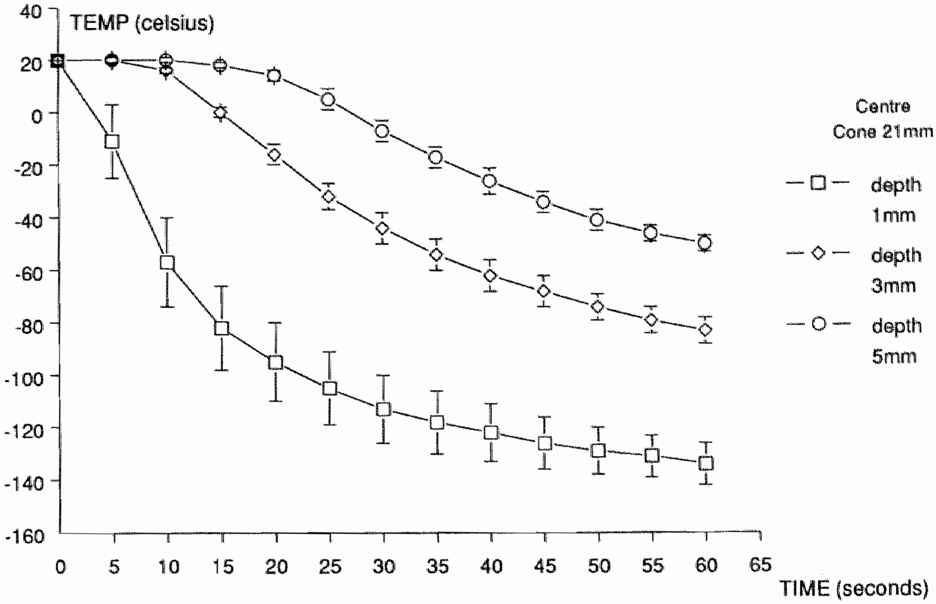


Figure 4.23. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying from a distance of 15 mm).

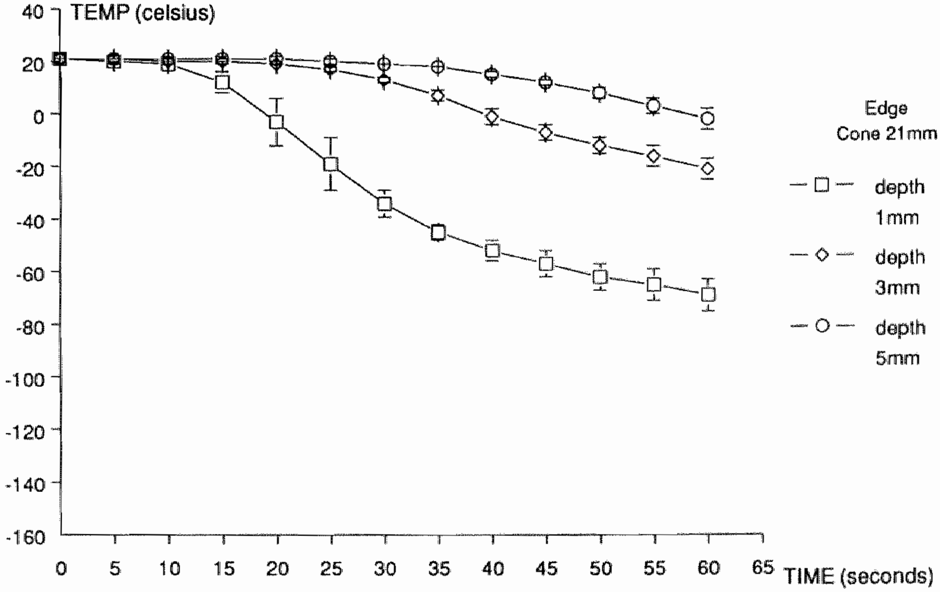


Figure 4.24. Temperature course (mean±sd, n=4) of the spraytip diameter of 1.7 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central sparying from a distance of 15 mm).

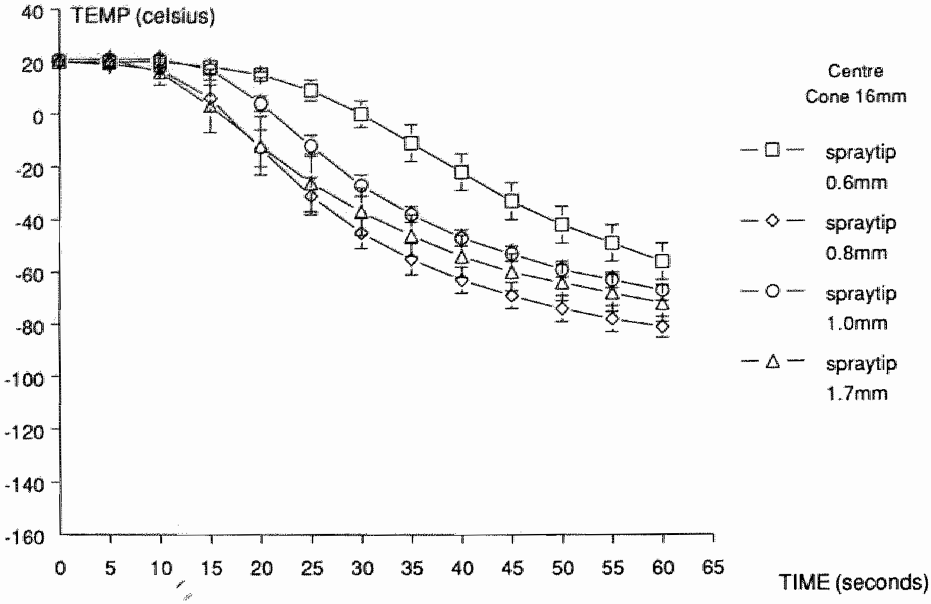


Figure 4.25. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

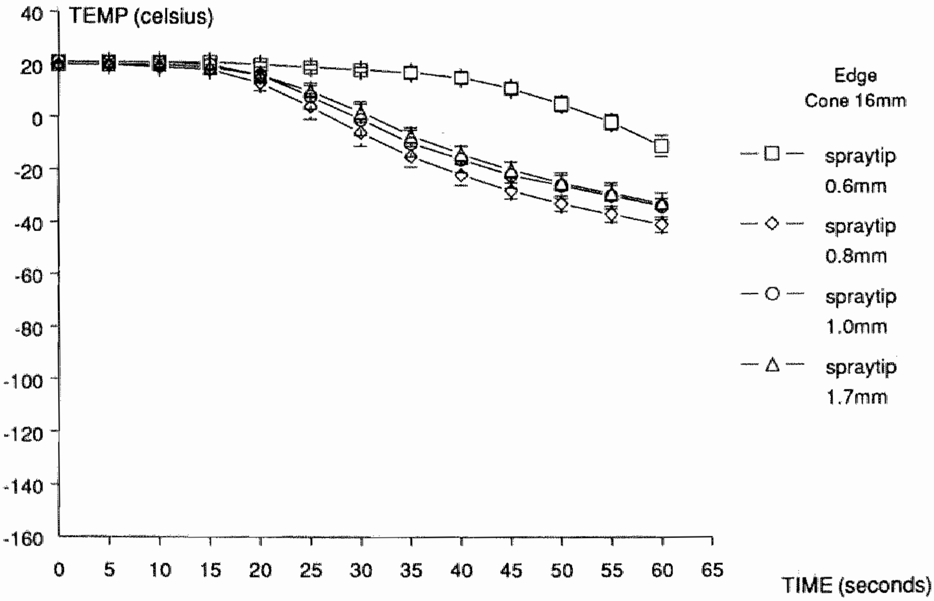


Figure 4.26. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

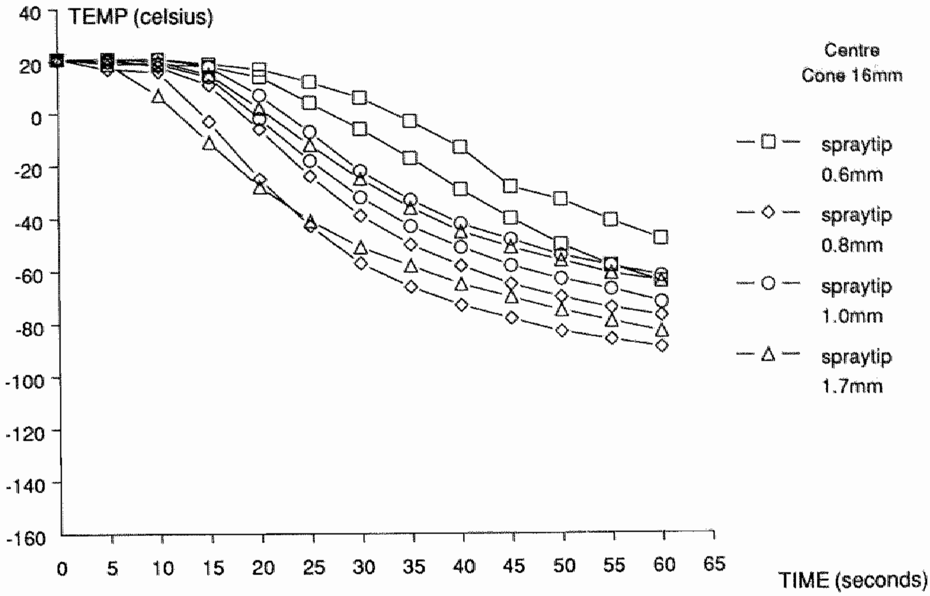


Figure 4.27. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

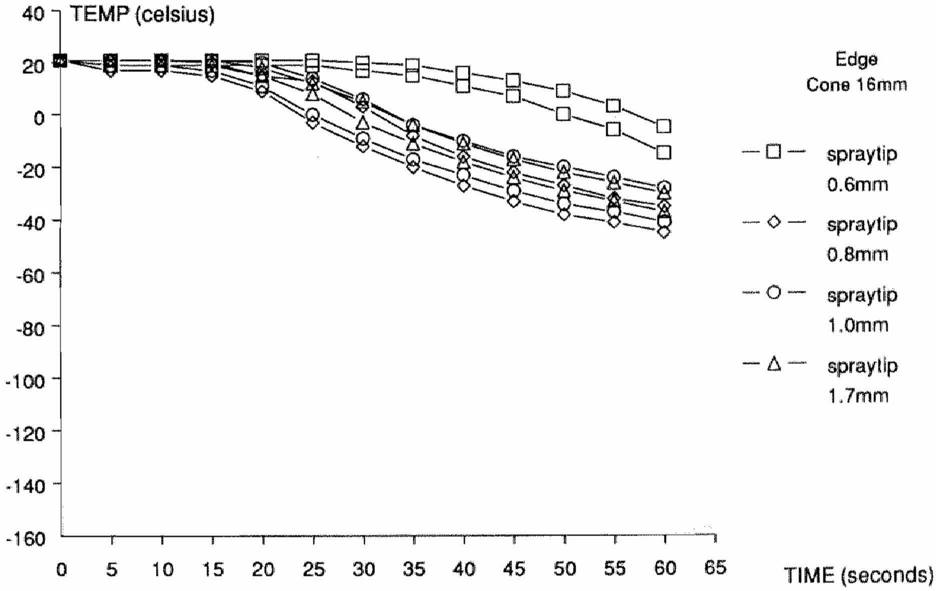


Figure 4.28. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

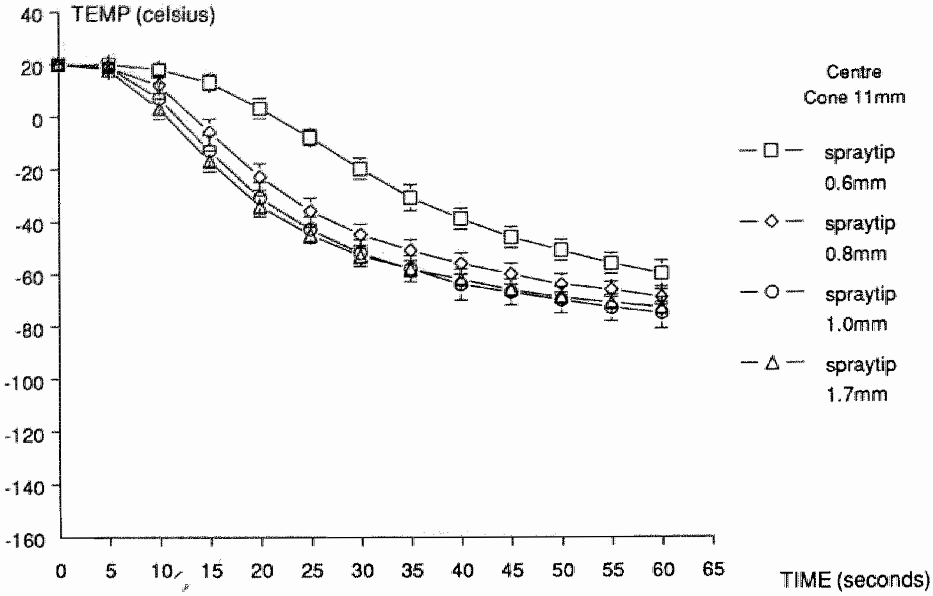


Figure 4.29. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

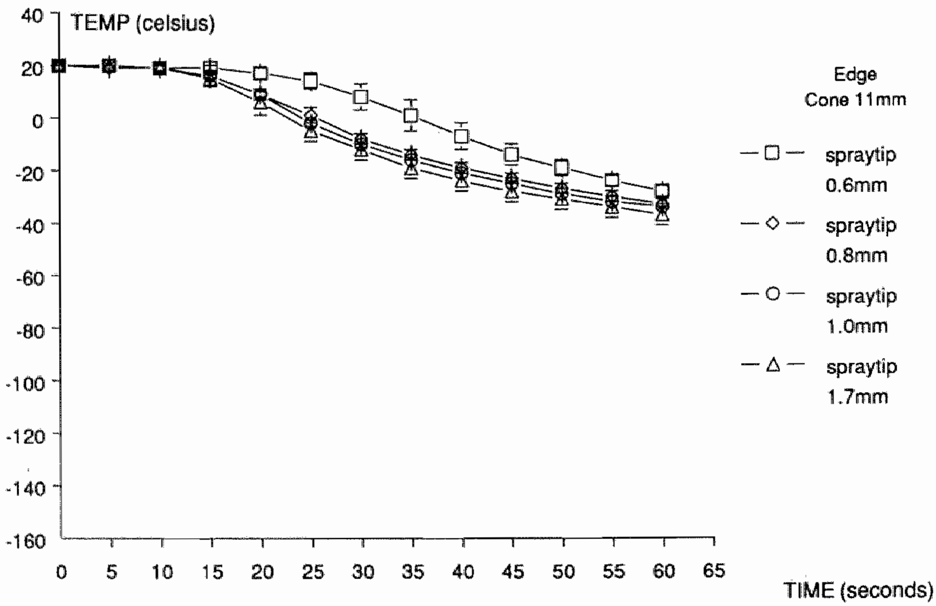


Figure 4.30. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

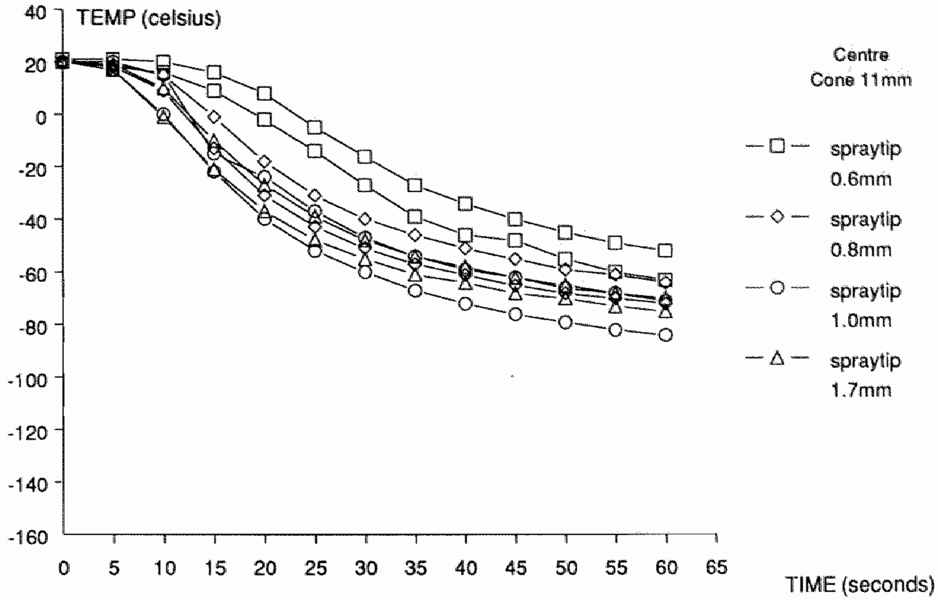


Figure 4.31. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

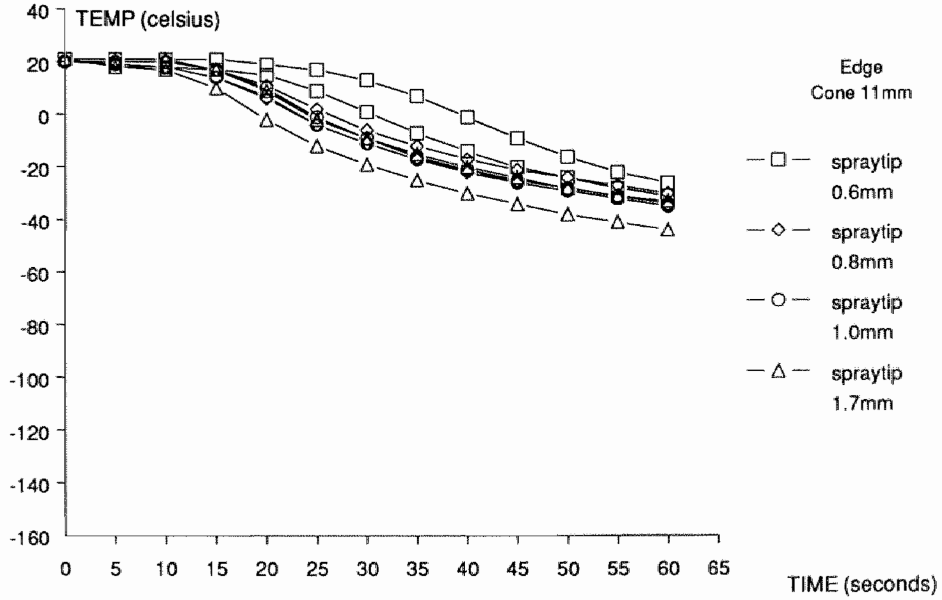


Figure 4.32. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

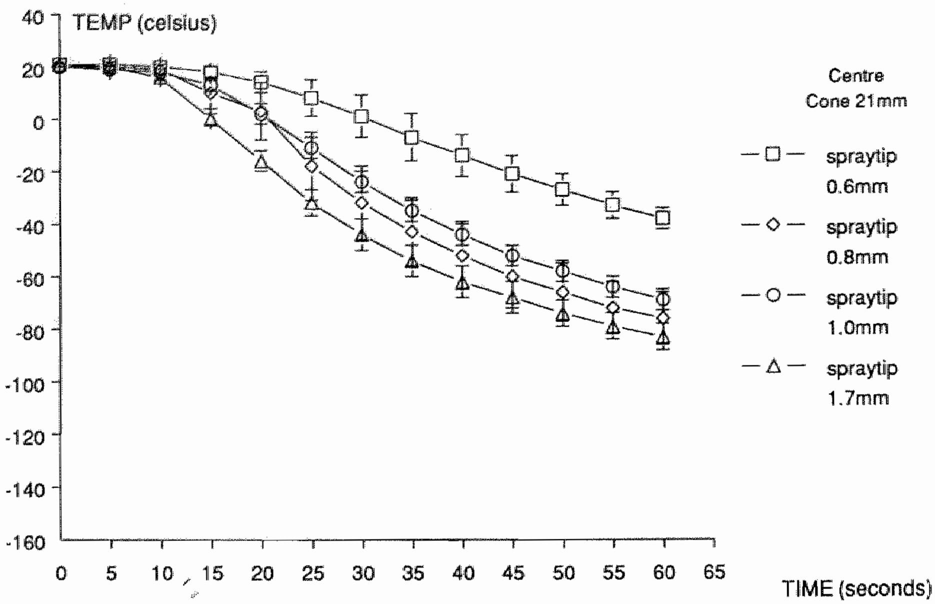


Figure 4.33. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

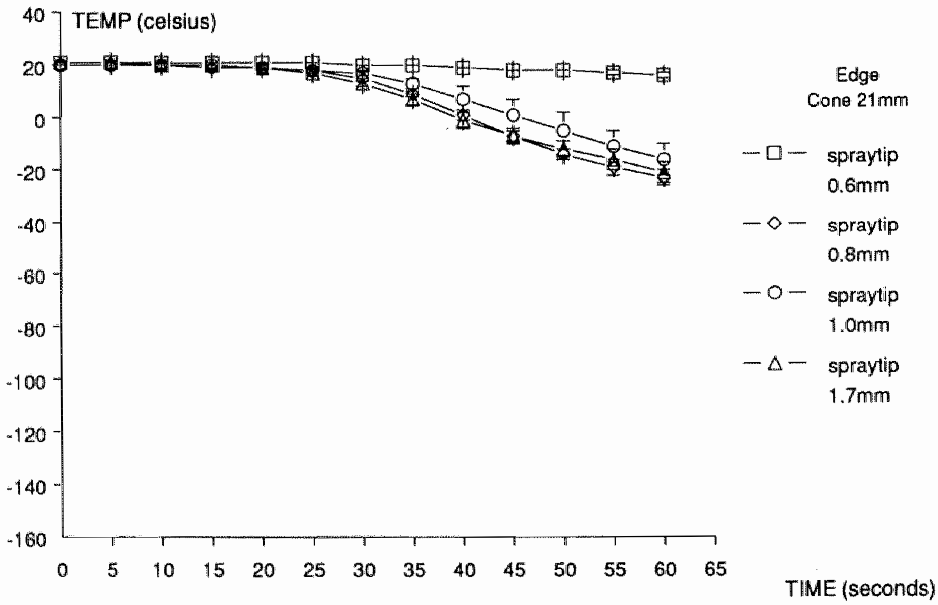


Figure 4.34. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

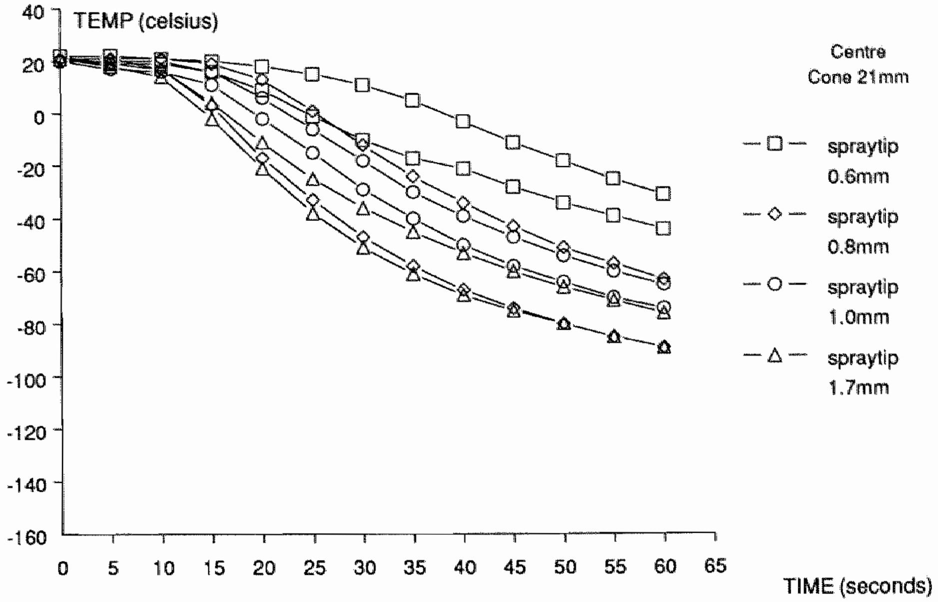


Figure 4.35. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm in the centre of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

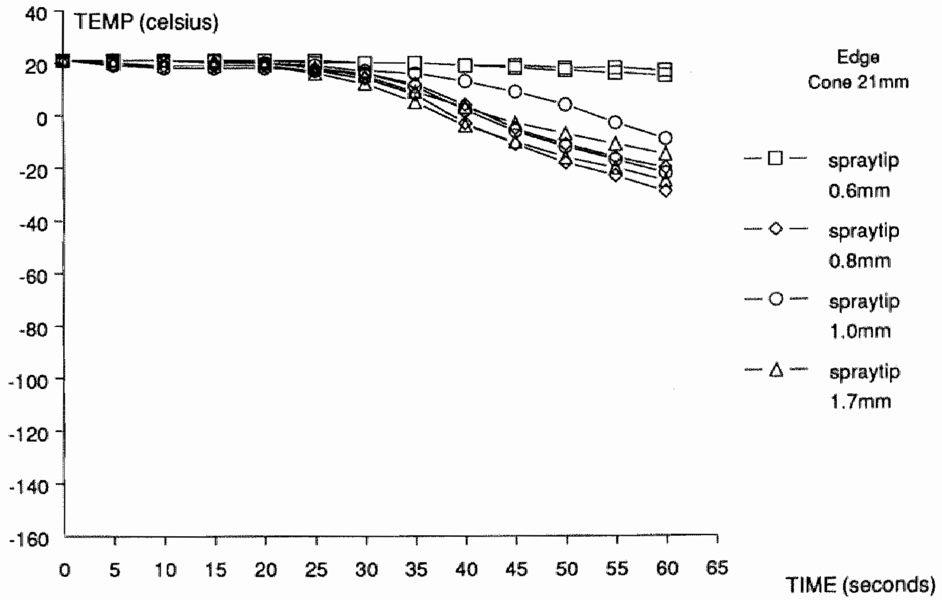


Figure 4.36. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm, 0.8 mm, 1.0 mm and 1.7 mm at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

Figures of chapter 5

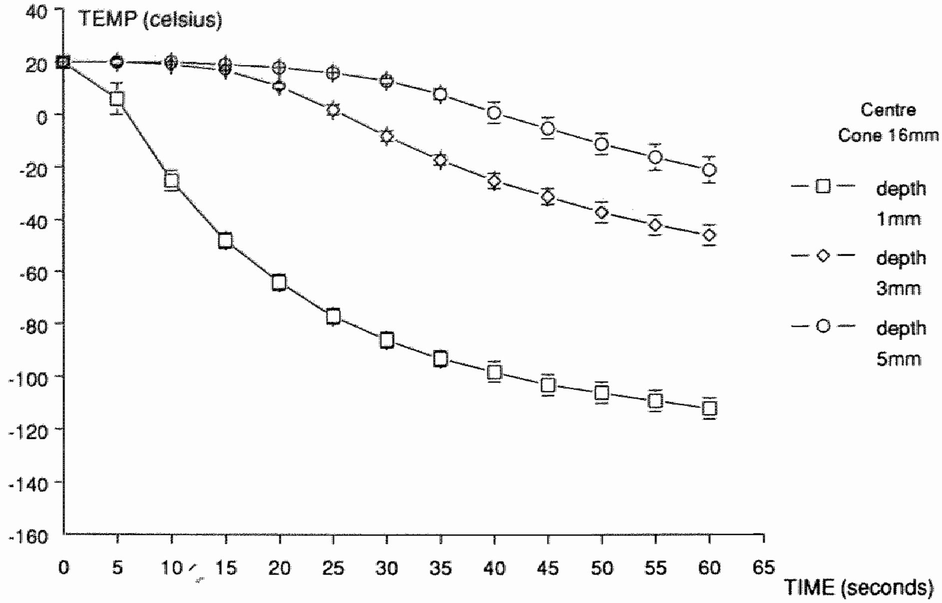


Figure 5.1. Temperature course (mean±sd, n=4) of the spray distance of 5 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

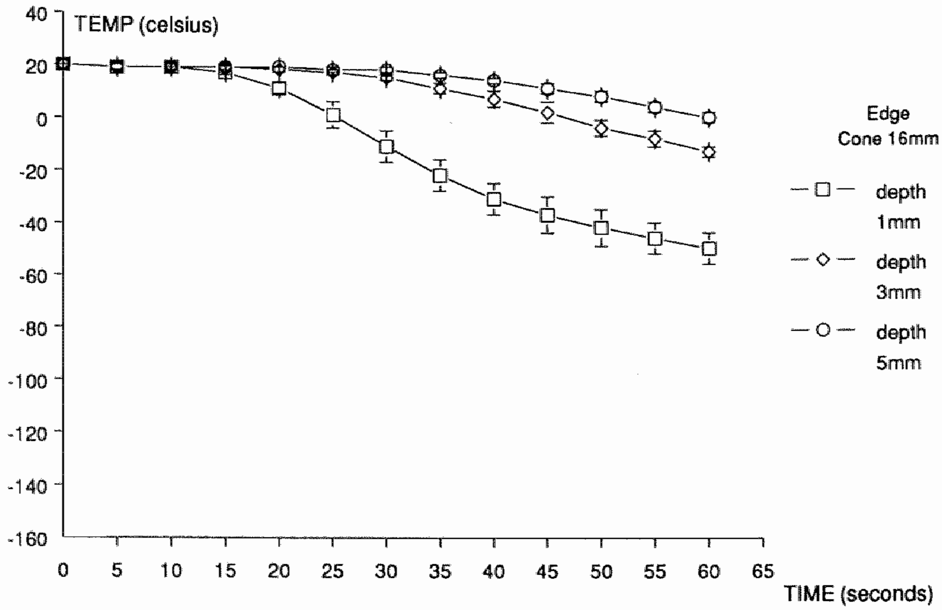


Figure 5.2. Temperature course (mean±sd, n=4) of the spray distance of 5 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

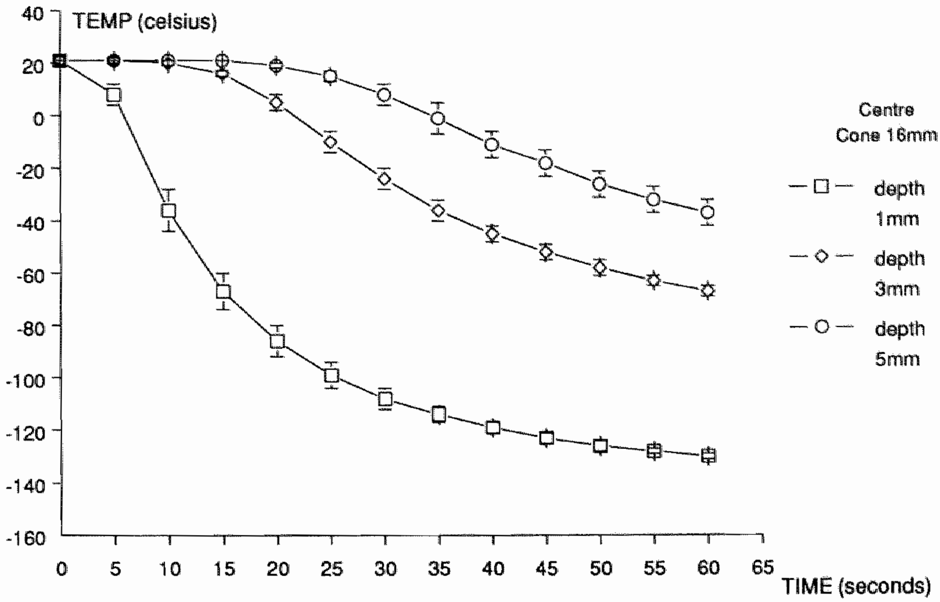


Figure 5.3. Temperature course (mean±sd, n=4) of the spray distance of 10 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

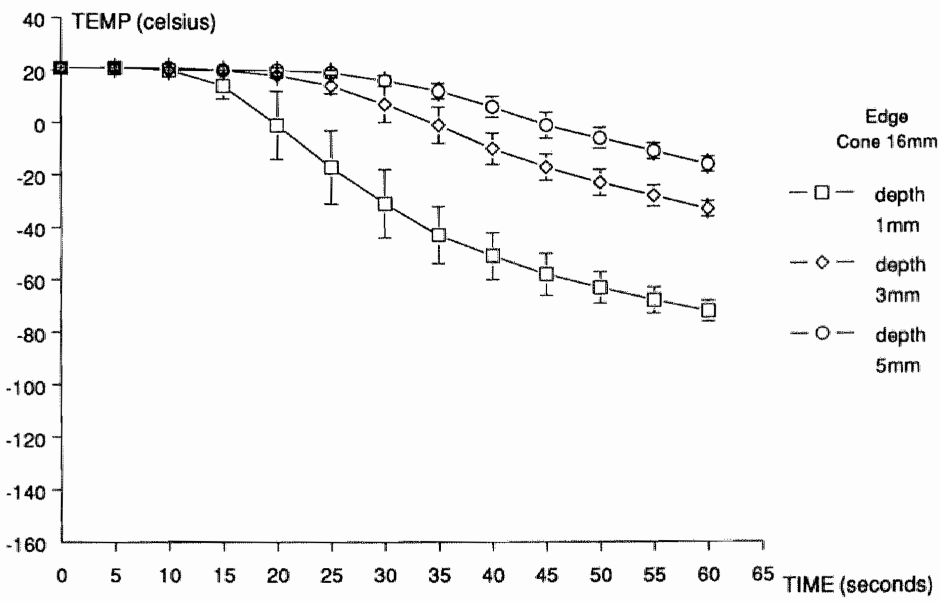


Figure 5.4. Temperature course (mean±sd, n=4) of the spray distance of 10 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

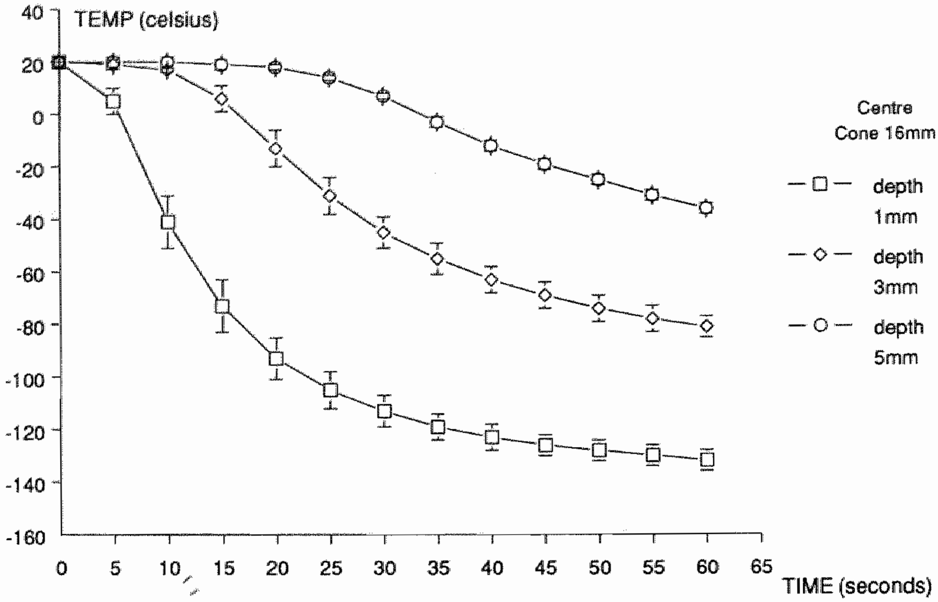


Figure 5.5. Temperature course (mean±sd, n=4) of the spray distance of 15 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

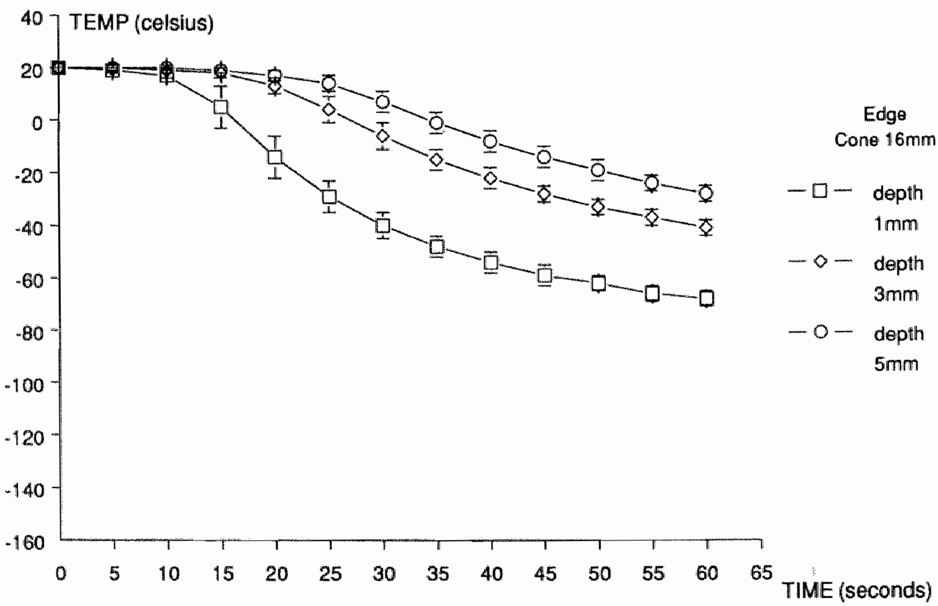


Figure 5.6. Temperature course (mean±sd, n=4) of the spray distance of 15 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

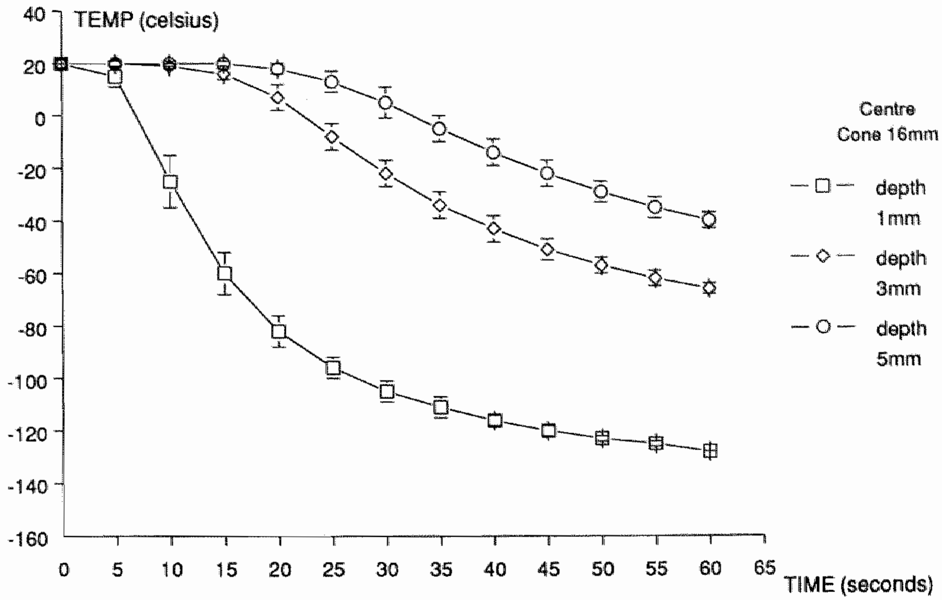


Figure 5.7. Temperature course (mean±sd, n=4) of the spray distance of 20 mm in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

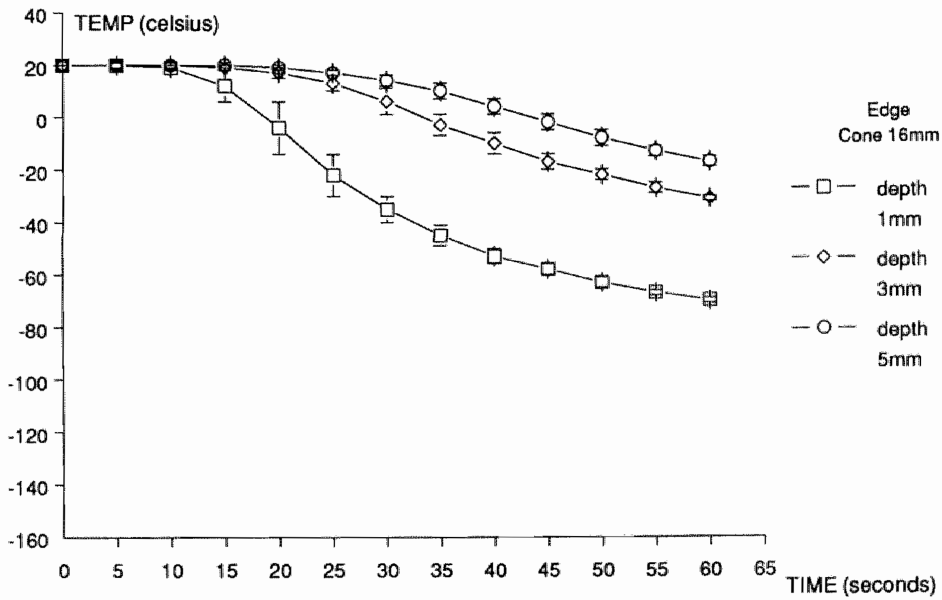


Figure 5.8. Temperature course (mean±sd, n=4) of the spray distance of 20 mm at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

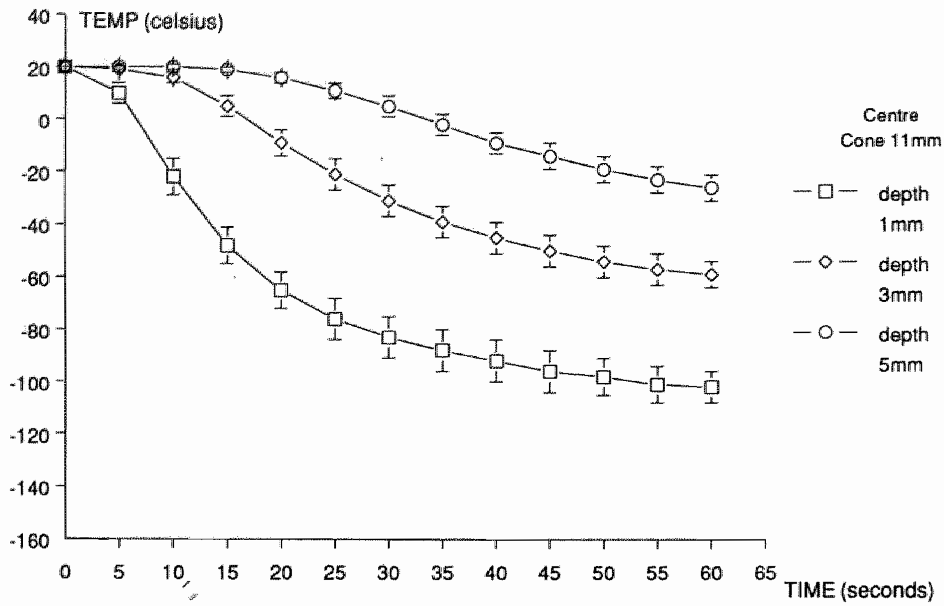


Figure 5.9. Temperature course (mean±sd, n=4) of the spray distance of 5 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

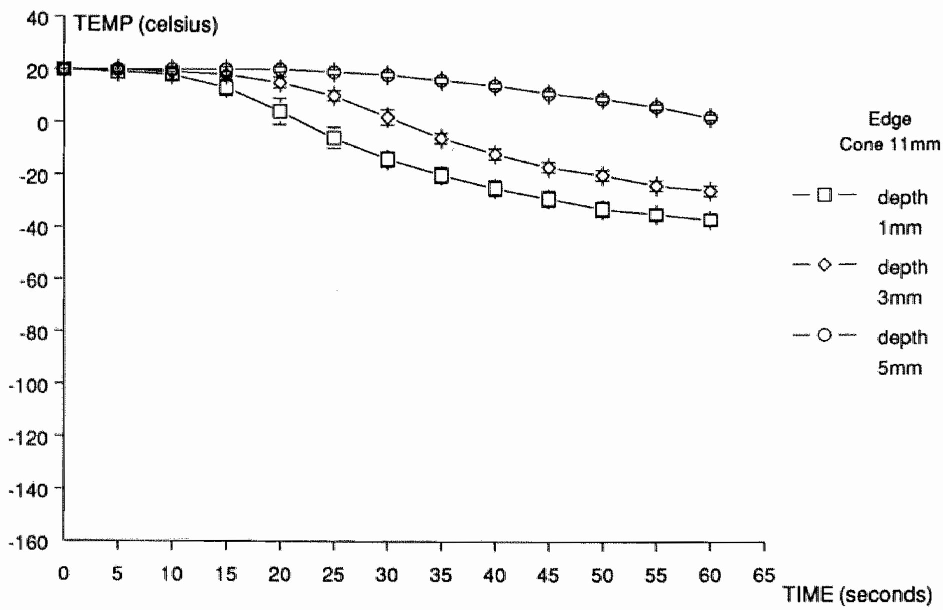


Figure 5.10. Temperature course (mean±sd, n=4) of the spray distance of 5 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

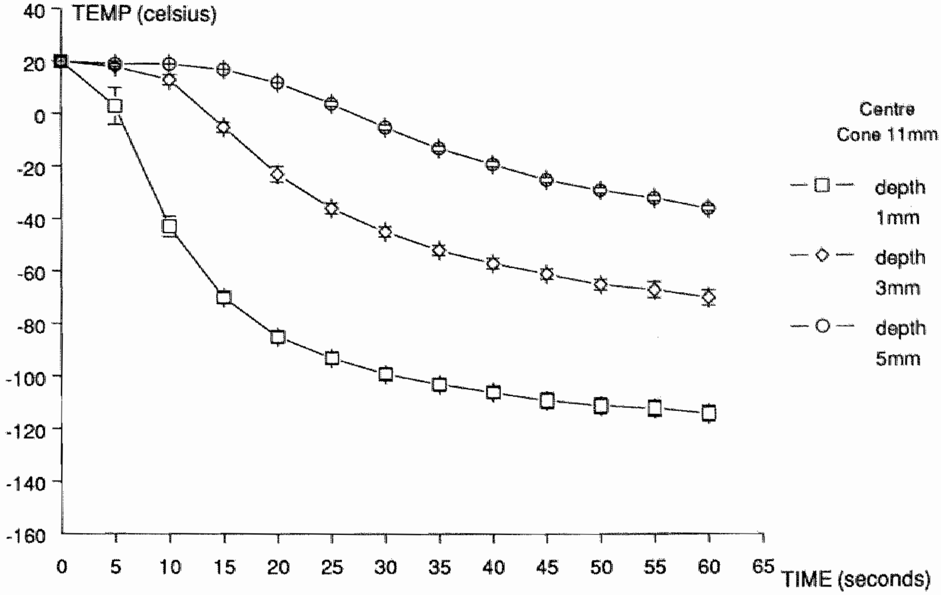


Figure 5.11. Temperature course (mean±sd, n=4) of the spray distance of 10 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

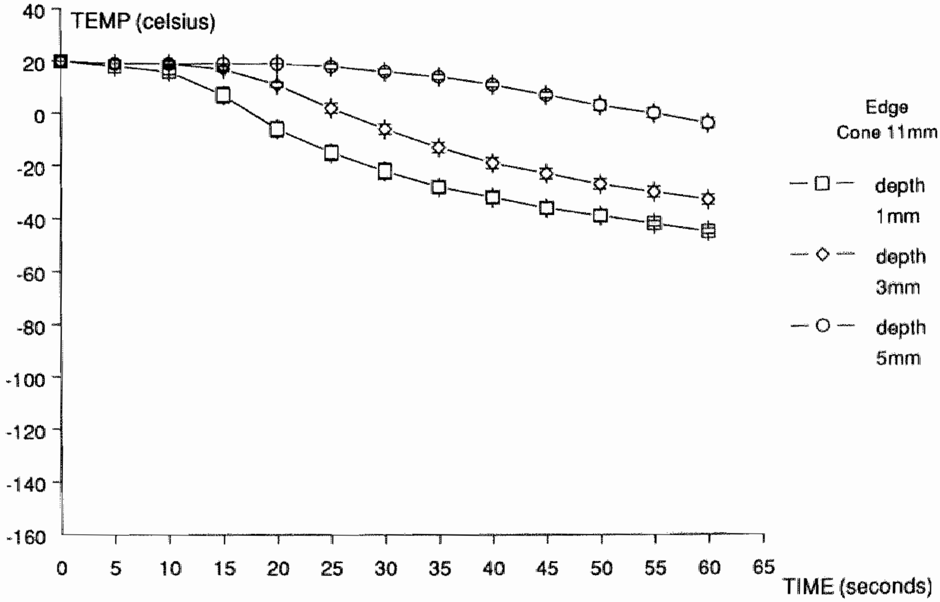


Figure 5.12. Temperature course (mean±sd, n=4) of the spray distance of 10 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

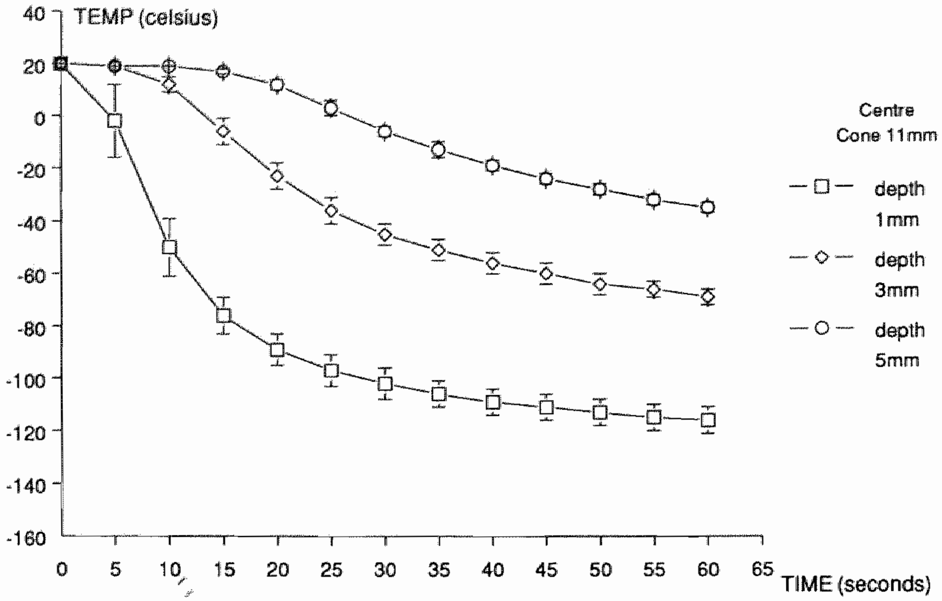


Figure 5.13. Temperature course (mean±sd, n=4) of the spray distance of 15 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

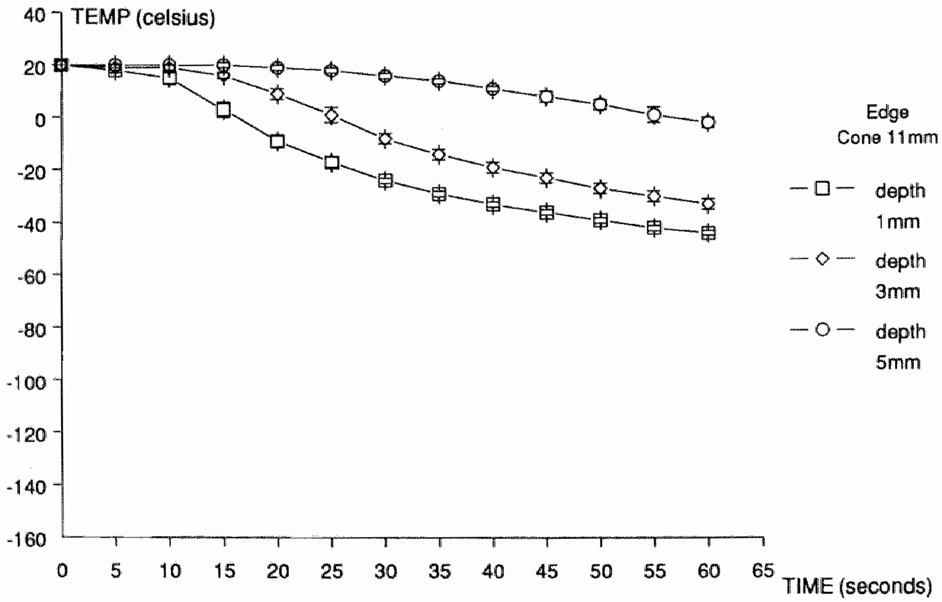


Figure 5.14. Temperature course (mean±sd, n=4) of the spray distance of 15 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

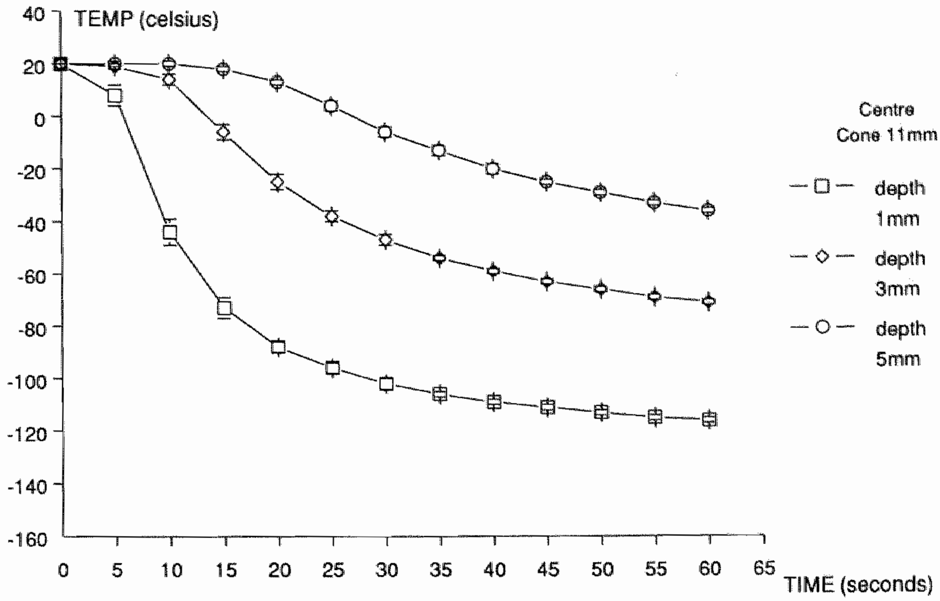


Figure 5.15. Temperature course (mean±sd, n=4) of the spray distance of 20 mm in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

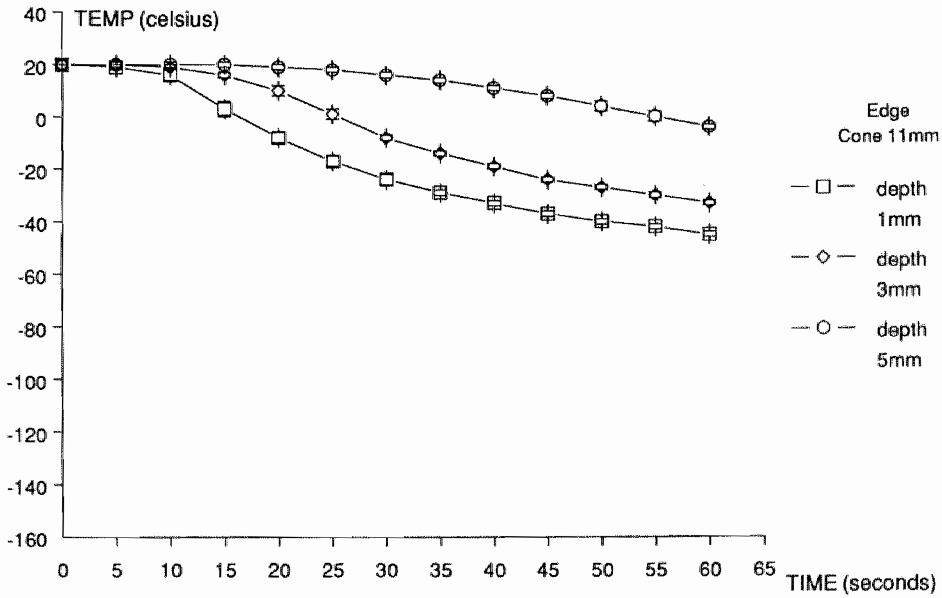


Figure 5.16. Temperature course (mean±sd, n=4) of the spray distance of 20 mm at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

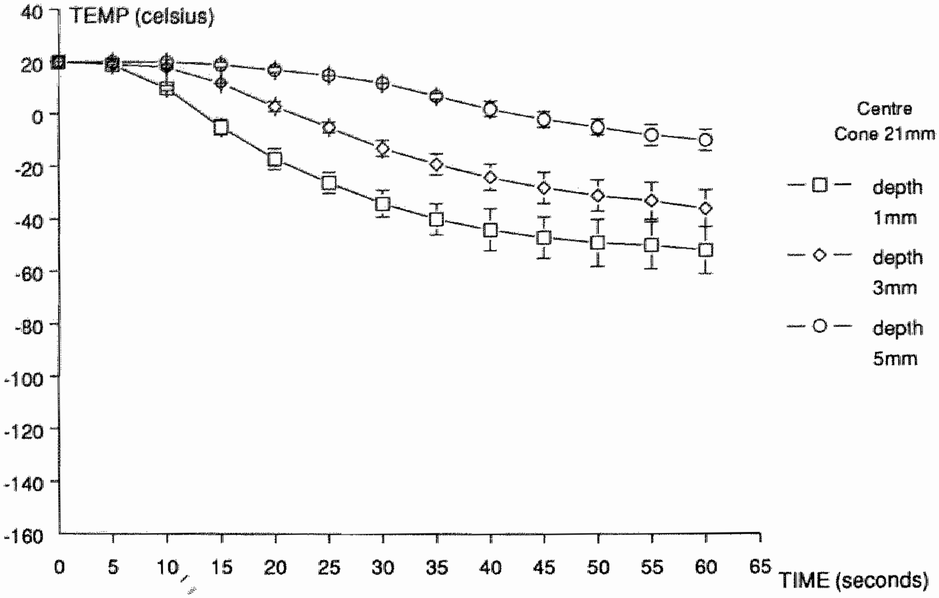


Figure 5.17. Temperature course (mean±sd, n=4) of the spray distance of 5 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

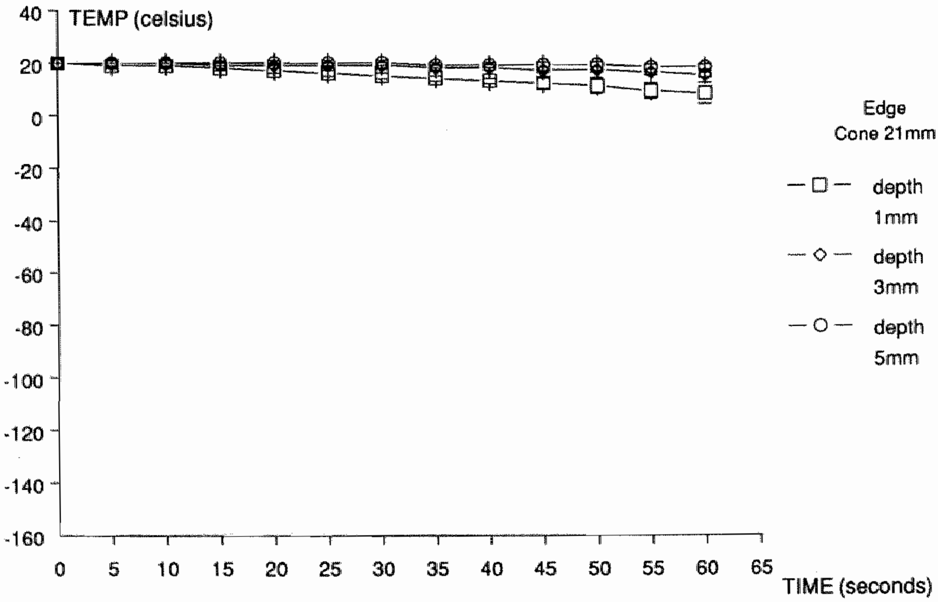


Figure 5.18. Temperature course (mean±sd, n=4) of the spray distance of 5 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm-and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

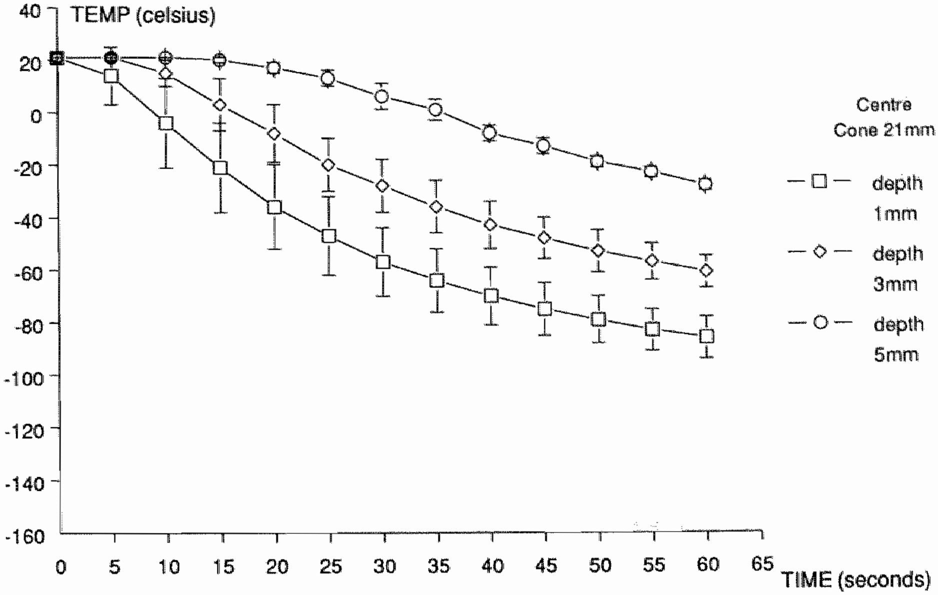


Figure 5.19. Temperature course (mean±sd, n=4) of the spray distance of 10 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

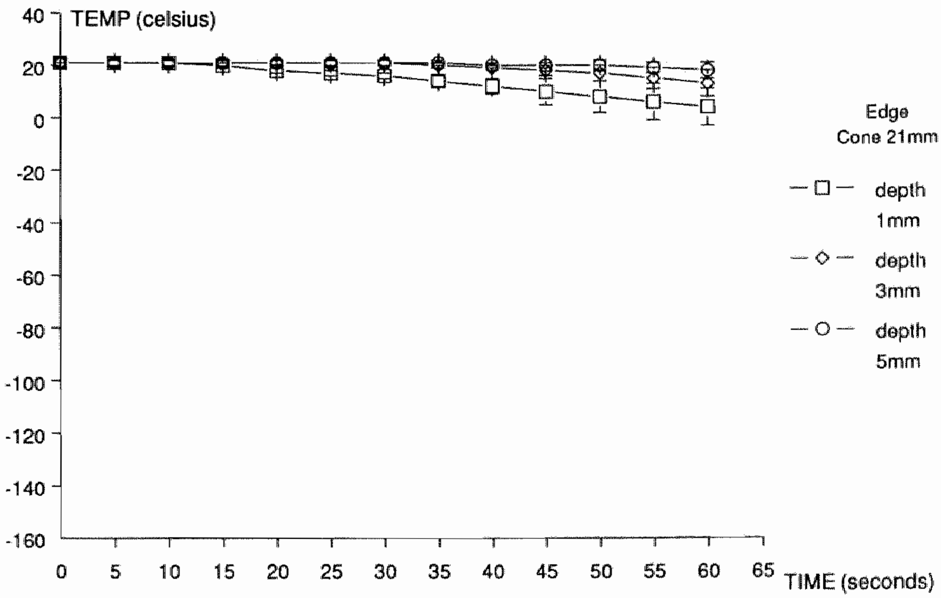


Figure 5.20. Temperature course (mean±sd, n=4) of the spray distance of 10 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

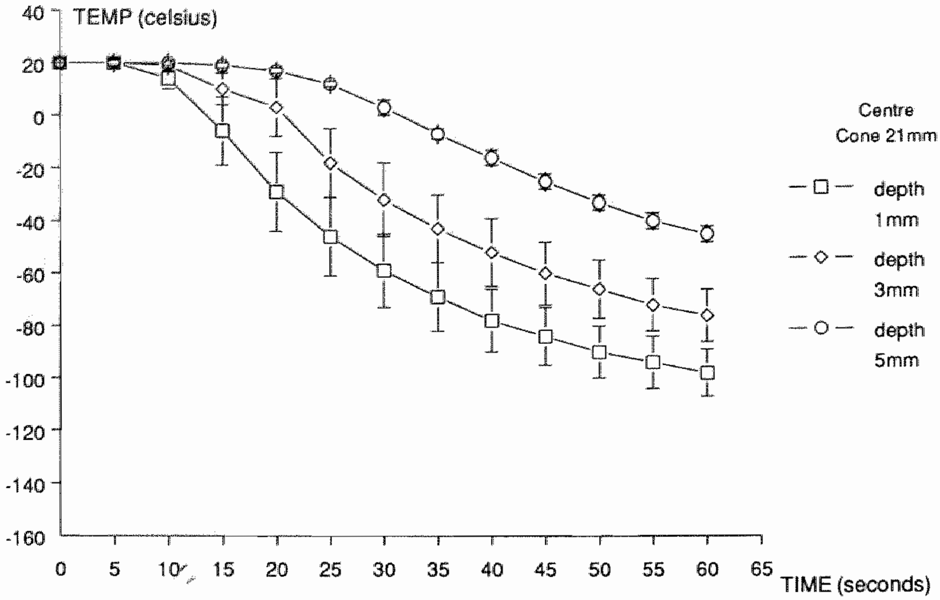


Figure 5.21. Temperature course (mean±sd, n=4) of the spray distance of 15 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

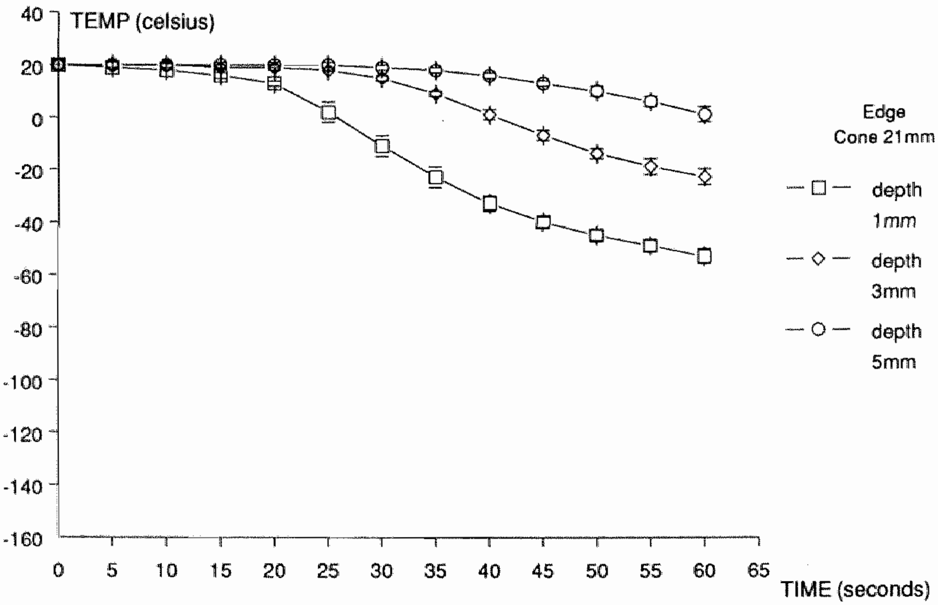


Figure 5.22. Temperature course (mean±sd, n=4) of the spray distance of 15 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

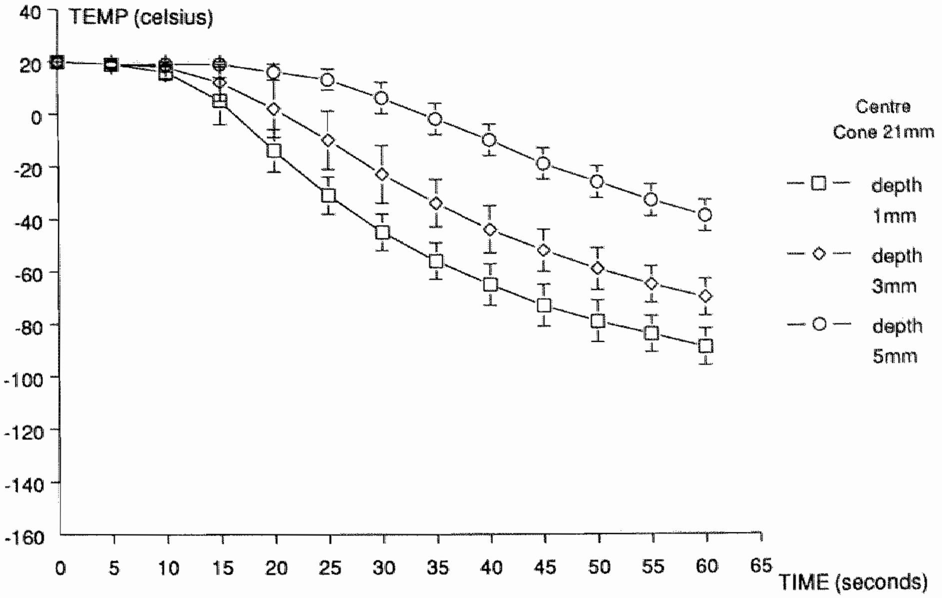


Figure 5.23. Temperature course (mean±sd, n=4) of the spray distance of 20 mm in the centre of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

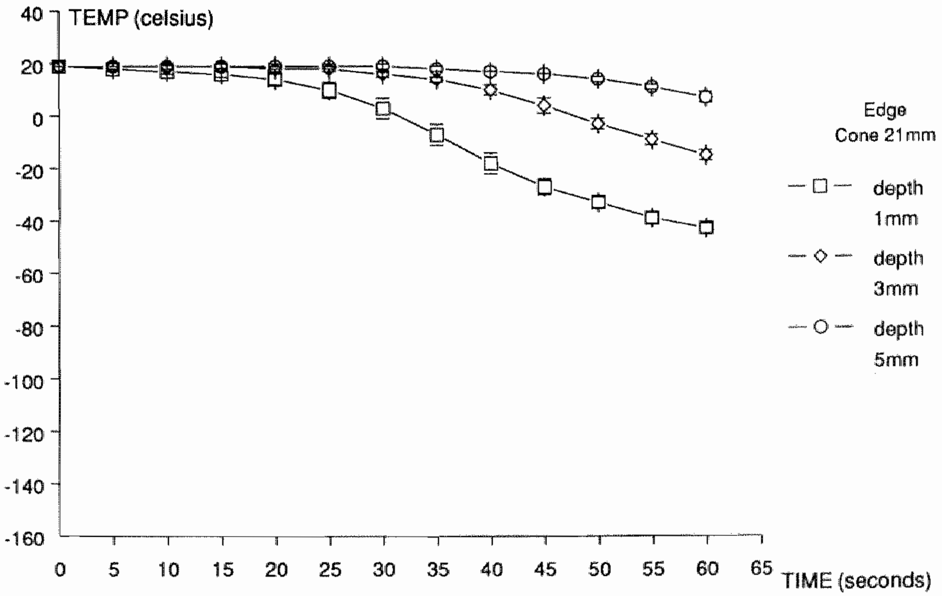


Figure 5.24. Temperature course (mean±sd, n=4) of the spray distance of 20 mm at the edge of the cone diameter of 21 mm at 1 mm-, 3 mm- and 5 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

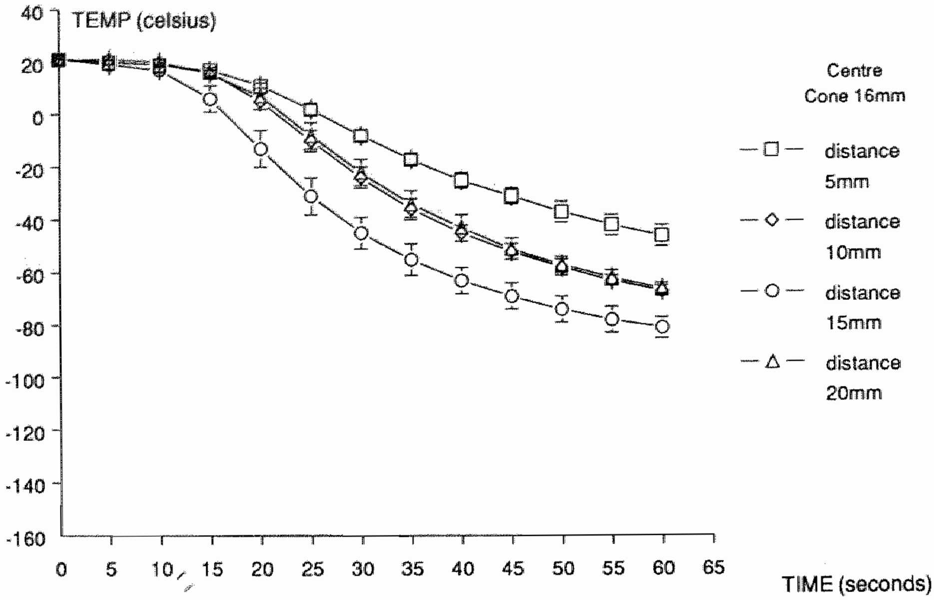


Figure 5.25. Comparison (mean±sd, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

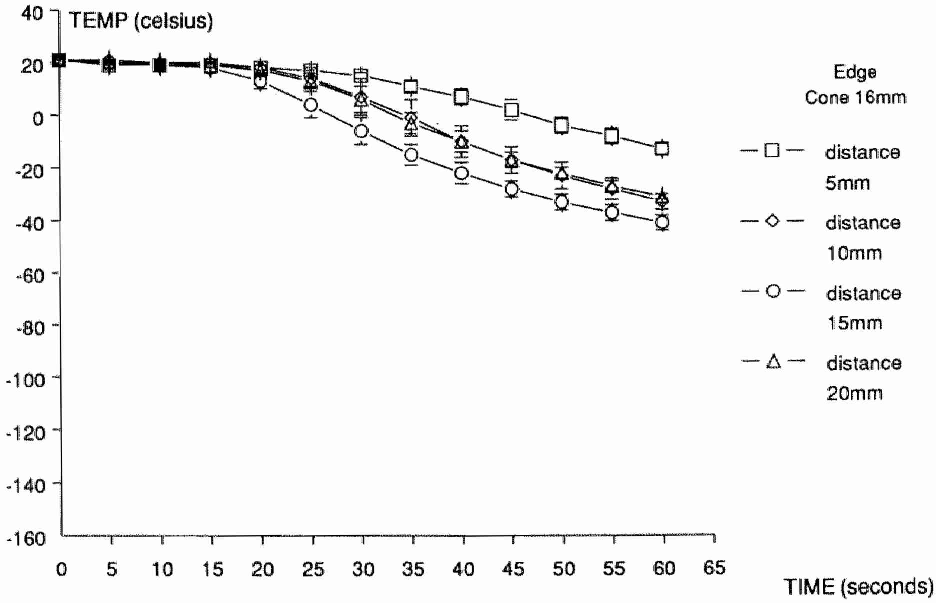


Figure 5.26. Comparison (mean±sd, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

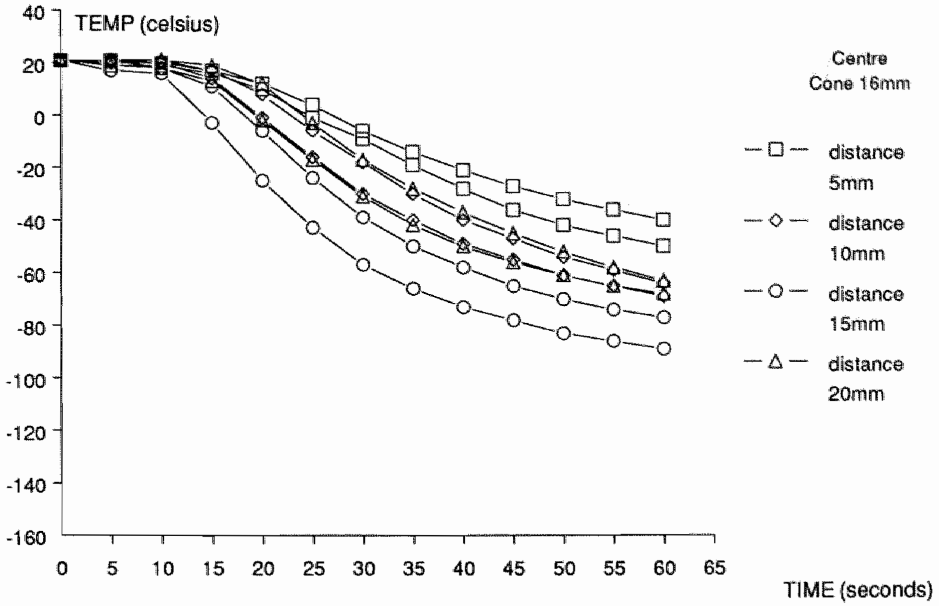


Figure 5.27. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm in the centre of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

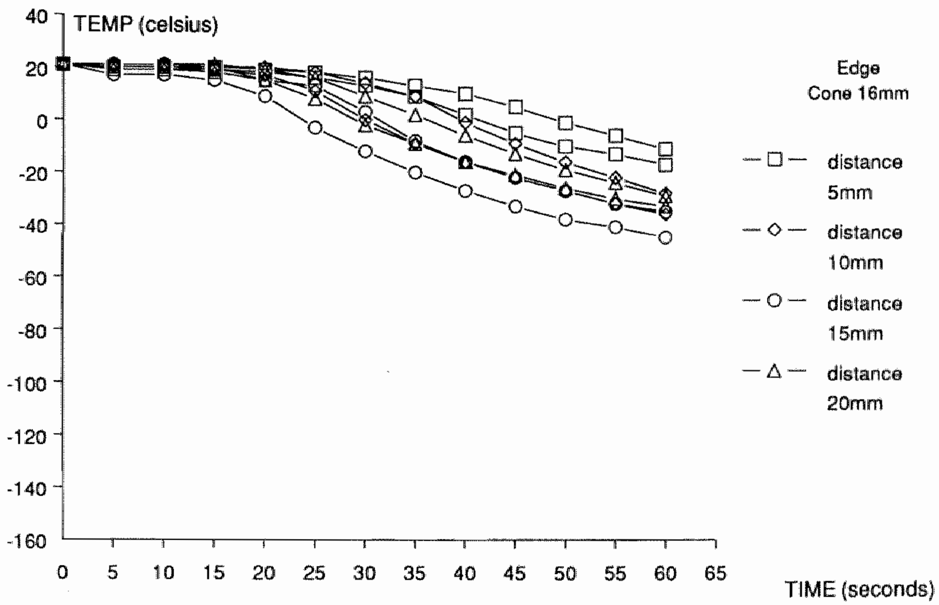


Figure 5.28. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 16 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

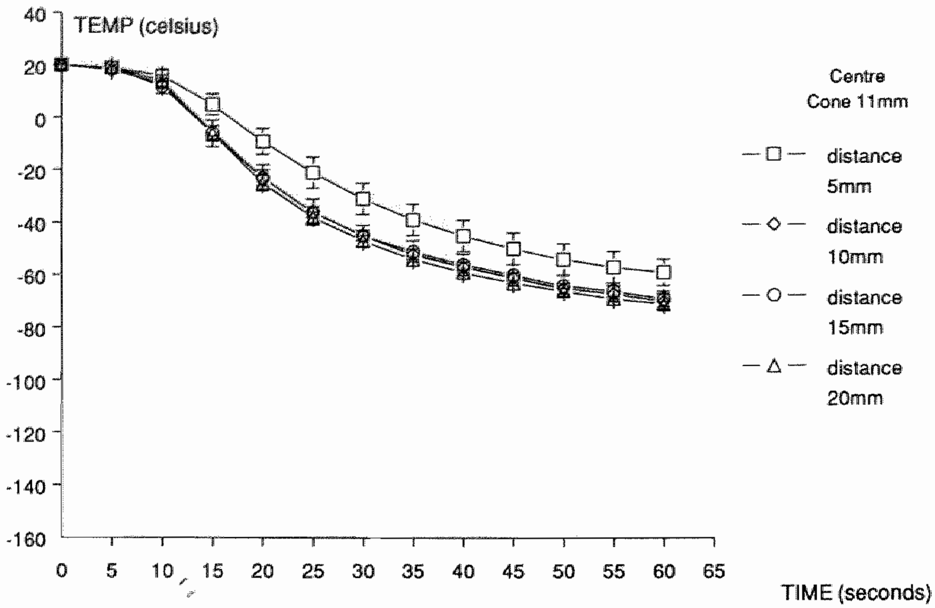


Figure 5.29. Comparison (mean±sd, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

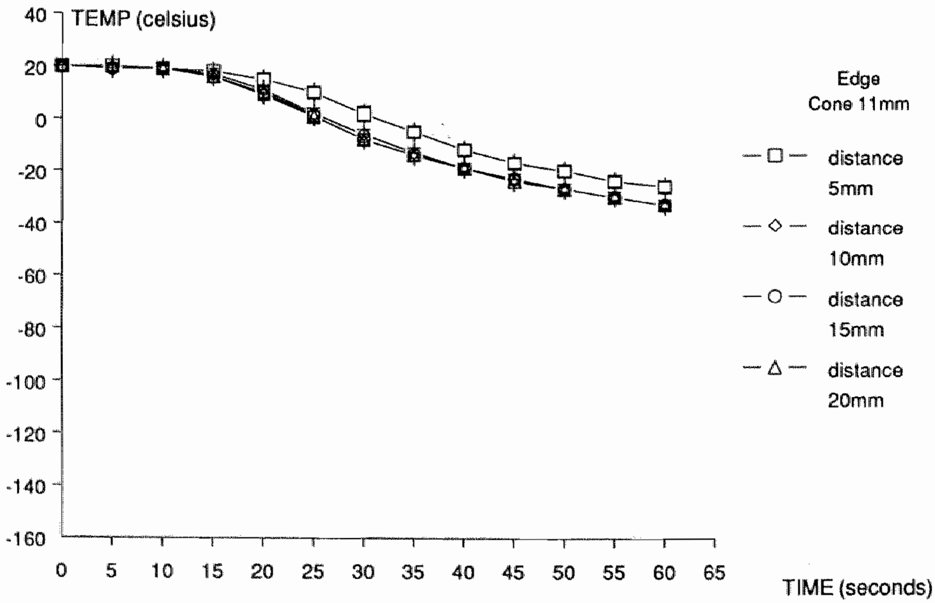


Figure 5.30. Comparison (mean±sd, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

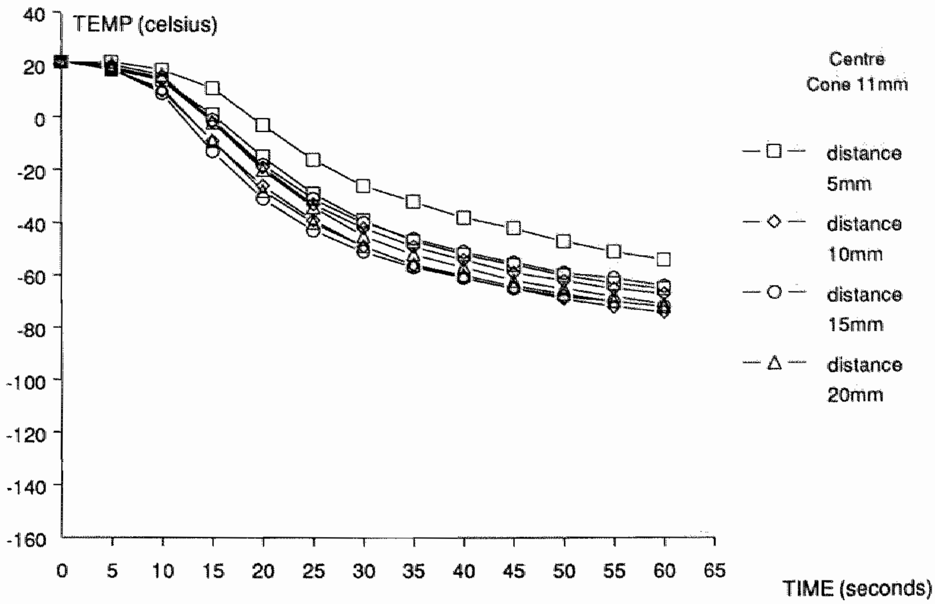


Figure 5.31. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm in the centre of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

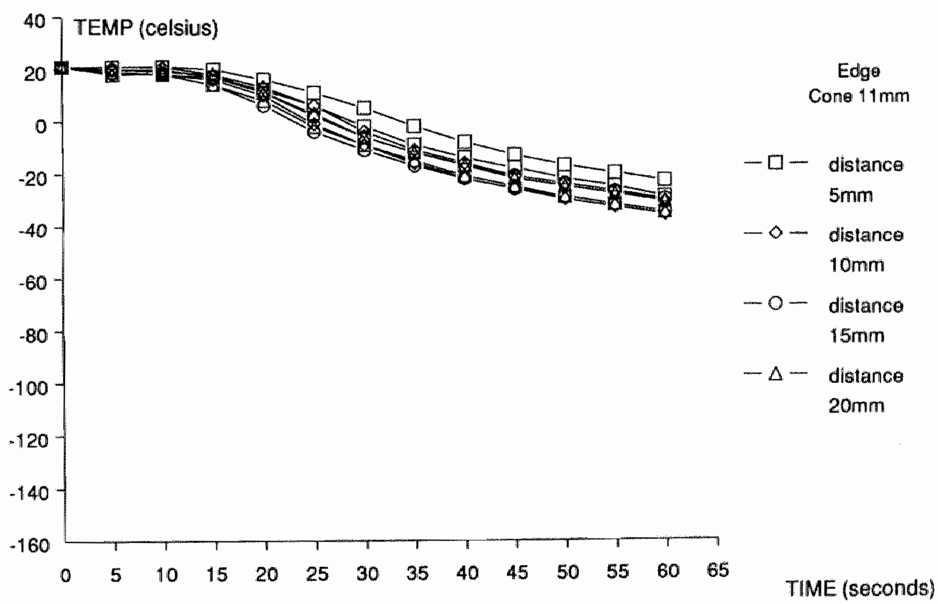
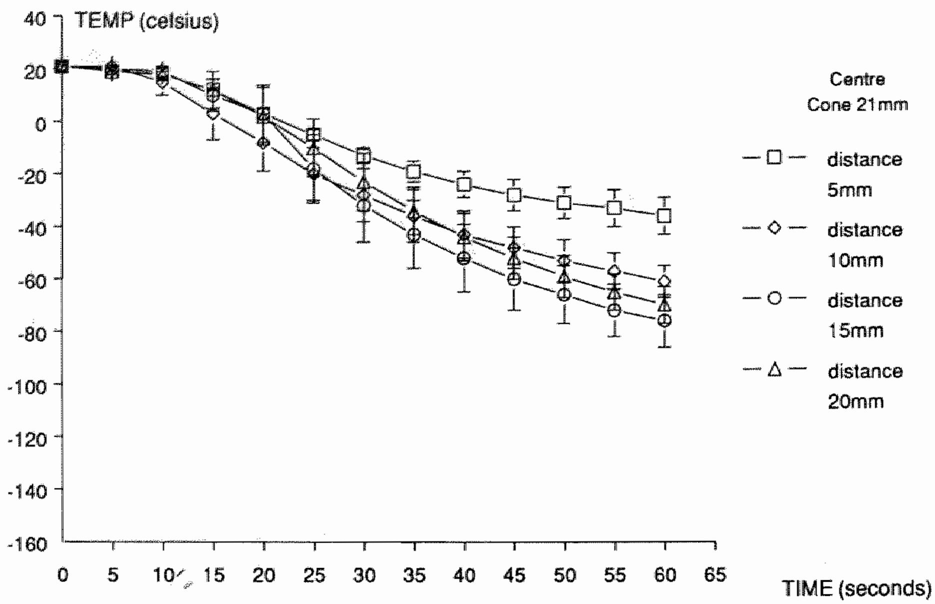


Figure 5.32. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 11 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).



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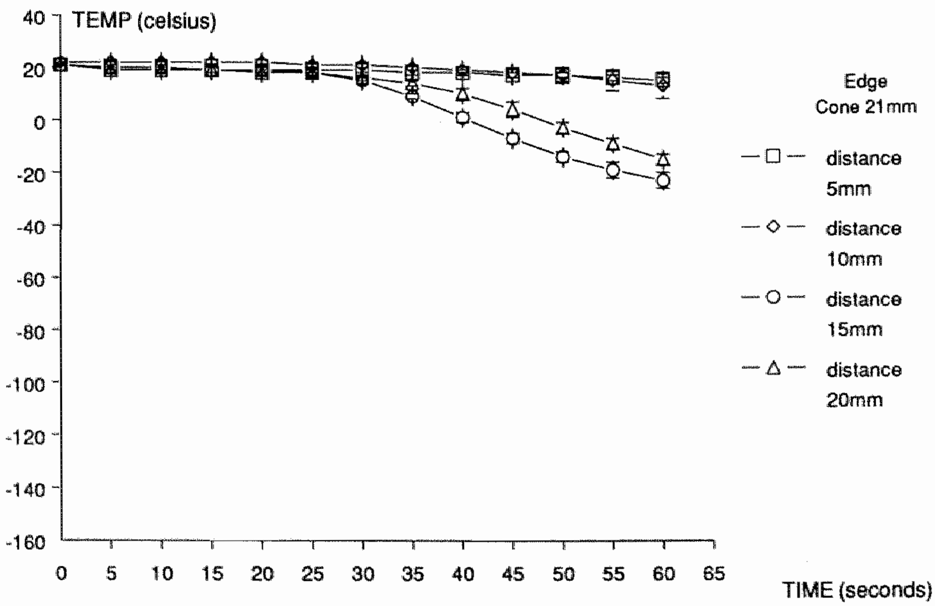

Figure 5.34. Comparison (mean±sd, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

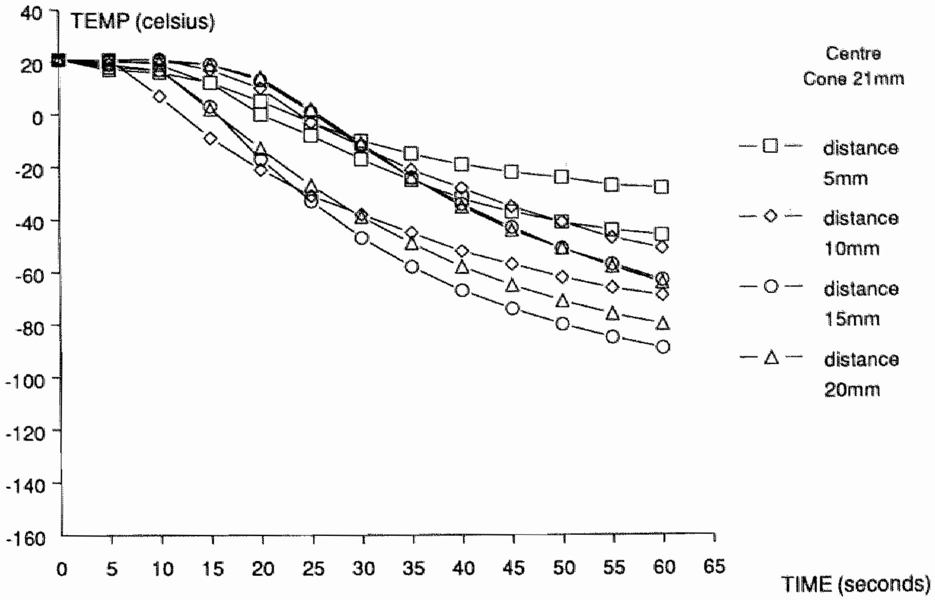

Figure 5.35. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm in the centre of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

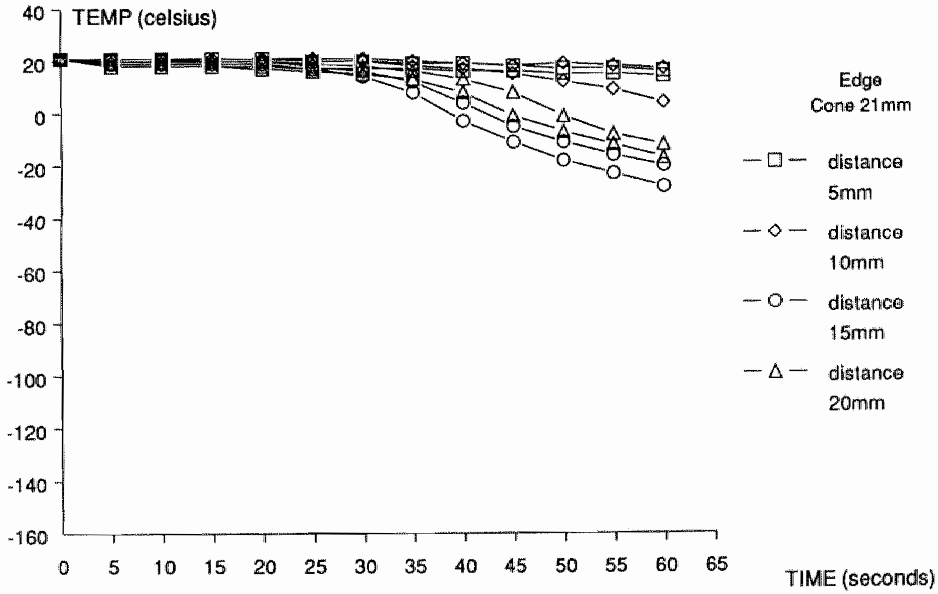

Figure 5.36. Comparison (intervals of minimum and maximum values, n=4) of the spray distances of 5 mm, 10 mm, 15 mm and 20 mm at the edge of the cone diameter of 21 mm at 3 mm-depth (continuous central spraying, using a spraytip of 0.8 mm).

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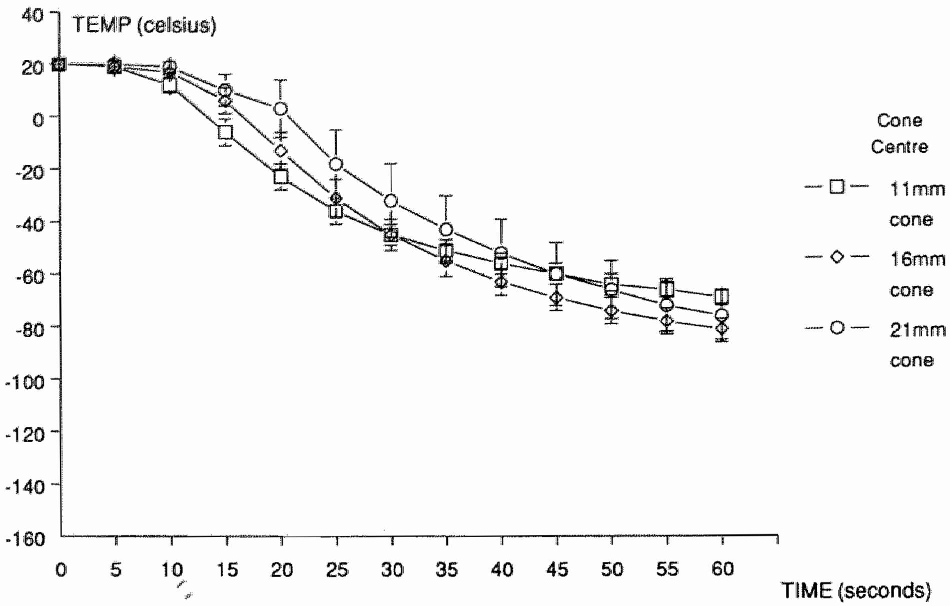

Figure 6.1. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

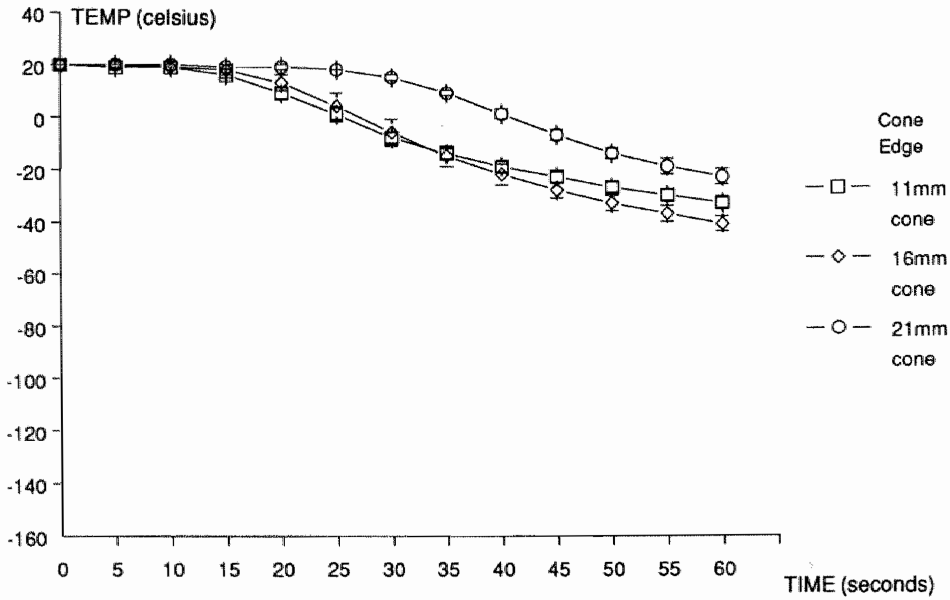

Figure 6.2. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

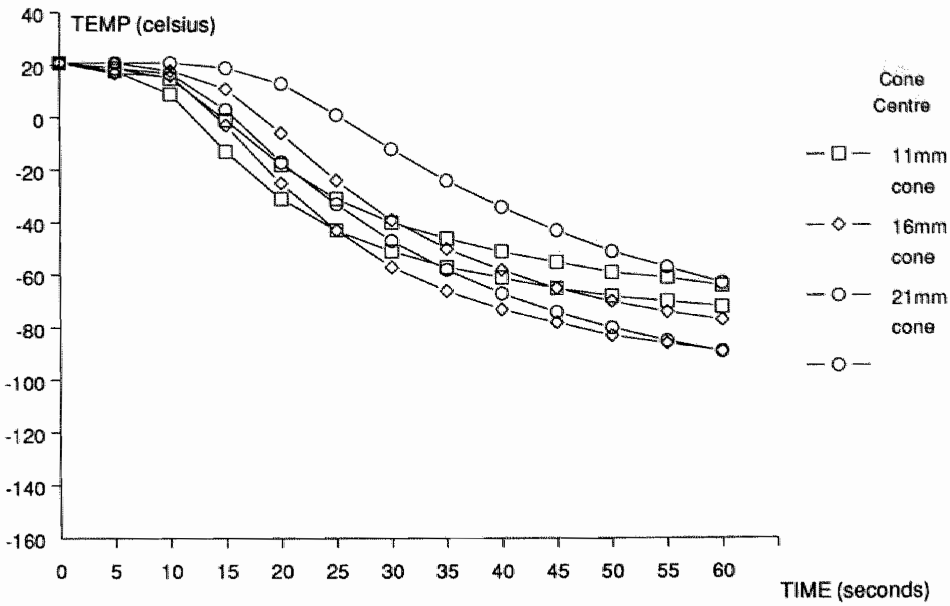

Figure 6.3. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

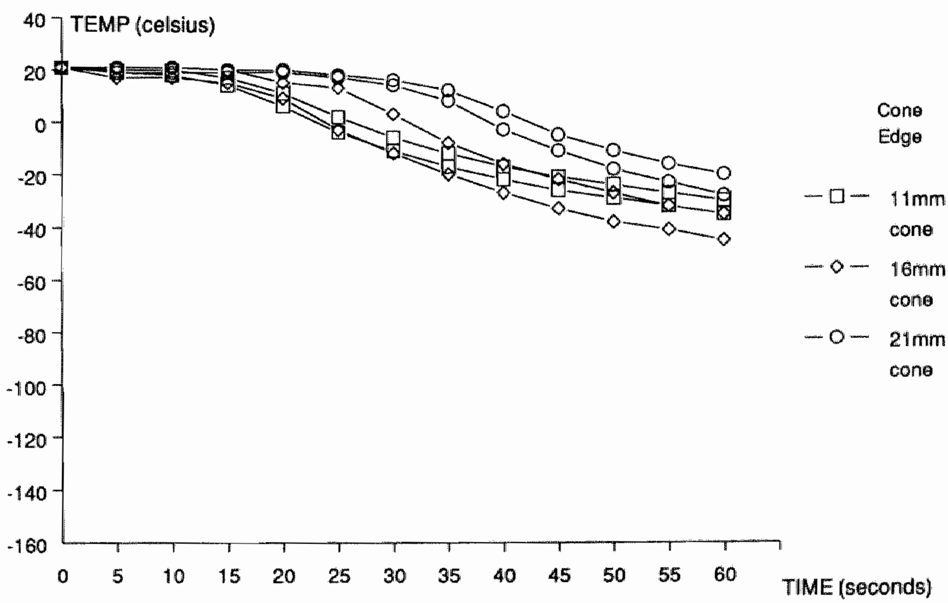

Figure 6.4. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm-depth (continuous spraying, using a spraytip of 0.8 mm from a distance of 15 mm).

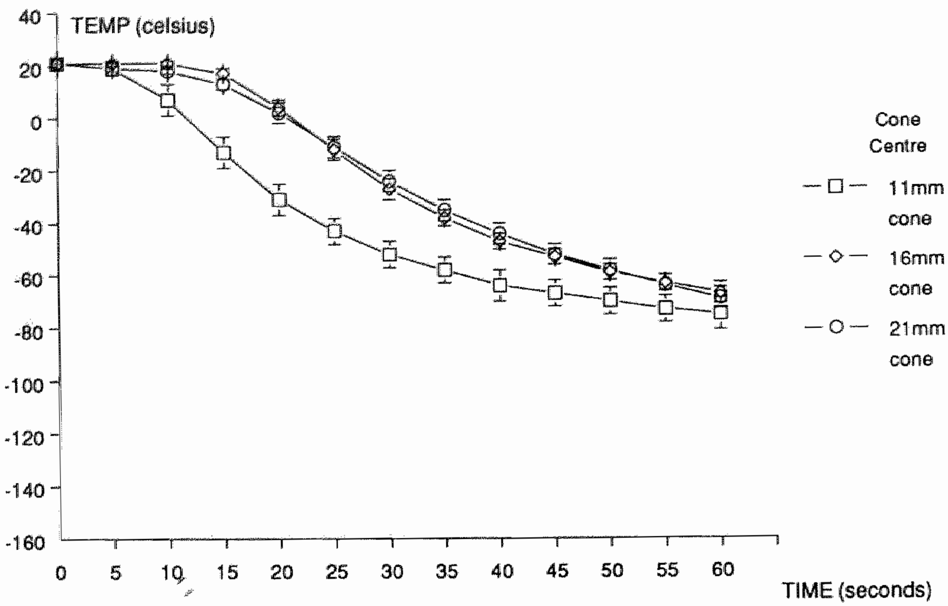

Figure 6.5. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.0 mm from a distance of 15 mm).

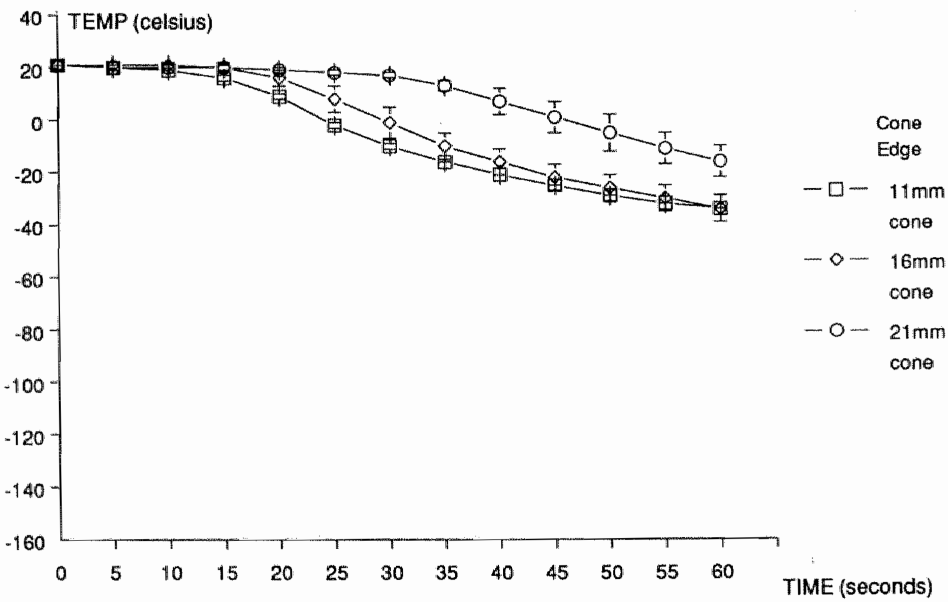

Figure 6.6. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.0 mm from a distance of 15 mm).

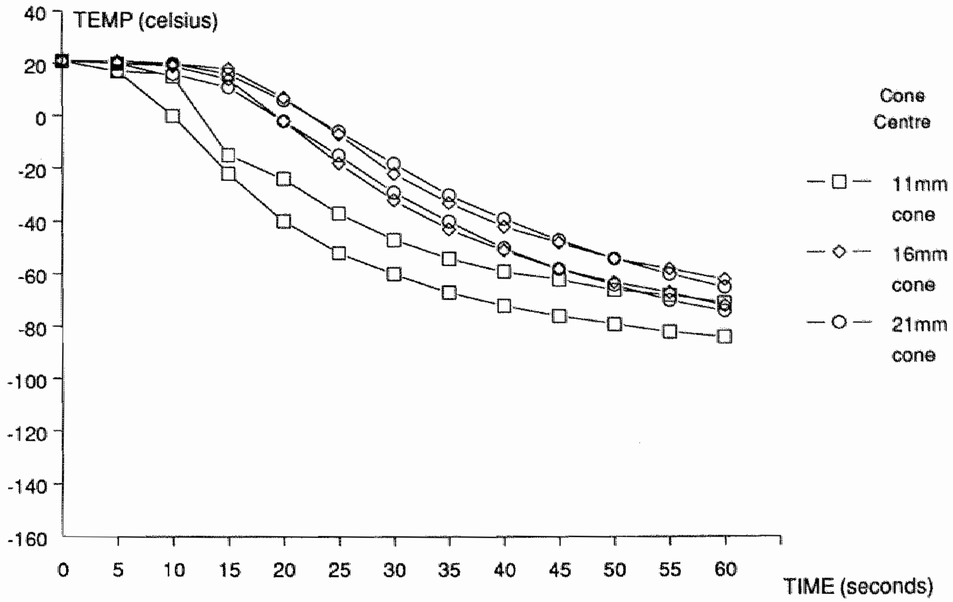

Figure 6.7. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.0 mm from a distance of 15 mm).

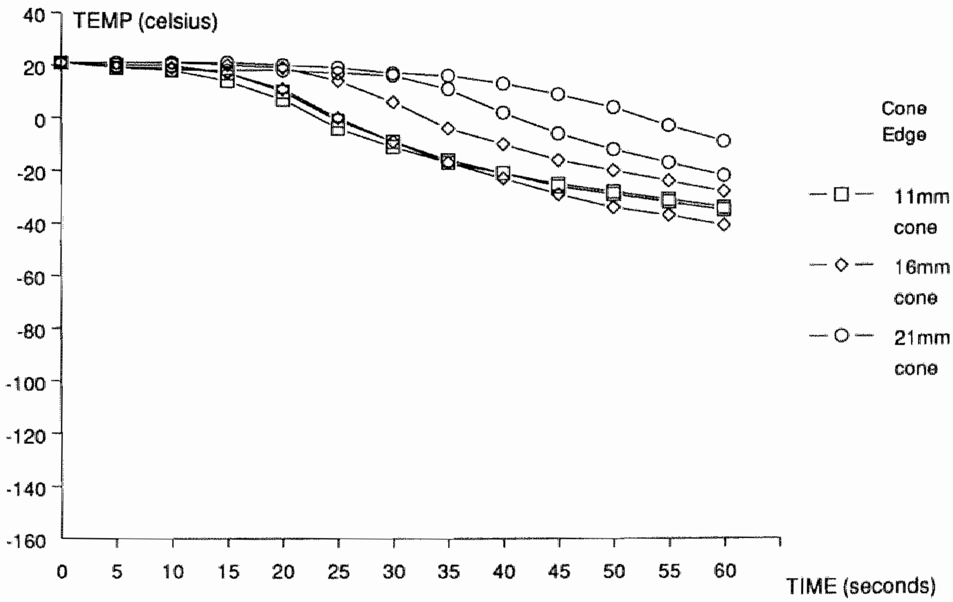

Figure 6.8. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.0 mm from a distance of 15 mm).

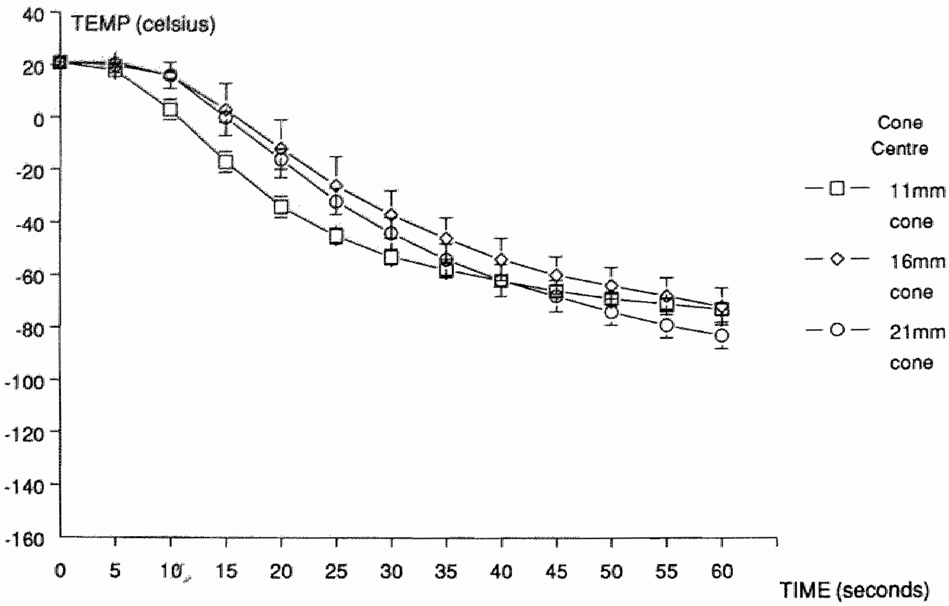

Figure 6.9. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.7 mm from a distance of 15 mm).

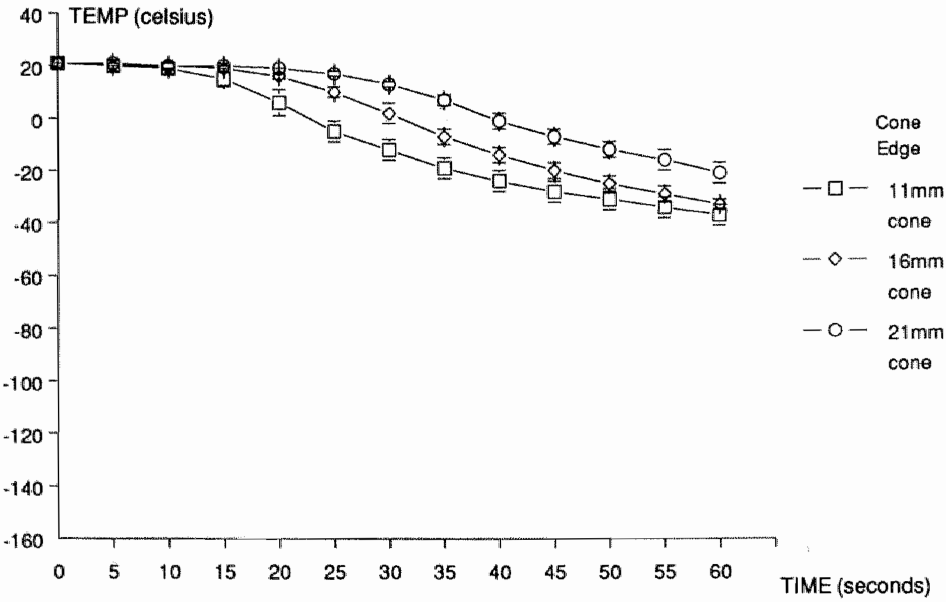

Figure 6.10. Comparison (mean±sd, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm depth (continuous spraying, using a spraytip of 1.7 mm from a distance of 15 mm).

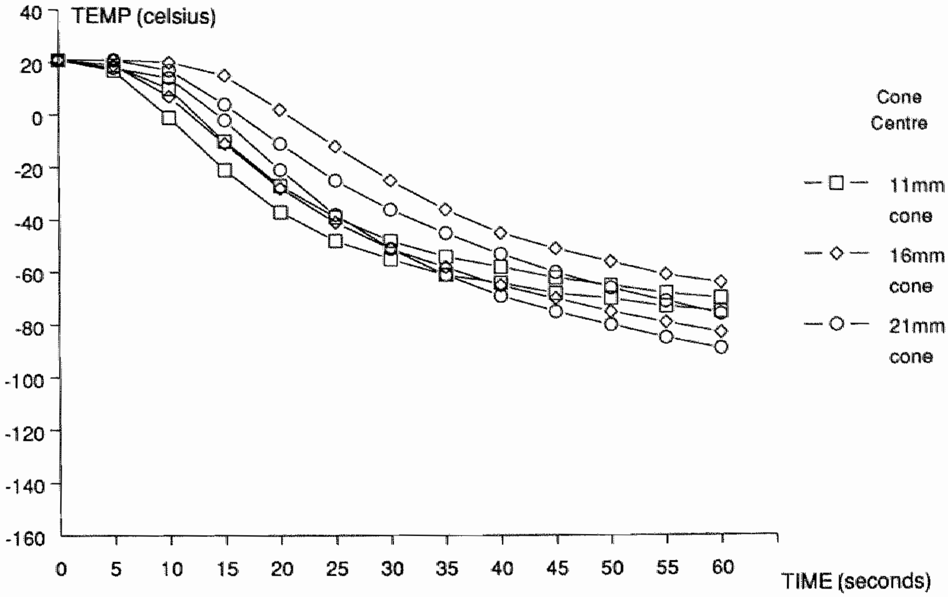

Figure 6.11. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm in the centre of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.7 mm from a distance of 15 mm).

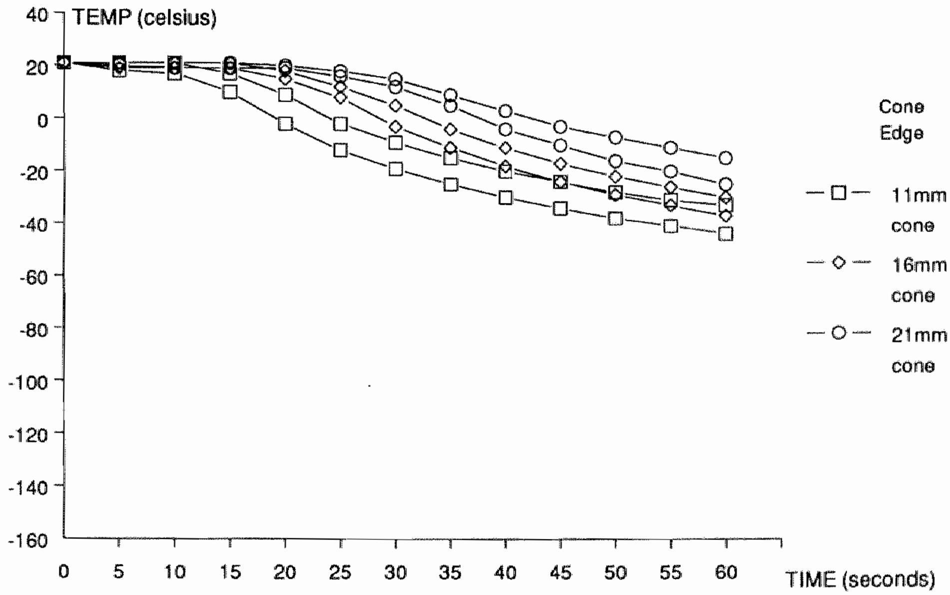

Figure 6.12. Comparison (intervals of minimum and maximum values, n=4) of the cone diameters of 11 mm, 16 mm and 21 mm at the edge of the cones at 3 mm-depth (continuous spraying, using a spraytip of 1.7 mm from a distance of 15 mm).

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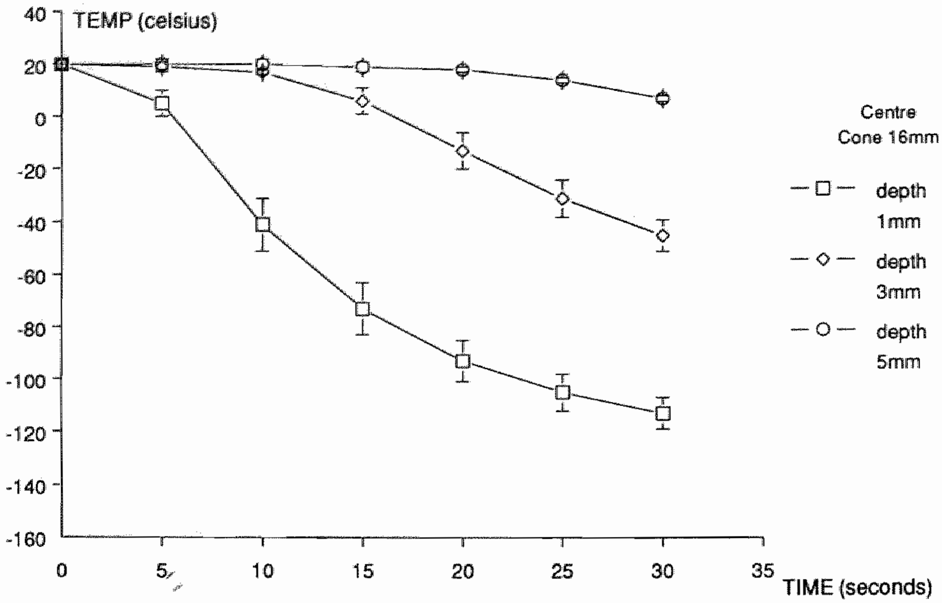

Figure 7.1. Temperature course (mean±sd, n=4) of continuous spraying in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

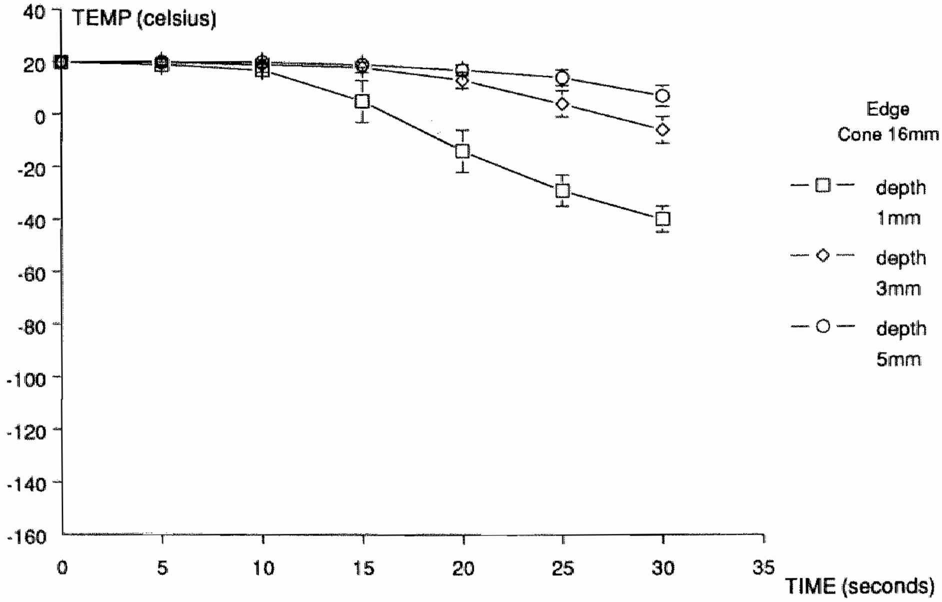

Figure 7.2. Temperature course (mean±sd, n=4) of continuous spraying at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

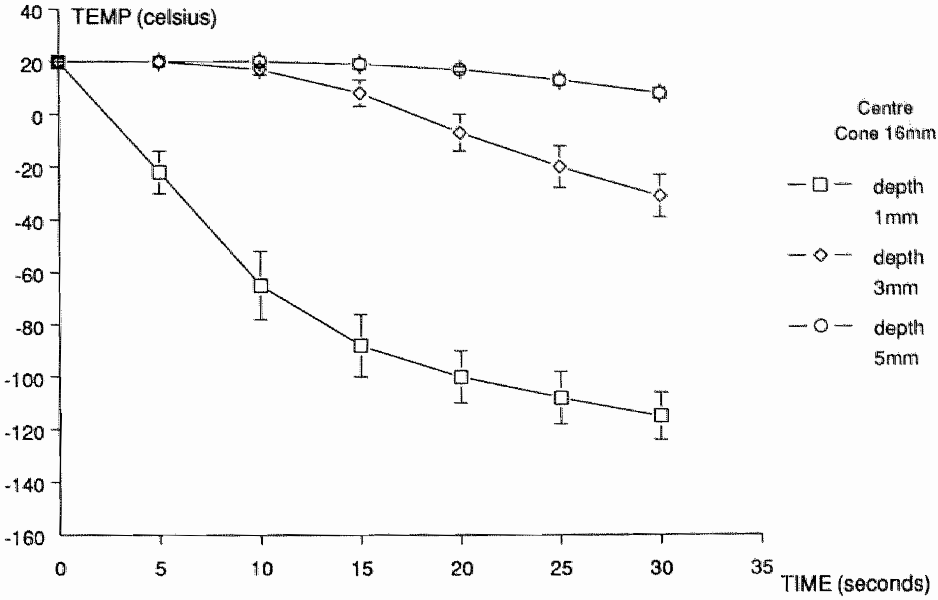

Figure 7.3. Temperature course (mean±sd, n=4) of intermittent spraying of once a second in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

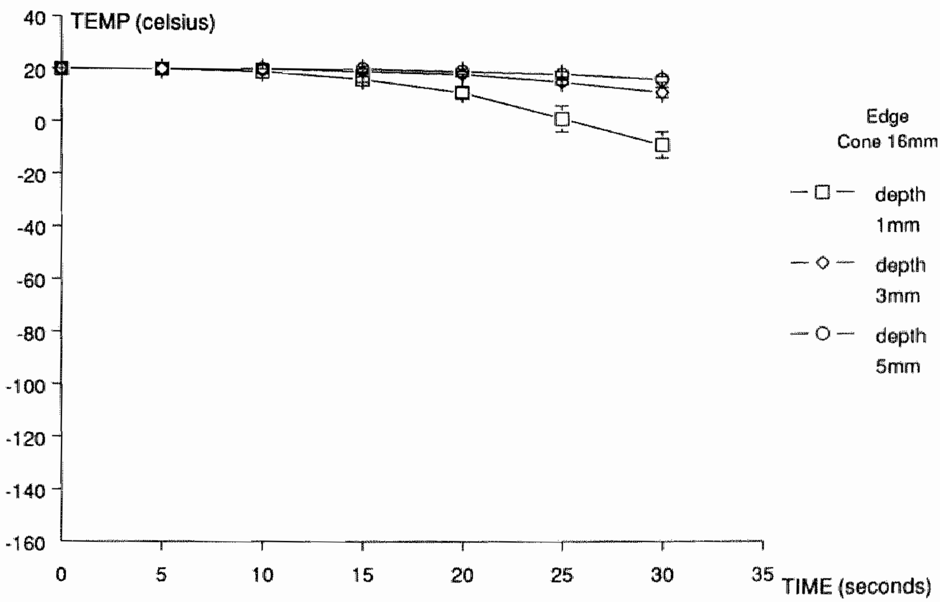

Figure 7.4. Temperature course (mean±sd, n=4) of intermittent spraying of once a second at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

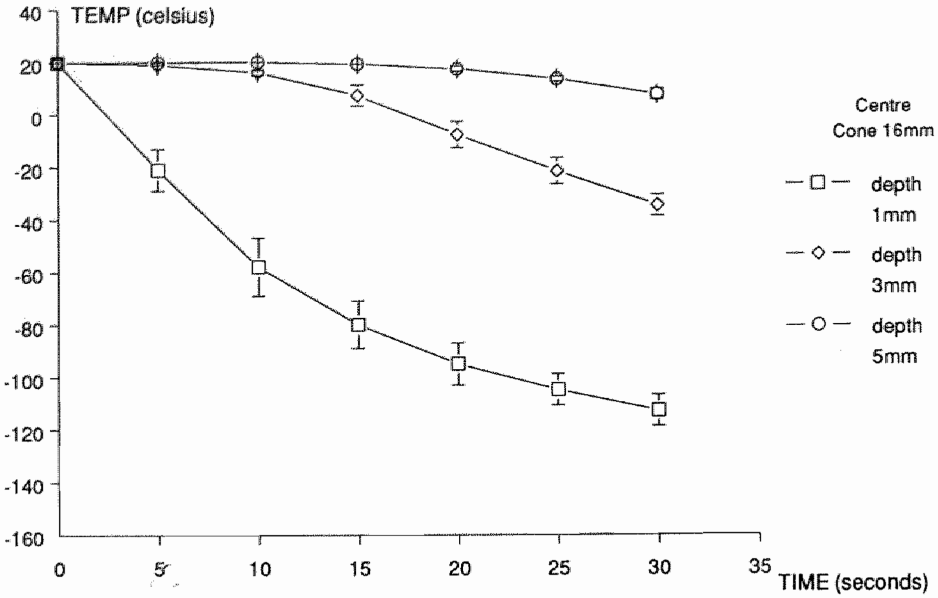

Figure 7.5. Temperature course (mean±sd, n=4) of continuous spraying over 5 seconds, combined with intermittent spraying of once a second in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

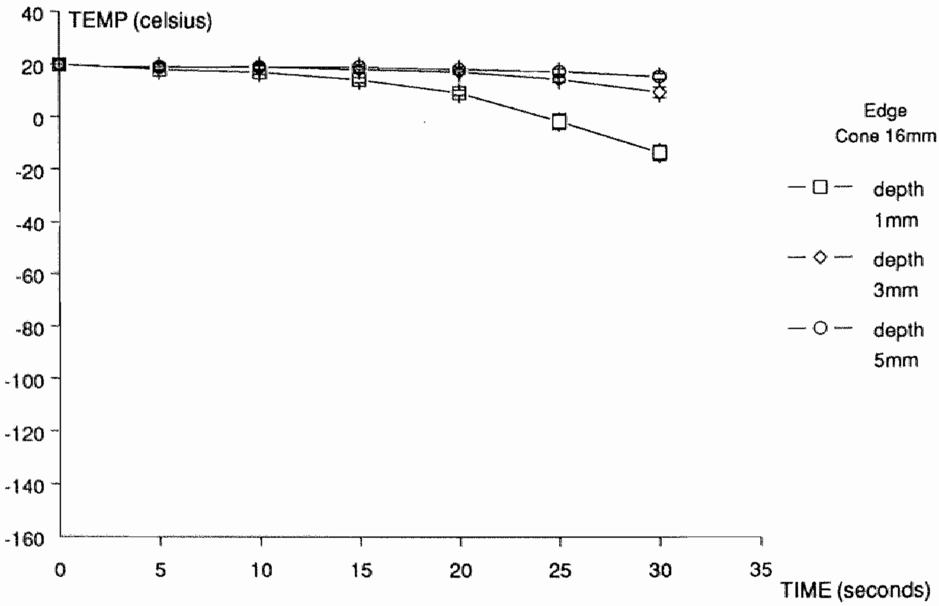

Figure 7.6. Temperature course (mean±sd, n=4) of continuous spraying over 5 seconds, combined with intermittent spraying of once a second at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

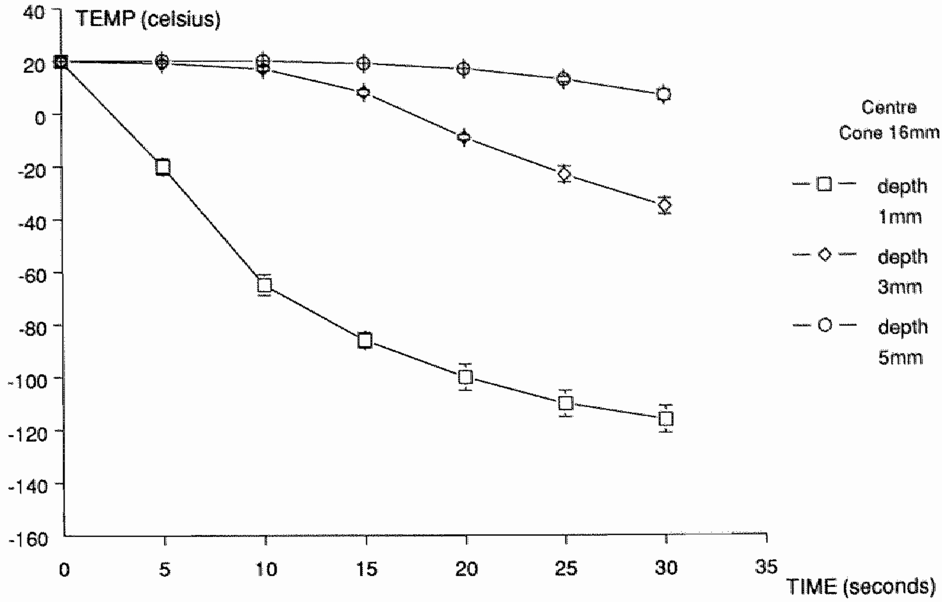

Figure 7.7. Temperature course (mean±sd, n=4) of continuous spraying over 10 seconds, combined with intermittent spraying of once a second in the centre of the cone diameter of 16 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

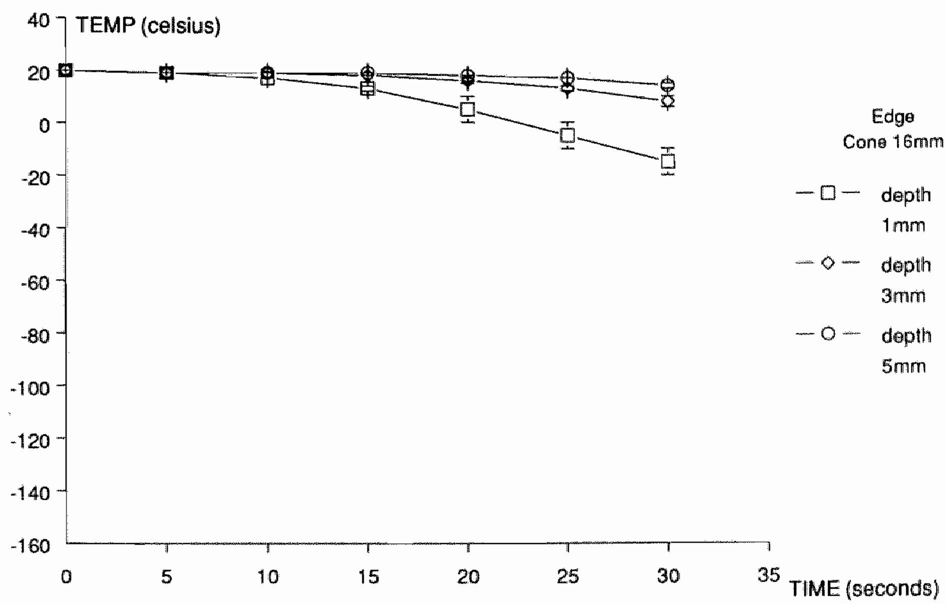

Figure 7.8. Temperature course (mean±sd, n=4) of continuous spraying over 10 seconds, combined with intermittent spraying of once a second at the edge of the cone diameter of 16 mm at 1 mm-, 3 mm-and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

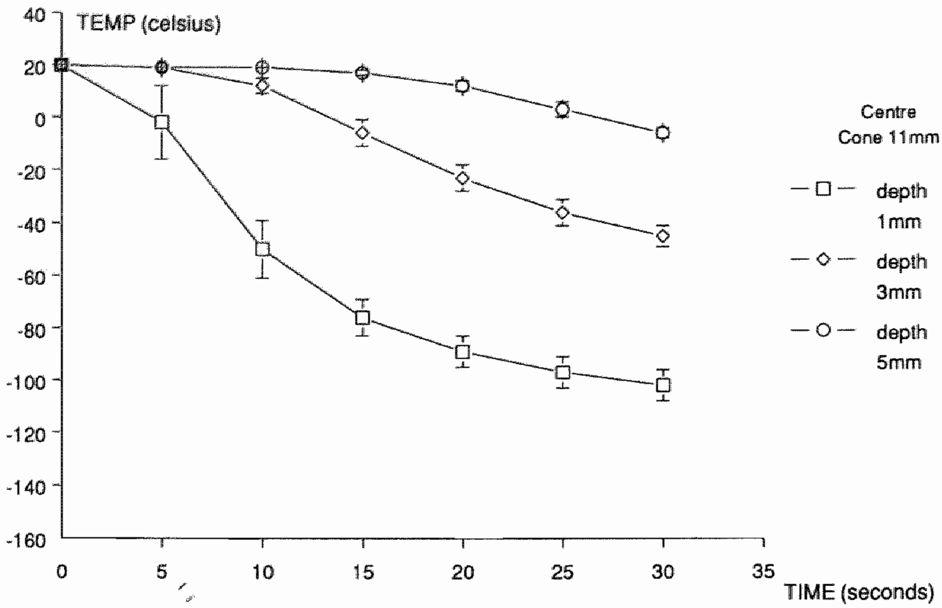

Figure 7.9. Temperature course (mean±sd, n=4) of continuous spraying in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

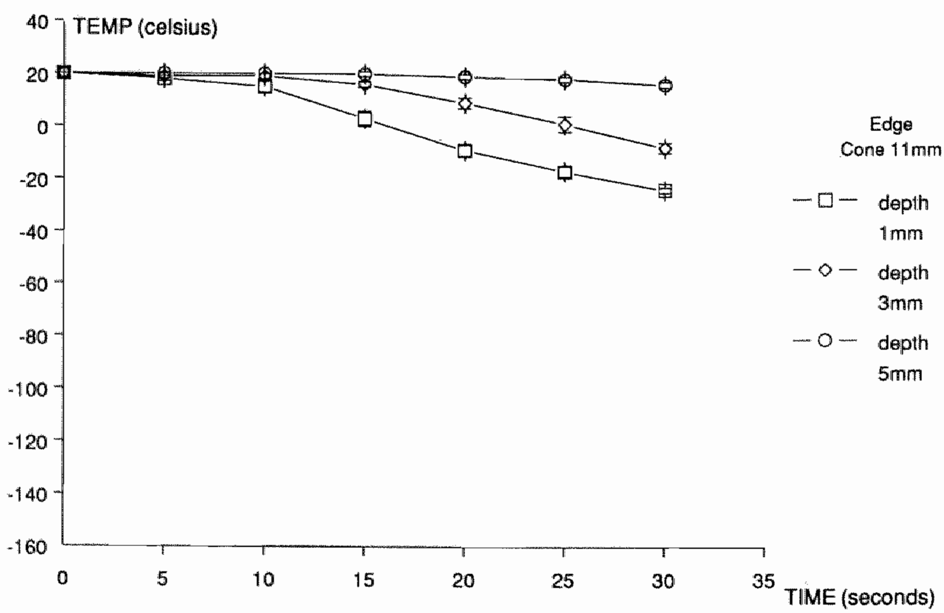

Figure 7.10. Temperature course (mean±sd, n=4) of continuous spraying at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

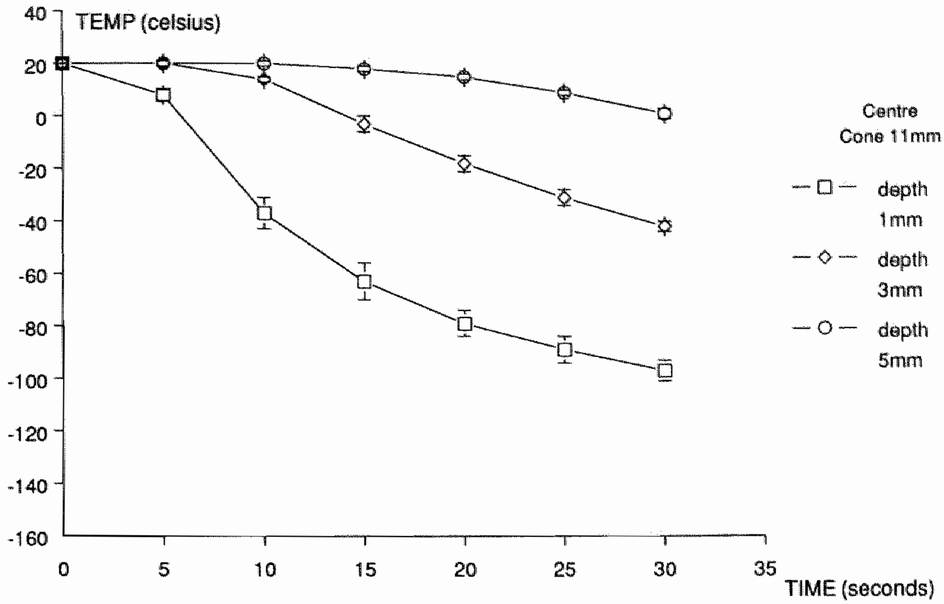

Figure 7.11. Temperature course (mean±sd, n=4) of intermittent spraying of once a second in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

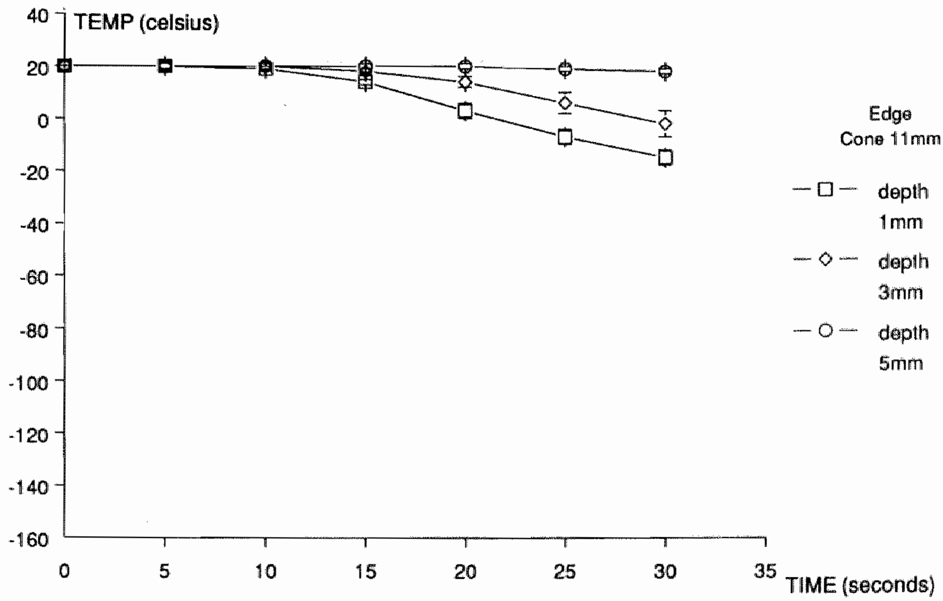

Figure 7.12. Temperature course (mean±sd, n=4) of intermittent spraying of once a second at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

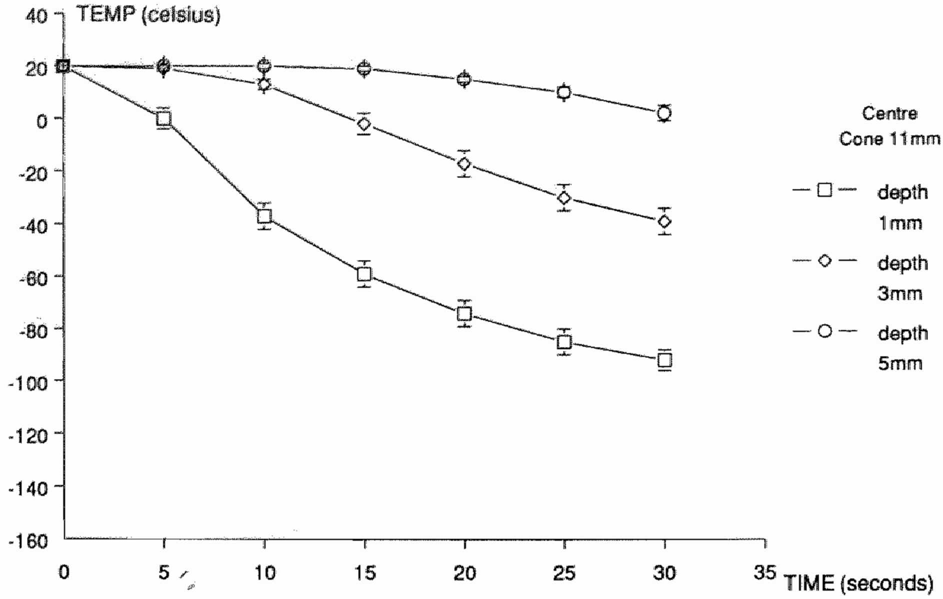

Figure 7.13. Temperature course (mean±sd, n=4) of continuous spraying over 5 seconds, combined with intermittent spraying of once a second in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

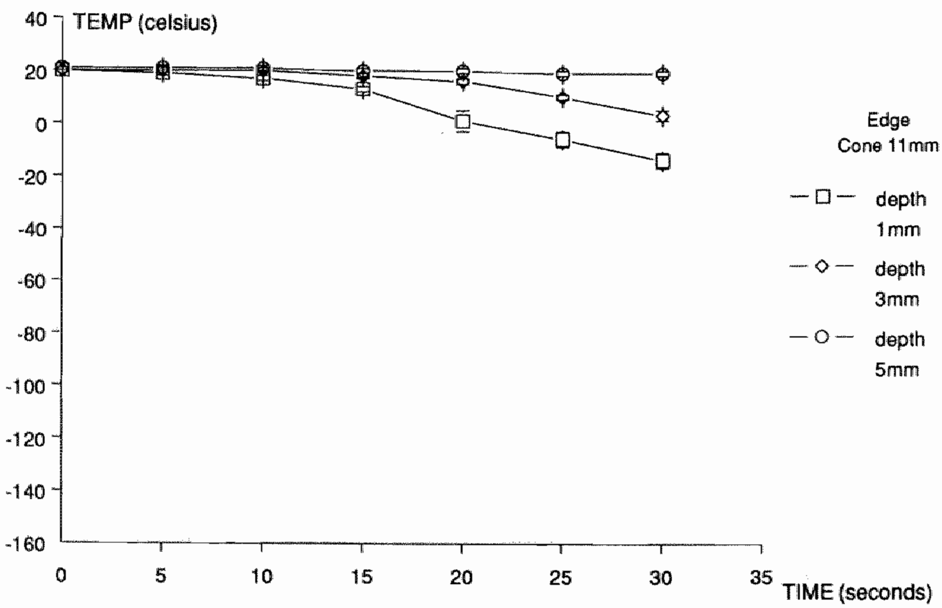

Figure 7.14. Temperature course (mean±sd, n=4) of continuous spraying over 5 seconds, combined with intermittent spraying of once a second at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm-and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

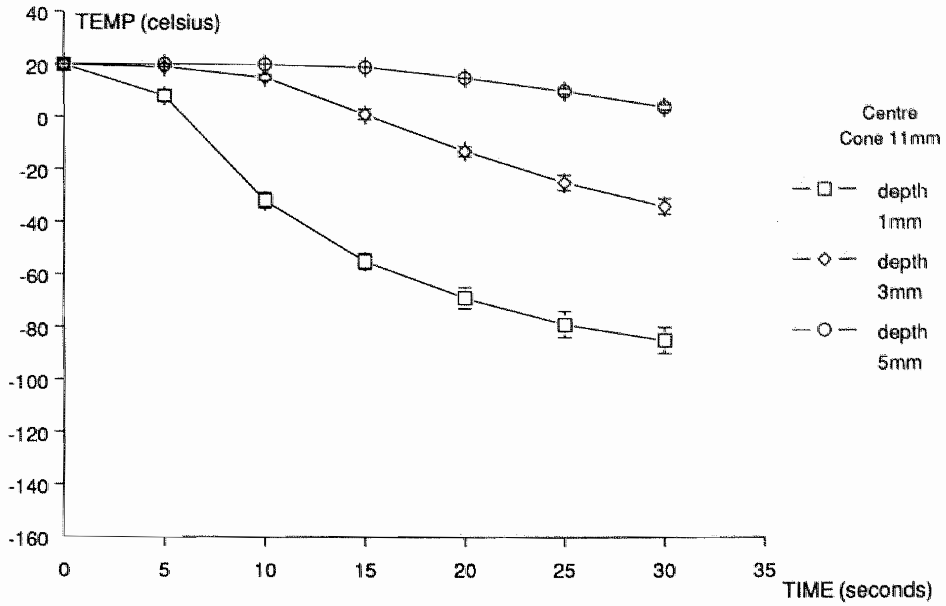

Figure 7.15. Temperature course (mean±sd, n=4) of continuous spraying over 10 seconds, combined with intermittent spraying of once a second in the centre of the cone diameter of 11 mm at 1 mm-, 3 mm- and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

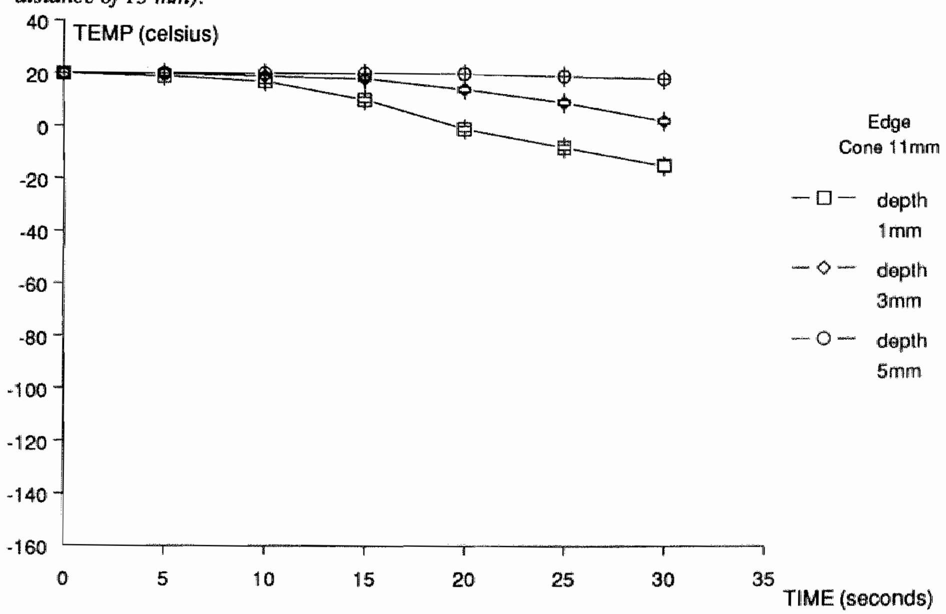

Figure 7.16. Temperature course (mean±sd, n=4) of continuous spraying over 10 seconds, combined with intermittent spraying of once a second at the edge of the cone diameter of 11 mm at 1 mm-, 3 mm-and 5 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

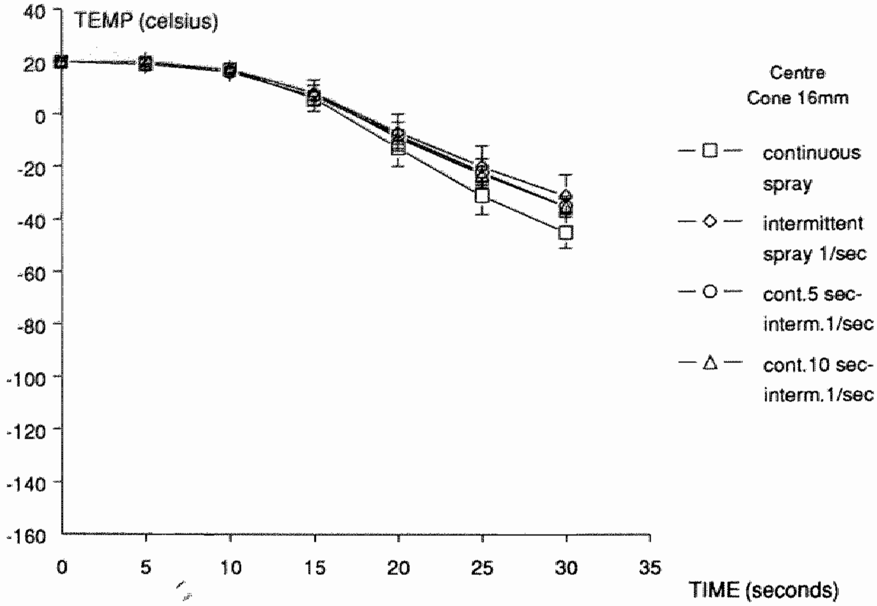

Figure 7.17. Comparison (mean±sd, n=4) of the four spraying techniques in the centre of the cone diameter of 16 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

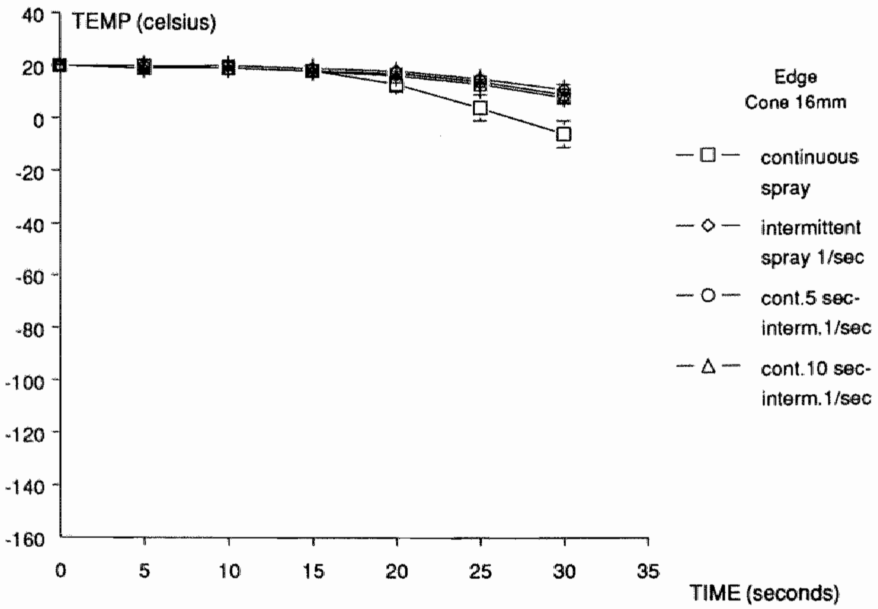

Figure 7.18. Comparison (mean±sd, n=4) of the four spraying techniques at the edge of the cone diameter of 16 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

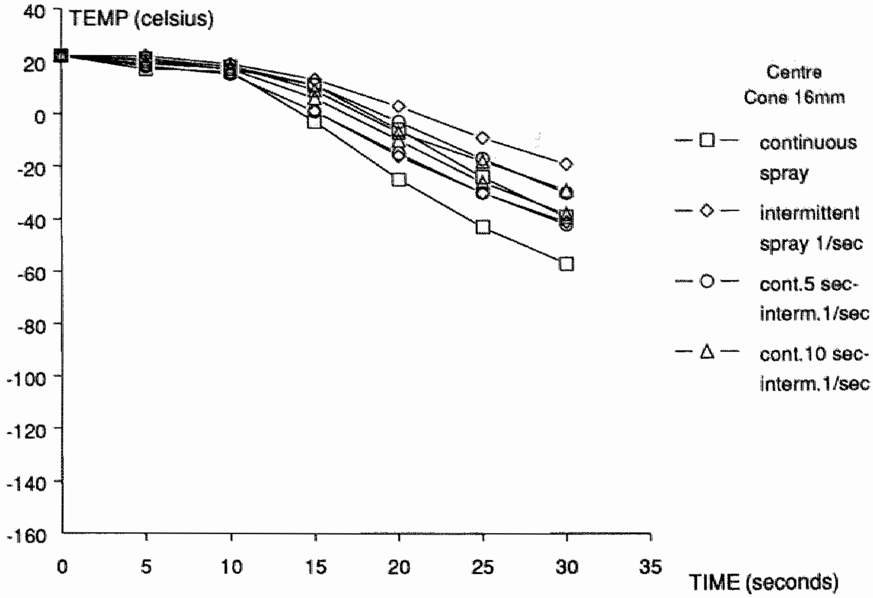

Figure 7.19. Comparison (intervals of minimum and maximum values, n=4) of the four spraying techniques in the centre of the cone diameter of 16 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

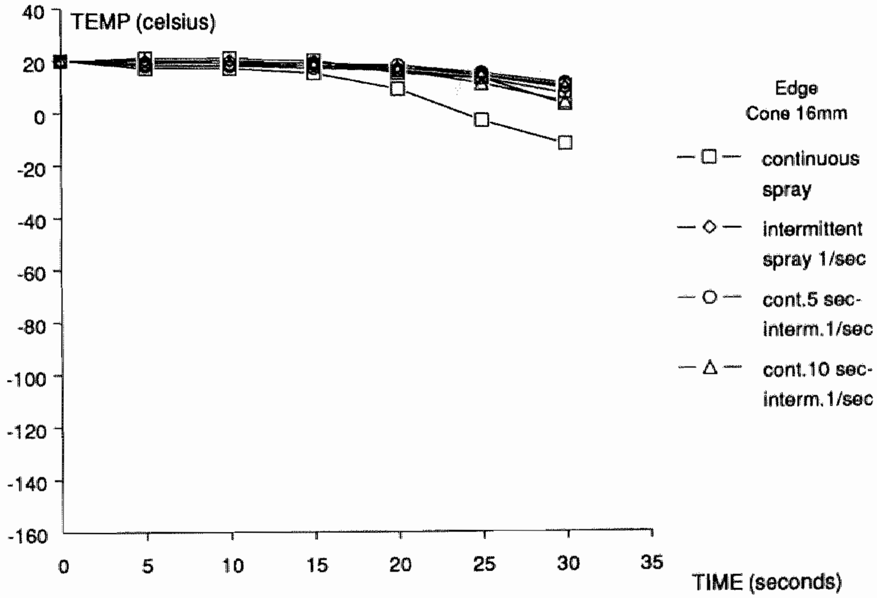

Figure 7.20. Comparison (intervals of minimum and maximum values, n=4) of the four spraying techniques at the edge of the cone diameter of 16 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

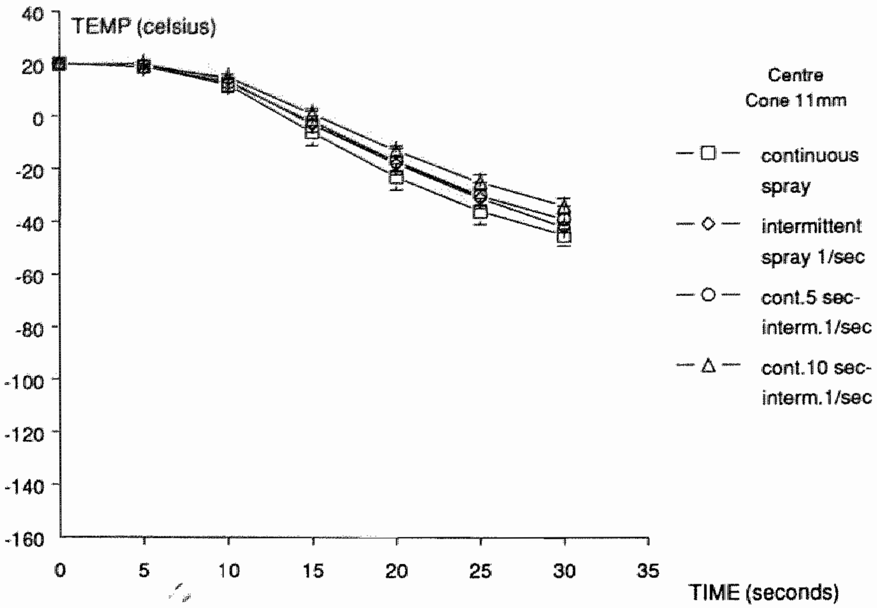

Figure 7.21. Comparison (mean±sd, n=4) of the four spraying techniques in the centre of the cone diameter of 11 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

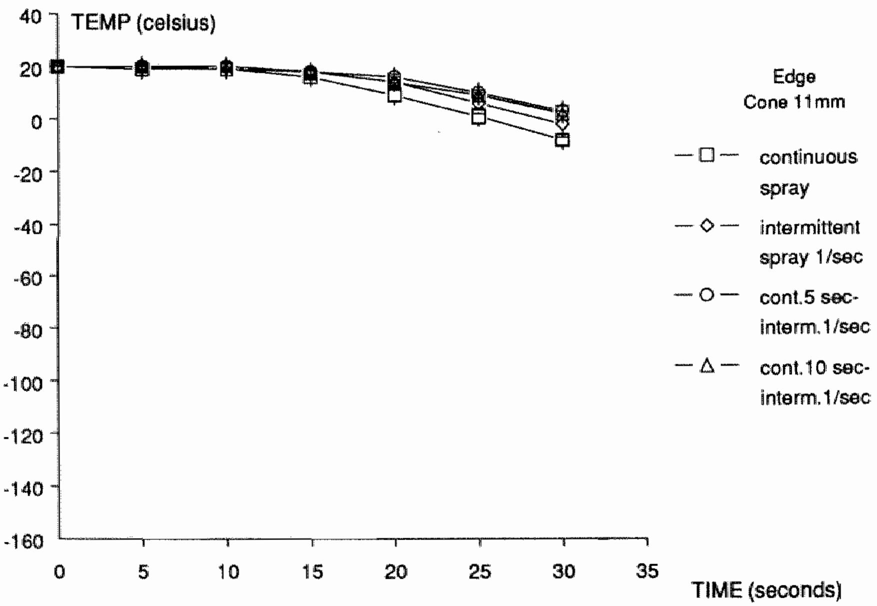

Figure 7.22. Comparison (mean±sd, n=4) of the four spraying techniques at the edge of the cone diameter of 11 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

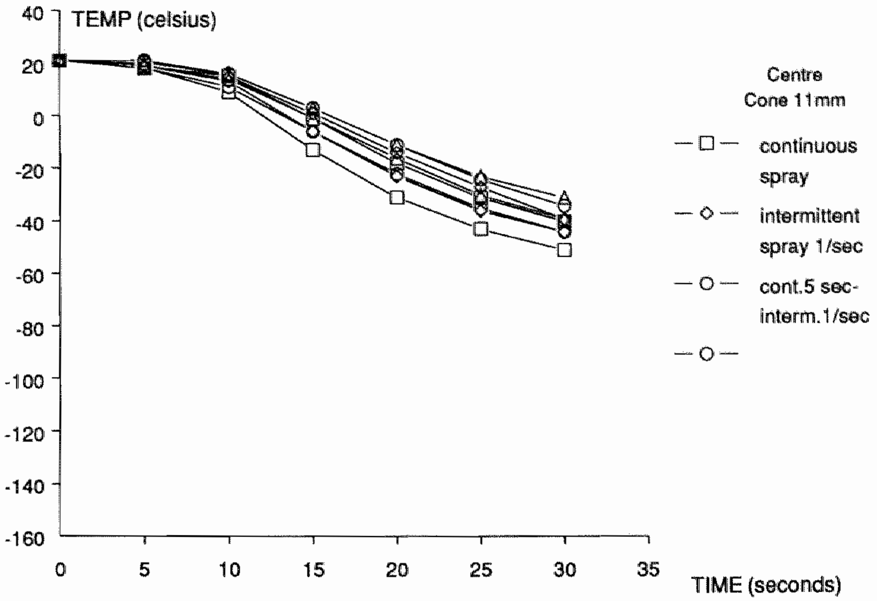

Figure 7.23. Comparison (intervals of minimum and maximum values, n=4) of the four spraying techniques in the centre of the cone diameter of 11 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

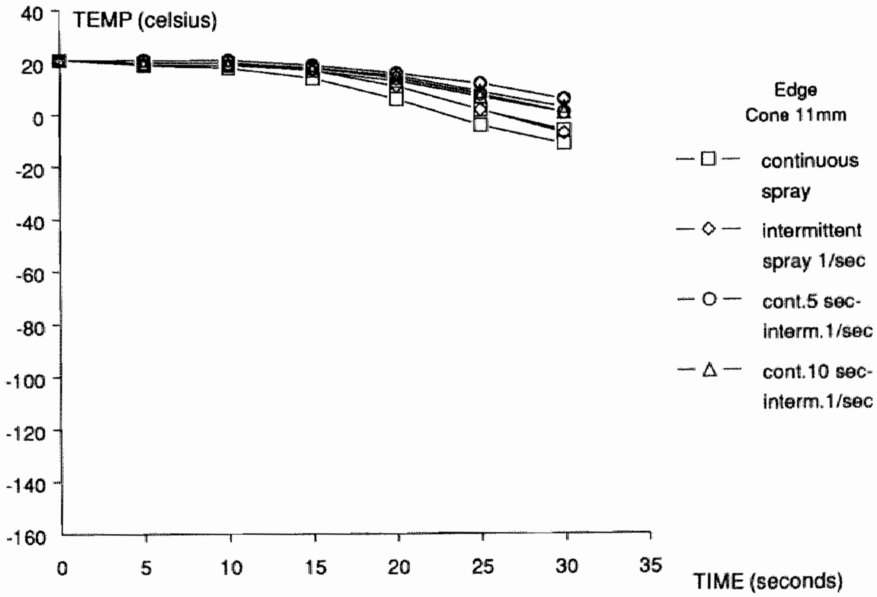

Figure 7.24. Comparison (intervals of minimum and maximum values, n=4) of the four spraying techniques at the edge of the cone diameter of 11 mm at 3 mm-depth (central spray pattern, using a spraytip of 0.8 mm from a distance of 15 mm).

Figures of chapter 8

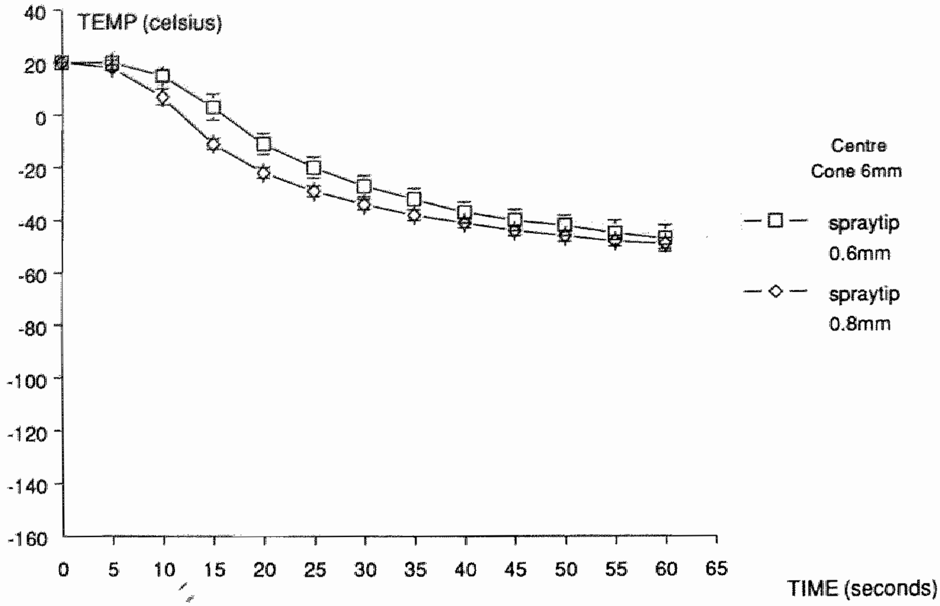

Figure 8.1. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm and 0.8 mm in the centre of the cone diameter of 6 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

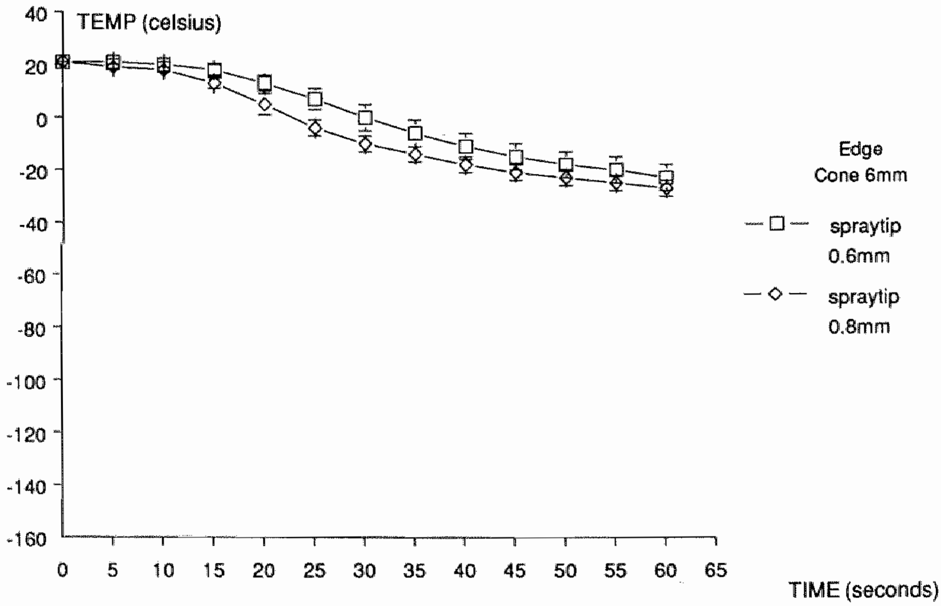

Figure 8.2. Comparison (mean±sd, n=4) of the spraytip diameters of 0.6 mm and 0.8 mm at the edge of the cone diameter of 6 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

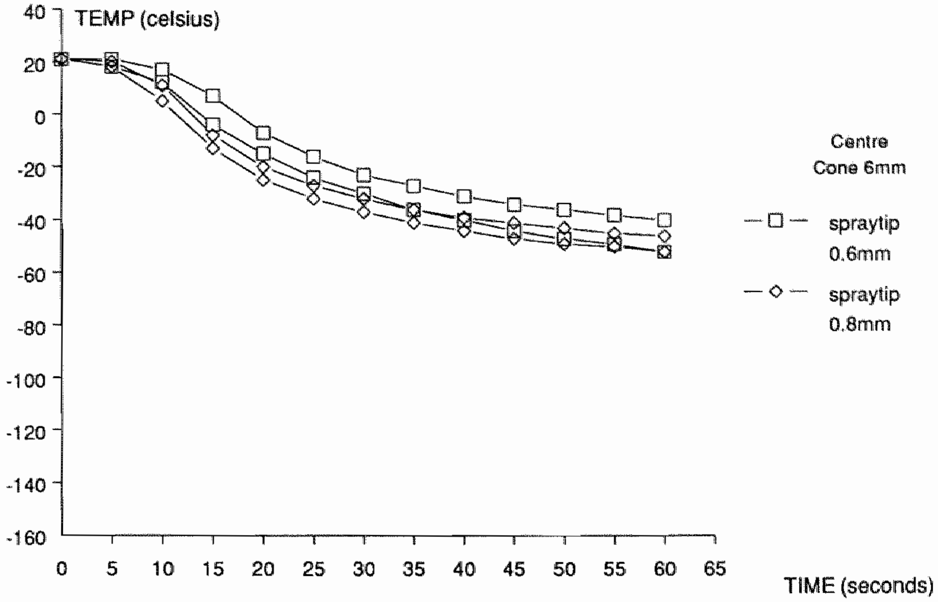

Figure 8.3. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm and 0.8 mm in the centre of the cone diameter of 6 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).

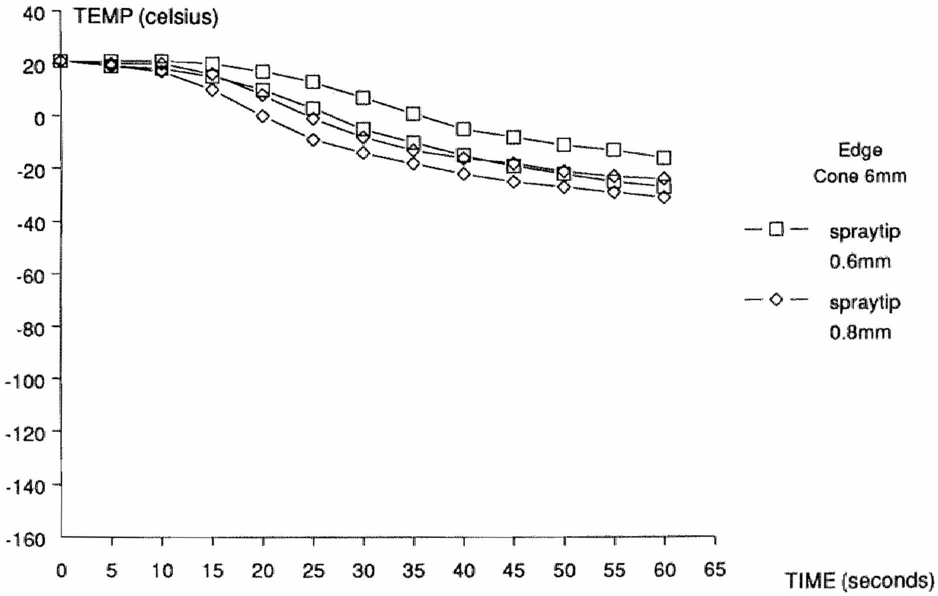

Figure 8.4. Comparison (intervals of minimum and maximum values, n=4) of the spraytip diameters of 0.6 mm and 0.8 mm at the edge of the cone diameter of 6 mm at 3 mm-depth (continuous central spraying from a distance of 15 mm).