AUTOLOGOUS SKIN TRANSPLANTS IN THE TREATMENT OF WOUNDS ON THE DISTAL PARTS OF THE LIMB IN CATTLE.

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A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN VETERNARY SURGERY TO THE UNIVERSITY OF NAIROBI.

MAY - 1985

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University Supervisors.

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ABSTRACT

Autologous free full-thickness and pinch skin grafts have been used in man, horse and the dog in the repair of both fresh and granulating skin wounds. The literature available contained little information on skin grafting as a technique of wound repair in cattle. The technique of full-thickness skin grafting in cattle has, however, been described. It has been reported that dehorning wounds in cattle healed faster when treated with full-thickness skin grafts than when treated conventionally. It was found desirable to investigate the effect of autologous free full-thickness and pinch skin grafts on fresh and granulating lower limb wounds in cattle. The parameters studied were: the ease of take of the autologous free-full-thickness and pinch skin grafts in fresh and granulating wounds; the acceptance of the full-thickness and pinch grafts in fresh compared to granulating wounds, and the effects of the full-thickness and pinch skin grafts on the healing time. Wound contraction and hair growth were also studied as well as the density of hair follicles, sebacious glands and sweat glands in the histological sections of grafted healed wounds.

A total of thirty six full-thickness skin wounds measuring 60 mm x 60 mm were surgically created in the metatarsal and metacarpal regions in bovine calves. Eight of these wounds were grafted with autologous free full-thickness skin grafts while the other eight fresh wounds were grafted with autologous pinch skin. Four wounds were treated without grafting and studied as the controls while the remaining sixteen wounds were let to granulate for fourteen days before being subjected to further treatment. Eight of the granulating wounds were then treated with autologous free full-thickness skin grafts and the other eight granulating wounds treated with autologous pinch skin grafts.

The healing times hair growth and appearance of the wounds after healing were evaluated. Wound contraction was determined by measuring the wound sizes using calipers. Graft acceptance and the density of hair follicles, sebacious and sweat glands was also evaluated in a histological section from the healed wounds.

Autologous full-thickness skin grafting in fresh wounds had a shorter healing time compared to the granulating wounds and the control wounds. The healing time of pinch skin grafted fresh and granulating wounds was similar and less than that of the

control wounds. Better acceptance of full-thickness skin grafts was observed in fresh wounds compared to the granulating wounds. The acceptance of pinch skin grafts in fresh and granulating wounds was good and similar. Wound contraction occured in both the ungrafted control wounds and the grafted wounds. The best hair growth, appearance and regeneration of structures associated with the skin was observed in healed wounds that had been grafted with full-thickness skin while fresh. Sparse hair growth and poor regeneration of the structures associated with the skin was found in the pinch-skin grafted wounds. No hair growth and no regeneration of skin appendages was observed in the healed control wounds and granulating full-thickness skin grafted wounds. The healed wounds in these cases were covered by a thin friable epithelial scar.

The use of autologous free full-thickness and pinch skin grafting in the treatment of large fresh full-thickness skin wounds in the lower limbs of cattle were found to enhance the healing of the wounds. Pinch skin grafting was also found to be useful in the treatment of similar granulating wounds in this species. Granulating wounds did not accept full-thickness grafts.

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisors Dr. S.M. Mbiuki and Prof. J.C. Kiptoon for their guidance and constructive criticism during the course of this study. I am most grateful to Prof. J.C. Kiptoon, Chairman, Department of Clinical Studies for allowing me to utilize facilities in the department for this study.

I should like to thank Drs, S.D. Byagagaire, J.A.N.-Mwangi and D. Kihurani for their unselfish help during the period I was involved with this investigation.

Thanks go to Messrs. J. Muraguri and P. Thuo for the laboratory assistance and Messrs. G.K. Njane and E. Kiarie for the theatre assistance they rendered to me.

I wish also to thank the animal hospital attendants namely, Messrs. Kariuki and Kiplang'at for their help in handling and looking after the experimental animals.

Miss C.W. Kariuki's assistance in typing the final draft of this thesis is gratefully acknowledged.

DEDICATION

I hereby dedicate this thesis to my wife Adelaide and to my sister Reverend Maria Mwikali.

INTRODUCTION

In cattle, cases of skin injuries may be caused by barbed wire, dog bites, traumatic injuries and burns which could be caused by fire or electrical accidents.

Many wounds below the knee and the hock in the large animals are often lacerated and extensive (Frank, 1964). Lacerations involving the lower limbs are complicated by the production of exuberant granulation tissue with poor healing qualities (Hogle, Kingrey and Jensen, 1959; Frank, 1964; Hanselka, 1974). Lower limb wounds are subject to increased contamination and infection and may often involve a considerable amount of skin loss. Excessive movement of the lower limbs delays healing of these wounds.

Extensive wounds below the knee and the hock in large animals leave little or no skin for suturing. Such wounds are usually left to heal by second or third intention (Frank, 1964).

Healing of wounds by first intention is the closest approach to perfection that can be obtained in wound healing. The wound heals without infection and the tissues are approximated so accurately that the apparance of the defect is reduced or eliminated completely. Only enough granulation tissue is formed

to join the apposed surfaces (Frank, 1964).

Wound healing by second and third intention is dependent on epithelialization and wound contraction (Frank, 1964; Peacock and Van Winkle Jr.(1976).

Wound contraction can close a large defect if the wound is located in an area where the tissues are freely movable. The tissues of the lower limbs are fixed to the underlying structures. Wound contration does not occur in the lower limb wounds (Frank, 1964). Epithelialization by epithelial cell migration from the wound edges seems to be the factor in the healing of lower limb wounds. As the margin of epithelium advances, its thickness decreases until one cell thickness results. In large skin wounds, even coupled with contraction, the coverage of the entire wound by epithelium may not occur. Even if complete epithelialization does occur, specialized structures such as glands and hair follicles may not be regenerated adequately and the decreased thickness of the epithelial covering lends itself to repeated disruption (Frank, 1964; Peacock and Van Winkle Jr (1976).

Skin grafting allows a uniform epithelial covering to develop in a shorter time than is possible on lesions allowed to heal by natural cicatrix formation. Skin grafting also promotes the recovery of wounds that may otherwise be difficult to heal and the cosmetic appearance of the grafted wound following healing is

improved (Peacock and Van Winkle Jr (1976).

Researchers in veterinary medicine have attempted to adopt human skin grafting techniques to the Horse, the Dog, Sheep and Goat as well as the Buffalo and Monkey. Literature is scanty concerning skin grafting as a method of wound treatment in cattle.

The technique of autologous free full-thickness skin grafting in cattle has been described by Rumawaz and Ressang (1979) and Spooner, Miller and Olliver (1980).

Bose and Mukherjee (1982) carried out experiments to determine the effects of skin grafts on the healing of dehorning wounds in adult cattle. They observed a shorter healing time than that observed in conventional methods of dehorning in cattle. They subsequently recommended the grafting of skin on dehorning wounds as a method of quickening healing.

In general practice, a conservative approach employing conventional methods of wound treatment has often led to failure in healing, necessitating the slaughter or euthanasia of highly valuable and productive cattle.

Skin grafting on granulated wounds may be indicated in cases where the conventional wound treatment has failed.

There are no published investigations reported on skin grafting as a method of treatment of large wounds in the lower limbs of cattle. Despite this anomaly, there is loose redundant skin especially in the brisket, lateral thorax and the ventral abdomen of cattle. This skin could be harvested and used for grafting.

This project was therefore designed with the following objectives in mind:-

1. To determine the ease of 'take' of autologous free full-thickness and pinch skin grafts in the lower limbs of cattle.

2. To compare the ease of acceptance of autologous free full-thickness skin and pinch skin grafts in fresh and granulating wounds in cattle.

3. To determine the effects of autologous free full-thickness and pinch-skin grafts on healing time, wound contraction, hair growth and the density of sebacious and sweat glands in histological sections of the grafted, healed wounds.

LITERATURE REVIEW

History of tissue transplantation

According to Hanselka (1974) tissue transplantation was being practiced at a highly developed scale by the Egyptians as far back as 3500 B.C.

The process of skin grafting, like science in general followed the rise and fall of empires. In the 15th Century the art of tissue transplantation by means of flaps was established in Europe. However, the art of free tissue transplantation was not used until the middle of the 19th Century when a Swiss surgeon demonstrated the possibility of successful transplantation of living pieces of epithelium to a granulating area. Others improved on this original technique but it was not until the first world war that tissue transplantation was given a real stimulus. Today, the art of tissue transplantation has reached a high level of efficiency in clinical application (Fomon, 1960; Hanselka, 1974).

Classification of Grafts (Transplants)

The genetic compatibility between the host and the transplant has much to do with the success or failure of tissue grafting (Brown and McDowell, 1958; Carpenter, 1968). . On this basis

transplants have been classified as:-

1) Autotransplants (autografts) i.e. one made to a new site in the same animal.

2) Homotransplants (Homografts) i.e. transplants made from one animal to another of the same species.

3) Heterotransplants (Heterografts) i.e. those transplants made from one animal to another of different species.

According to Peacock 1976; autotransplantations does not stimulate detrimental immune response such as that seen in heterotransplantation and homo-transplantation.

The skin has been transplanted autologously either as free skin grafts or as skin flaps (Pedicle grafts) (Epstein, 1962; Woodruff, 1960).

Peacock and Van Winkle (1976) and Woodruff (1960) described the technique of skin grafting as consisting of an isolated piece of tissue which at the time of grafting was completely devoid of vascular and nervous connections. The free skin grafting was apparently made possible by the fact that the skin could retain its viability for a short time without any blood supply and even for a longer time by osmotic interchange with intercellular fluids until capillaries

invaded it and supplied nutrients.

A pedicle graft remains connected to its donor-site, at least for sometime by a pedicle containing the blood vessels. When a new blood supply has developed the pedicle is usually severed and the graft is then adjusted to fit the defect (Woodruff, 1960; Dhablania, Rama and Tyagi, 1978).

Autologous free full-thickness skin grafts.

Autologous full-thickness skin grafting was being practiced as far back as 1871 when Lawson described the use of whole thickness skin grafts of up to 3cm in diameter. According to Lawson (1871) for the graft to be successful it should consist of skin only without subcutaneous fat and should be firmly held to a healthy granulating surface without interruption. More recently, Woodruff (1960) and Frankland (1979) have recommended Lawson's methods of skin grafting.

Krause (1893) reported on 100 cases of human patients in which full-thickness skin grafts were used to treat ulcers on the legs and face. Full-thickness skin grafts of up to 8 cm in diameter had been used in another report at about the same time by Olliver (1872)

When free of subcutaneous fat, full-thickness skin grafts were more likely to survive on fresh clean surfaces than on granulation tissue (Greeley, 1948; Woodruff, 1960; Frankland, 1979). Due to the presence of fat in the deep surface of full-thickness skin grafts, the latter survived less readily than split-thickness skin grafts Hynes, 1950; Woodruff, 1960; Converse, 1970).

Frankland (1979) observed that for full-thickness skin grafts to be successful they should be cut to the size of the defect by means of a pattern and maintained in position by means of sutures and local pressure.

If hair was required, a little subcutaneous fat was deliberately included in full-thickness skin grafts. Inevitably, however, the successful proportion of the grafts was reduced (Greeley 1952).

Adams (1949) and Spina (1949) reported the use of free full-thickness skin grafts for repairing small defects on the face, the flexor surface of the hands and the finger tips of human patients. The same authors also reported the use of pigmented skin graft of the labia minora to replace areolar skin.

The use of autologous free whole-thickness skin grafts for treatment of wounds in horses, particularly on the lower limbs has been well established (Hogle, Kingrey and Jensen, 1959; Boyd, 1967; Meagher and Adams, 1971).

Wallace, Spruell and Hamilton (1962) have recorded the successful treatment of a chronic inflammatory skin lesion in a dog by widely excising the lesion and repairing the subsequent defect by an autologous free full-thickness skin graft. Four months after surgery the transplanted skin appeared secure and showed good hair growth on its superficial surface.

Dam, Boot and Donk (1978) reported good success when they transplanted autologous full-thickness skin in goats. 25mm^2 wounds were surgically created at the backs of the goats and grafted with skin from the medial aspect of the thighs. All grafts were accepted and hair growth was observed at 15 days after the operation.

The technique of full-thickness skin transplantation on calves and the effects of antibovine lymphocyte serum on graft survival has been studied by Rumawaz and Ressang (1979). Full-thickness skin 2.5 cm by 3 cm was harvested and grafted onto surgically created wounds on the backs of the same calf.

The autografts were accepted in all ten cases. The epidermal layer of the grafts became dry and sloughed off leaving the dermal layer which covered the graft bed. After the sloughing off of the epidermal layer, hair started to grow indicating that the grafts had been accepted. Rumawaz and Ressang (1979) had used Dutch-friesian calves 70 to 130 kg and at ages of 3-6 months. The donor skin had been harvested by dissection and excision and the donor wound had been immediately closed by suturing. The authors had then placed the grafts in Hanks Solution containing antibiotics and cleaned all subcutenous fat from them.

Kumar, Prasad, Singh and Sharma (1979) have reported a case of bovine diaphragmatic herniaplasty in a Buffalo (Babalus bubalis) using an autologous full-thickness skin graft to compensate for the lost fascia.

Frankland (1979) observed that when used successfully, full-thickness skin grafts produced a pliable and strong area of skin which was functionally and cosmetically acceptable. He noted that although no special tools were required during their harvesting, the use of full-thickness skin grafts should be limited to small wounds because of the limited availability of full-thickness skin

and the unfavourable results obtained when using large grafts.

Autologous Pinch skin grafts

Pinch skin grafts which have been used in human and equine surgery consist of small discs of skin about 3-5 mm in diameter of full-thickness skin at the centre and tapering towards the periphery (Woodruff, 1960; Neal, 1961; Converse, 1970).

Hogle et al. (1959) observed that when firmly implanted on the surface of a recipient wound in the horse, pinch skin grafts epithelialize the wound by providing small areas of epithelium in the raw surface that grow outwards and eventually coalesce to cover the whole recipient bed. Neal (1961) and Hoffer and Alexander (1976) made similar observation in the Horse while the same was found to occur in man (Peacock, 1976).

Frankland (1979) asserted that the time taken by Pinch skin grafts to completely epithelialize a wound defined the healing time.

Reverdin (1870) had showed that small pieces of autologous human skin could survive and grow when transplanted onto different parts of the same body. He had transplanted the pieces of skin onto a granulating surface. In the same year, Dobson

modified Reverdin's technique by inserting pieces of skin into granulating human skin wounds. Reverdin's grafts evolved finally to the modern pinch grafts (Davis, 1927; Woodruff, 1960; Neal, 1961).

Hanselka (1974) reported success when he implanted pinch skin grafts autologously and directly from the donor to recipient wounds in Horses but Mackay and Marks (1968) inserted pinch skin grafts into surgically prepared pockets scattered over a granulating surface in the same species.

A skin biopsy punch has been used to harvest pinch skin grafts as well as in preparation of the pinch graft recipient beds in horses (Boyd and Hanselka, 1971; Joanne, Mitten and Jackson, 1980; Pavletic, 1982). The technique consisted of trimming away all excessive granulation tissue from the recipient wound and creating small circular recipient beds in the granulating lesion using a biopsy punch. Small, uniform full-thickness skin grafts were then produced using the biopsy punch from the donor site. The circular pieces of skin were then placed in the recipient beds and a pressure bandage applied to keep the grafts in their respective beds.

Joanne et al. (1980) observed that grafts transplanted in this manner shed off their epidermal

layer and lost their pigment. The pigment was restored by the sixth week and the hair started growing by the end of the third month.

Pinch-skin grafts have been harvested by lifting the donor skin with Addison's forceps or a bent needle. The tissue was then cut at right angles to the tented portion of skin. This resulted in a cone shaped piece of tissue about 2mm in diameter which was placed directly onto a recipient wound (Hoffer and Alexander, 1976).

According to Hoffer and Alexander (1976) a pinch skin grafted area should be bandaged with vaseline and gauze cotton dressing or a Robert Jones dressing. The dressing was then applied without a lot of pressure and its function was to protect the surgical site from contamination. The authors recommended that dressings be changed every four days. By the fifth day, granulation tissue over the grafts dissappeared and the grafts appeared as small, whitish-pink spots. By the third week the grafted skin had spread and coalesced to produce a good epithelial cover when the grafts were placed 5 mm apart in the equine subject. Hoffer and Alexander (1976) asserted that the point at which the recipient site was completely epithelialized depended on the number of pinch-grafts that had 'taken' and the distances

between them.

It has been found important to restrain patients from self mutilation after skin grafting (Neal, 1961; Hoffer and Alexander, 1976).

Pinch skin grafts are more effectively used in areas where there is motion. Pinch-skin grafts could also be used when the recipient site is infected (Neal, 1961; Peacock, 1976).

Neal (1961) reported that a smooth healthy and fresh granulating bed was necessary for pinch-skin grafting. When burried in granulation tissue the latter supplied nutrients to the graft until a 'take' occurs. The smoother the recipient bed, the more easily would epithelial cells migrate from pinch-skin grafts.

Peacock and Van Winkle (1976) observed that pinch skin grafts gave a poor cosmetic appearance and left rather ugly marks at the donor site.

Other investigators have published reports in agreement with these observations (Woodruff, 1960; Neal, 1961; Hoffer and Alexander, 1976).

Other skin grafts

Autologous Split-thickness skin grafts.

Woodruff (1960) and Peacock (1976) noted that split skin grafts consisted of the epidermis and part of the dermis. They observed that those grafts were of uniform thickness and ranged from 30-80 percent of the thickness of skin so as to leave enough epithelial elements to epithelialize the donor area.

In the preparation of split-thickness skin grafts either a manual or an electric dermatome could be used (Frankland 1979). Sherinberg (1956) observed that split-thickness skin grafts could be prepared by removing full-thickness skin and shaving off the subcutis and part of the dermis. Skin grafting knives which removed only split thickness skin have also been used to prepare these grafts (Frankland, 1979).

According to Woodruff (1960) split-thickness skin grafts could be applied in large sheets, strips or small rectangles known as 'Postage stamp' grafts. Many incisions 4-5 mm long were made on split skin grafts to allow drainage of exudate forming under them (Frankland, 1979).

Hanselka and Boyd (1976) observed that an electric mesh dermatome could be used to expand split-skin grafts so as to cover a wider area of the recipient site.

Split-thickness skin grafts could be maintained in position by dressing, suturing, moulding or by the coagulum contact technique (Woodruff, 1960).

In the coagulum contact technique, an extract from the buffy coat of the patient's blood was applied to the deep surface of the graft and a little of his/her plasma to the defect. When the graft was placed in position, the plasma coagulated causing the graft to adhere firmly to its bed. In the moulding technique, a mould of stent or plastic material was used to maintain a graft in contact with a concave recipient surface.

McKeever and Braden (1978) and Booth (1982) reported that split-skin grafts took well on clean granulating surfaces, on surfaces prepared by excision of redudant granulation tissue and on freshly exposed subcutaneous tissue.

In dogs, split thickness skin grafts have a greater chance of survival than full thickness skin grafts (Fox, 1982). Converse (1970) reported similar findings in man.

Frankland (1979) reported that split-skin grafts measuring approximately 0.76 mm in thickness gave better results than thinner or thicker grafts.

Split-skin grafts have been known to reduce the healing time of grafted wounds (Meagher, 1970; Boyd, 1970). Similar findings were reported in the dog by McKeever and Braden (1978), and Fox (1932). Converse (1970) and Peacock (1976) showed that the healing time of human skin wounds was reduced by split-skin grafting.

According to Frankland (1979) split-thickness skin grafts were more readily accepted in fresh than in granulating wounds in horses. Epstein (1962) and Converse (1970) have also reported that split-thickness skin grafts were more readily accepted in fresh than in granulating wounds of man.

Frankland (1979) has described a method of bandaging skin grafted tissues on the lower limbs of horses. This technique consisted of covering the grafted wounds with a non-adherent dressing, then a layer of cotton wool held firmly in place with a bandage which was further covered with a second layer of cotton wool and bandage and finally followed by a double layer of adhesive tape.

Dressings were removed every five to seven days

in most cases. If the first dressing was removed too early, viable grafts could be accidentally detached. On the other hand if it was delayed, pus accumulated from the regions where the graft failed and caused lysis of the grafts that had taken (Woodruff, 1960; Frankland, 1979).

Skin Flaps

A flap or a pedicle is a portion of skin or subcutaneous fat that has been raised from the underlying tissues but which remains, at least for some time connected at some part of its periphery to the donor site by a pedicle containing blood vessels and nerves (Peacock and Van Winkle Jr (1976).

According to Converse (1970) pedicled flaps could either be obtained from tissues adjacent to the recipient site (local flaps) or from a distant region (distant flaps).

Skin flaps have been used singly or in combination with other grafts depending on the size and location of the defect to be repaired (Converse, 1970).

Garrion (1929) and Woodruff (1960) reported that Indian native surgeons had used skin flaps as early as 2000 years ago, principally in rhinoplasty. Similar reports were recorded by Koch (1941) and Maltz (1946).

The use of local flaps for repairing lesions of the ears, nose and lips of man has also been reported. (Epstein, 1962).

Staged transfer of flaps, also called 'waltzing' of flaps was described by Halsted (1896).

Tubed pedicle skin grafts have been used to reconstruct the upper eyelid in man (Filatow, 1917).

Blair (1921) has also reported on the use of a delayed flap technique saying that its use reduced the risk of necrosis.

Various modifications of pedicled grafts have found many applications in human plastic surgery especially in the treatment of defects involving the neck and the face (Ivy, 1949; Catlin, 1950; Hynes; 1950; Smith, 1951; Conway, Stark and Kavanough, 1952; Mustarde, 1953; Moore and Faulkner, 1954).

An experimental study on pedicle skin grafting for the management of extensive wounds on the parotid, mandibular and antlantal regions in buffalo calves has been reported (Dhablania et al., 1978). The investigators reported that pedicle tube grafts formed parallel and oblique to the vertebral column were rejected while uncomplicated acceptance was observed when vertical tubed pedicle grafts were used.

Cawley and Francis (1958) have reported that the use of tubular pedicle grafts required a careful and skilled design while the technique still remained a hazardous and lengthy procedure.

The presence of adequate blood supply was found to be important for survival of skin flaps because this supplied the nutrients and innervation required for regeneration of cells (Peacock, 1976).

A narrow pedicle offered greater flexibility to the flap and hence facilitated its transfer (Esser, 1971; Smith, 1951; Converse, 1962).

Wound contraction

Wound contraction is an active process which tends to close an excised wound. By this process the size of a full-thickness skin wound was reduced and was characterized by centripetal movement of the whole thickness of the surrounding skin (Heinze, 1979; Peacock, 1976; Johnston, 1977).

Peacock (1976) demonstrated that wound contraction started at between the fifth and the tenth day following the wounding of skin and could proceed upto the third week in cases of large skin wounds. This latter observation was also in agreement with those of other researchers who investigated wound

contraction (Frank, 1964; Heinze, 1976).

Heinze (1976) further reported that in horses and cattle wound contraction could reduce the size of a wound to an extent such that the final scar was 10-15 per cent of the length of the original open wound. According to Frank (1964), a large wound located in an area of freely moveable skin could close completely by wound contraction. Wound contraction, however was not as dramatic in areas where the skin was not freely moveable. Later findings by Heinze (1976) and Peacock (1976) agreed with this observation.

Frank (1964) also asserted that wound contraction did not occur in the lower limbs of cattle as the skin in these areas was fixed to the underlying tissues.

According to Peacock (1976) full-thickness skin grafts inhibited wound contraction when applied before wound contraction started. This inhibition was, however, not seen when the grafts were applied after wound contraction had started. Split skin grafts did not inhibit wound contraction.

The rate of wound contraction was found to be independent of the size of the wound and was recorded as 0.60-0.75 mm/day (Peacock, 1976).

Healing of full-thickness skin defects

Billingham and Medawar (1955) observed that when the skin was freely moveable, the healing of full-thickness skin defects was brought about by wound contraction and intussusceptive growth. Frank (1964) and Peacock (1976) observed similar changes in skin healing. Granulation tissue was formed within the perimeter of the defect and epithelialization from the margins occurred subsequent to this granulation.

According to Billingham and Medawar (1955), neither epithelialization nor fibroplasia made any substantive contribution to the final repair.

Linquist (1946) had argued that fibroplasia and epithelialization provided the tensile strength required for contraction.

Other investigators found that when the defect was large or occurred in an area where the skin was firmly attached to the underlying structures, contraction and intussusceptive growth did not suffice to close the defect (Woodruff, 1960; Frank, 1964; Peacock and Van Winkle Jr 1976).

In the absence of skin grafting, the end result was a fibrous scar covered by epithelium of migratory origin or a persistent ulcer (Woodruff, 1960). In either event, however, there ensued permanent distortion

of the sorrounding structures (Frank, 1964; Peacock, 1976).

These investigators therefore concluded that unless with very small full-thickness skin defects, early skin replacement was necessary to obtain good functional and cosmetic result.

It has been demonstrated in the rabbit that specialized structures such as hair follicles and sebacious glands were regenerated by differentiation of migrated epithelium (Billingham and Medawar, 1955; Woodruff, 1960; Peacock 1976). Peacock (1976) noted that the process of regeneration started late in the healing process and usually after surface epithelialization was completed. Hair follicles were the first to be regenerated and by the time hair production started sebacious glands had appeared.

Healing of free skin autografts

The healing of free-skin autografts has been divided into three stages, viz: the stage before revascularization, the stage of revascularization and the stage of organic union (Billingham and Medawar 1950: Cawley and Archibald, 1974).

Before revascularization, the graft was seen to be attached to its bed by coagulated plasma and was nourished by osmotic interchange of fluids with its bed (Billingham and Medawar, 1950; Cawley and Archibald, 1974; Peacock, 1976).

The process of revascularization began within twenty four hours and the development of vascular blood supply was completed within twelve days (Cawley and Archibald, 1974). The revascularization process depended on the development of anastomoses between vessels growing from the bed and preexisting vessels in the graft and partly on the invasion of the graft by vessels from the bed (Converse and Rapaport, 1956; Peacock and Van Winkle Jr (1976).

The stage of organic union was manifested by a firm fibrous union and the usual stages of wound healing. The union was evident by the tenth day but complete healing required as long as eighteen months (Converse and Rapaport, 1950; Woodruff, 1960; Cawley and Archibald, 1974).

Split-skin grafts tended to contract to an extended depended on the nature of the underlying tissue and the graft thickness. Contraction was minimal when the split skin grafts were placed on bone but ranged from 30-60% when the underlying tissue were loose. Full-thickness skin grafts showed negligible contraction (Padgett, 1942; Peacock, 1976).

According to Oghi (1950) a successful graft remained true to type and a full-thickness skin graft from a hair bearing area continued to grow hair after attaching to the recipient site.

Dressing of grafts

The major aims of dressing grafts have been stated to be to immobilize the grafted site, maintain a firm contact between the grafts and graft beds, reduce water loss from the grafts and to protect the grafts from contamination and injury (Medawar, 1944; Billingham and Medawar, 1951).

A convenient method of dressing grafts has therefore been developed consisting of dusting the grafted area with penicillin lactose powder, covering it with cotton gauze impregnated with petroleum jelly and then applying an ordinary bandage (Medawar, 1944; Billingham and Medawar, 1951; Woodruff, 1960). Frankland (1979) has described a method of bandaging skin grafted tissues on the lower limbs of horses. This technique consisted of covering the grafted wounds with a non-adherent dressing, then a layer of cotton wool held firmly in place with a bandage which was further covered with a second layer of cotton wool and bandage and finally followed by a double layer of adhesive tape.

MATERIALS AND METHODS

Location

Experimental work took place at the Veterinary Clinic Faculty of Veterinary Medicine, University of Nairobi at Kabete. Kabete lies at approximately $1^{o}16'$S and $36^{o}44'$E at an altitude of 1,932 metres above sea level.

Experimental animals

Experimental animals were bovine calves of mixed breeds bought from several farmers in Kabete and transported by lorry to the Veterinary clinic. Fourteen calves were used for the experiments; twelve being castrated and two being entire males. They were aged between one and two years.

Before the experiments were commenced, complete physical examination was done on each animal. Rectal temperatures and the pulse and heart rates were taken. The respiratory rates were also determined. A clinical examination was also carried out to assess the other body systems and their functions. All the calves were proved to be in their normal clinical state before the commencement of the experimental procedures.

Housing, Feeding and Routine Treatments:

The calves were housed in twos in unit stalls of concrete walls and floor and corrugated asbestos sheeting roofs. The stalls were of uniform area measuring 3 x 3.75 metres of floor space and 2.5 metres as the shortest height from the ground to the slanting roof. Each stall had at least one open window measuring 0.6 x 0.85 metres located above half door for adequate ventillation.

The feeding regime consisting of dry Rhodes' grass (Chloris gayana) hay and commercial maize bran (Unga Ltd., Nairobi) was fed twice daily. Water and commercial salt licks (Maclic - Twiga chemicals, Nairobi) were available ad libitum. Each calf was drenched with the appropriate dose of an antihelmintic (Thibendazole - Merck - Sharpe and Dohme, Hertfordshire, England) to remove any worms from their digestive systems. The deworming with Thibendazole was repeated every two months.

Experimental procedure:

Full-thickness skin and Pinch-skin grafting experiments were carried out on surgically produced fresh and granulating skin wounds. The fresh wounds were made surgically by removing full-thickness skin of dimensions 60 mm x 60 mm on the lateral side of

the metatarsi and or metacarpi. Bleeding was controlled and the wounds were immediately grafted. Eight fresh wounds were grafted with full-thickness skin and eight other fresh wounds with pinch-skin.

Granulating wounds were made by removing full-thickness skin from the lateral aspect of the metatarsi and or the metacarpi. The wounds so created were then bandaged and allowed to granulate for thirteen days. The granulated wounds were then grafted on the fourteenth day. Eight granulating wounds were grafted with full-thickness skin and eight other granulating wounds with pinch-skin.

The donor sites for the full-thickness grafts were the lateral thorax and or the brisket where there was loose skin. Pinch-skin grafts were harvested from the lateral aspects of the neck between the region of the ligamentum nuchae and the jugular groove.

Preparation of the calves.

The calf to be operated on was removed from the stalls and walked to the large operating theatre of the veterinary clinic a distance of about 100 metres.

The calf was sedated with an intramuscular

injection of 0.2 mg Rompun[R-1]. The calf was then cast with ropes onto lateral recumbency with the operation sites uppermost. The limbs were restrained with ropes while the head was kept down by means of a head halter tied to the operating table.

Full thickness skin grafting in fresh wounds.

a) Preparation of full-thickness skin grafts:

An area 80 mm x 80 mm on the donor site was clearly shaved using a scalpel blade, soap and water, then thoroughly washed with soap and water and disinfected with 70 percent ethyl alcohol.

Meanwhile, the surgeon and his assistant prepared for aseptic surgery by putting on-head caps, mouth and nose masks, scrubbing and gloving.

Then, using full aseptic technique an area 65mm x 65 mm of the prepared donor site was measured with a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the points of demarcation to outline a square area of 65mm x 65 mm. One of the edges of the incisions was picked up with a thumb

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1 Rompun[R] - (xylazine hydrochloride) 1 ml contains 23.32 mg of 2-(2,6 xylidino) 5,6 dihydro-4H-1, 3-thiazine hydrochloride (equivalent to 20mg active ingredient.) - Bayer-Leverkusen, Germany.

forceps and the piece of skin undermined by bluntly dissecting the subcutaneous tissues using the blunt side of a scalpel blade, and the skin was removed. The piece of skin (full-thickness skin graft) was placed on a sterile cotton gauze swab soaked in sterile physiological saline solution and then placed aseptically at one corner of the surgical table. Any adhering fat and connective tissue was removed from the graft by scraping it with a scalpel blade. The graft was then placed in a sterile petri-dish containing about 50 millilitres of physiological saline solution (Figure 1).

b) Treatment of the donor wounds:

Any bleeding from the donor skin wounds was controlled by application of gentle pressure with sterilized cotton gauze swabs and/or clamping larger blood vessels with hemostats. The edges of the wound were then undermined to obtain triangular skin sections which were removed, one from each of two opposite sides of the square defect. The bases of the triangles were formed by the two sides of the wound. The wound edges were apposed using number two nylon suture material (ETHILON^{R-2}) in a simple

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2 ETHILON^{R}- Monofilament polyamide.
ETHICON LTD. Bank Head Avenue, Edinburgh, SCOTLAND.

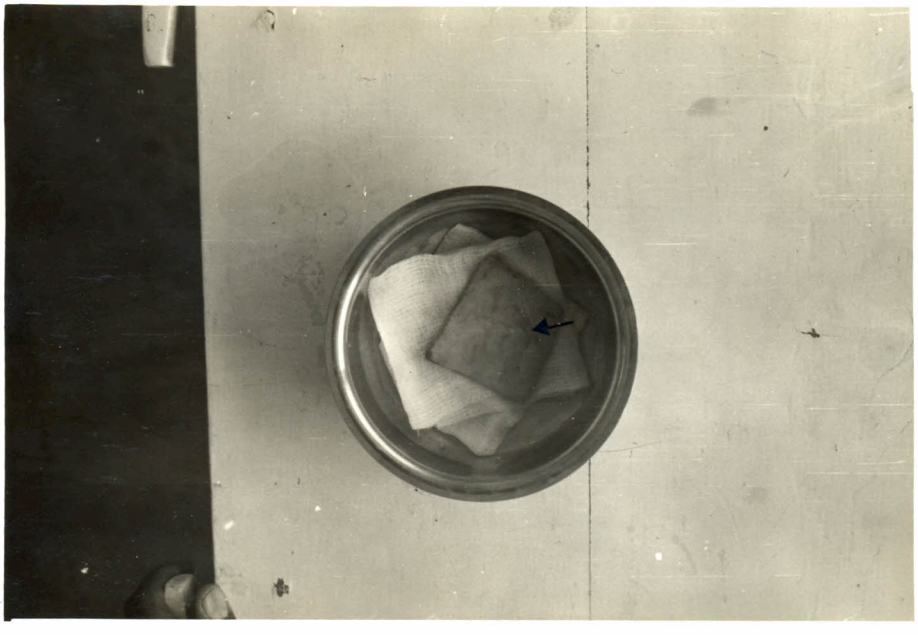

Figure 1: A full-thickness skin graft (arrow) prepared and ready for grafting.

interrupted pattern. The sutures were placed 1 cm apart and the knots tied 1 cm away from the wound edges. The wound was then sprayed with Terramycin Spray^{R-3} (Figure 2).

c) Preparation of full-thickness skin graft recipient wounds:

An area 80mm x 80mm on the recipient site was clearly shaved using a scalpel blade, soap and water and thoroughly washed with soap. The site was then disinfected with 70% ethyl alcohol. An area 60x60 mm of the prepared site was measured with a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the demarcation points to outline a square area of skin 60mm x 60mm. One edge of the incisions was picked up with a thumb forceps and the piece of skin undermined by blunt dissection with the blunt side of a scalpel blade and then removed. Any bleeding from the wound was controlled by application of gentle pressure with cotton gauze swabs and the wound was then grafted (Figure 3).

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3 TERRAMYCIN SPRAY^{R} - Contains 4g oxytetracycline hydrochloride E.P. (2% w/w and 375mg Gentian violet per 200 g pack.
Pfizer LTD. Sandwich, England.

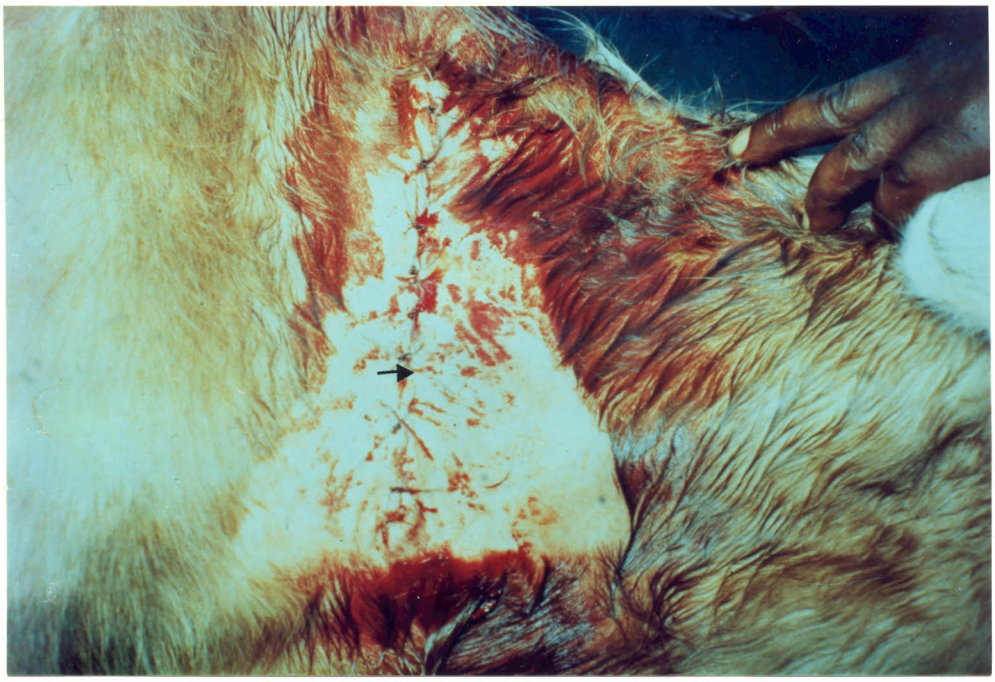

Figure 2: A full-thickness skin graft donor site (arrow) after graft removal and suturing the wound.

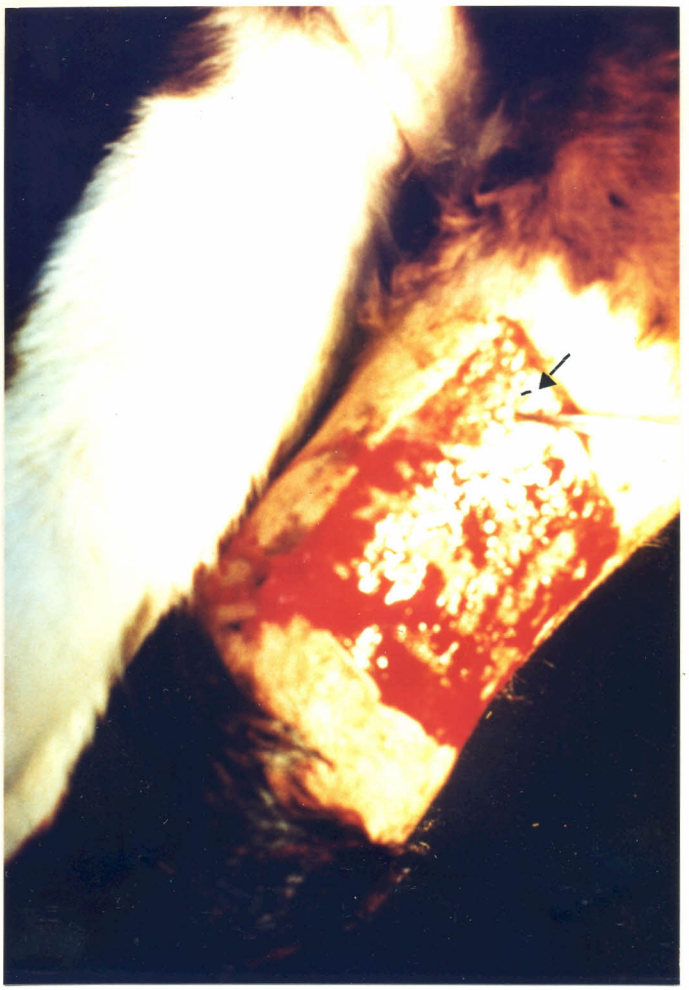

Figure 3: A fresh full-thickness skin wound (arrow) ready for grafting.

d) Full thickness skin grafting - grafting technique:

The full-thickness skin graft was picked from the petri-dish and placed onto the surface of the recipient wound. The hairy side of the graft faced outwards and adjusted so that the direction of the hair was the same as that of the skin sorrounding the recipient site. The edges of the graft were sutured to those of the recipient wound using number two nylon suture material in a simple interrupted pattern. When suturing, the needle was passed through the edges of the graft and then through the edges of the recipient wound in order not to dislodge the graft from the recipient bed. The sutures were placed 1 cm apart and the knots tied 1 cm away from the incision edges (Figure 4).

Stab incisions about 3 mm long were made on the graft using the tip of a scalpel blade, to prevent seroma formation under the graft. A thin film of antibiotic cream, (Strypen^{R-4}) was applied on the graft. The graft was then covered with a

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4/ STRYPEN^{R} - Each 5 ml contain 300 mg procaine penicillin G and 250 mg dihydrostreptomycin. MAY and BAKER Ltd. England.

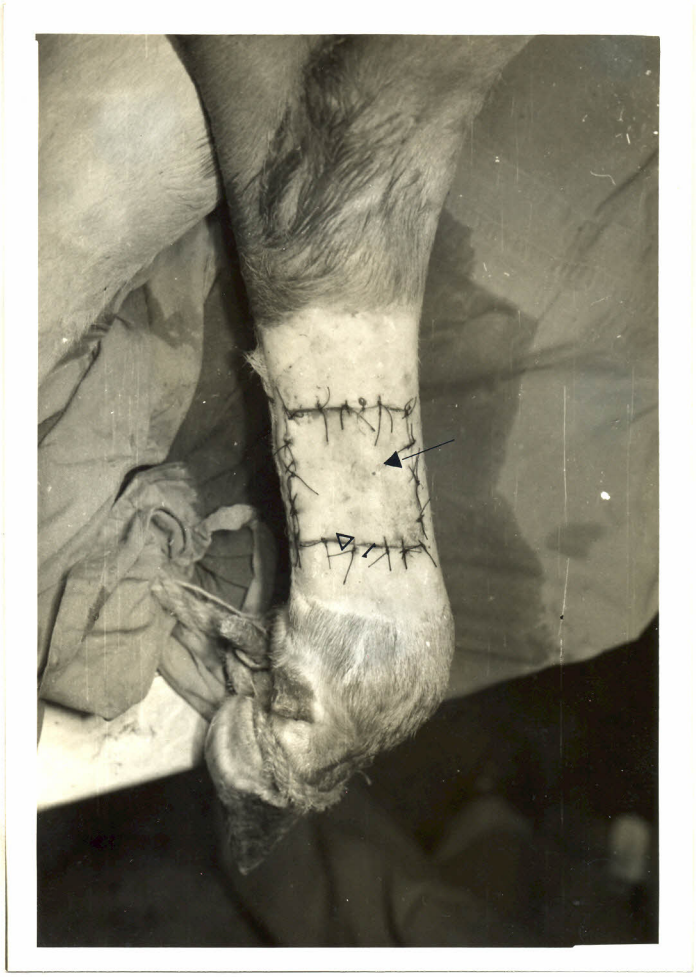

Figure 4: A full-thickness skin (arrow) grafted on a fresh wound.

non-adherent dressing (N-A[R-5]) which was followed by a layer of cotton gauze swabs. A layer of cotton wool was passed over the gauze swabs. Another layer of gauze bandage was applied and the procedure completed with an outer layer of waterproof elastic adhesive tape, (Leukoplast[R-6]) (Figure 5). The calf was then given an intramuscular injection of 15 ml combiotic[R-7]

Full-thickness skin grafting in granulating wounds.

a) Preparation of granulating full-thickness skin recipient wounds:

An area of skin 80mm x 80mm on the recipient site was clearly shaved using a scalpel-blade, soap and water. The shaved area was then thoroughly washed with soap and water and disinfected with 70% ethyl alcohol. An area 60mm x 60mm of the prepared.

5/ N-A[R] - Non-adherent dressing. - Johnson & Johnson Ltd., Slough England.

6/ LEUKOPLAST[R] - Beirsdorf, Germany.

7/ COMBIOTIC[R] - (200,000 i.e. procaine penicillin G plus 250 mg dihydrostreptomycin sulphate per millilitre of aqueous suspencion. PFIZER INC. AGRICULTURAL DIVISION, NEWYORK - U.S.A.

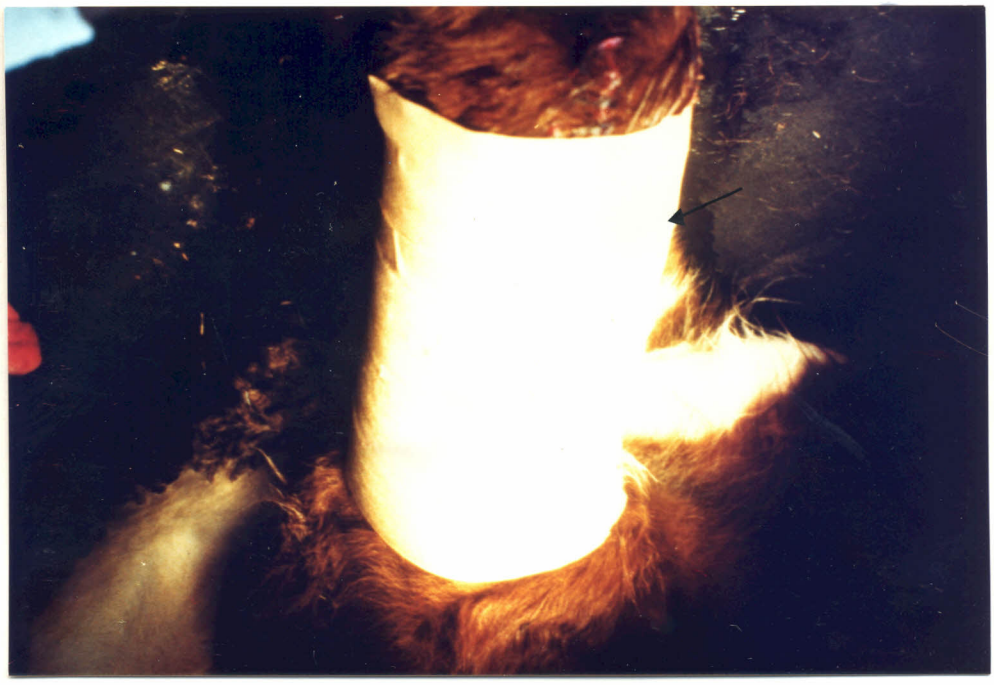

Fig. 5: A fresh wound grafted with a full thickness skin and fully dressed (arrow).

recipient site was measured using a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the demarcation points to outline a square area of skin 60mm x 60mm.

Using thumb forceps, one edge of the incisions was picked up and the piece of skin undermined and removed. Any bleeding from the wound thus created was controlled by applying gentle pressure with cotton gauze swabs and/or clamping larger vessels with hemostats. A thin film of Strypen was applied on the surface of the wound. Non-adherent dressing was applied onto the wound followed by a layer of sterile cotton gauze swabs on top of which gauze bandage was applied. The procedure was completed with an outer layer of waterproof adhesive elastic tape. The calf was given 15 ml of Combiotic, then untied and walked back to the stall.

From then on, dressings were changed every four days. On the thirteenth day after surgery, the dressings were removed and the wound was washed with physiological saline solution. The granulation tissue that had formed on the wound was trimmed to a level just below the wound edges. New dressings were then applied.

On the following day, the calf was tranquilized with an intramuscular injection of 0.2 mg of Rompun and cast on lateral recumbency with the limb bearing the granulating wound uppermost. The limbs were restrained by tying with ropes and the head kept down with a halter tied to the operating table. Dressings on the wound were removed. The wound surface was covered with a cotton gauze swab soaked in sterile physiological saline solution. The edges of the wound were then shaved. The cotton gauze on the wound surface was removed and the wound edges debrided. The surface was smoothened by scraping it with a scalpel-blade and flushing it with physiological saline solution. The wound was then covered with another cotton gauze swab soaked in physiological saline solution.

b) Preparation of full-thickness skin grafts :

An area 80mm x 80mm on the donor site was shaved using a scalpel blade, soap and water and then thoroughly washed with soap and disinfected with 70% ethyl alcohol. On the prepared donor site, an area of skin 65mm x 65mm was measured using a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the demarcation points and then the piece of skin was undermined and removed. The piece

of skin, (full-thickness skin graft) was placed on a saline soaked cotton gauze swab with the hair bearing side down and placed at one corner of the surgical table. Any adhering fat was removed from the subcutis by scraping with a scalpel-blade. The graft was then placed in a petri-dish containing about 50 ml of physiological saline solution.

c) Full-thickness skin graft donor wounds:

Any bleeding from the donor wounds was controlled. The edges of the wound were then undermined. Two triangular pieces of skin were removed, one from each of two opposite sides of the square defect. The sides of the defect formed the bases of the triangles. The edges of the wound were apposed using number two nylon suture in a simple interrupted pattern. The wound was then sprayed with Terramycin Spray.

d) Grafting technique:

The cotton swab was removed from the surface of the recipient wound and the full thickness skin graft placed onto the wound with the hair growing side facing upwards. The graft was then adjusted, to fit the wound and lie in such a manner that the

direction the hair on the graft was the same as that of the skin in the recipient site. The edges of the graft were then sutured to the wound edges using number two nylon suture and in simple interrupted fashion. The suture knots were placed 1 cm apart while the sutures were 1 cm from the wound edges. Stab incisions 3 mm long were made on the graft using a scalpel blade.

A thin film of antibiotic cream (Strypen) was applied on the graft and the suture lines. The graft was then covered with a non-adherent dressing (NA) followed by a layer of cotton gauge swabs. A layer of cotton wool was placed on the gauze swabs. Another layer of gauze bandage was applied and the procedure completed with an outer layer of waterproof adhesive elastic tape (Leukoplast). The calf was then given an intramuscular injection of 15ml of Combiotic.

Pinch-skin grafting in fresh wounds.

a) The Preparation of pinch skin grafts:

The calf to be operated on was cast down on the operating table with ropes and the legs restrained by tying them to the operating table. A square area 120mm x 120mm on the lateral side of the neck was

clearly shaved using a scalpel blade, soap and water. The area was then thoroughly washed with soap and disinfected with 70% ethyl alcohol.

Meanwhile the surgeon and his assistant prepared for aseptic surgery.

Pinch skin grafts were harvested from the prepared area on the neck by lifting the skin with a double curved needle and cutting the tissue at right angles to the tensed portion of skin. This resulted in a cone-shaped piece of tissue approximately 3mm in diameter. The pinch graft was then put in a petridish containing about 50 ml of sterile physiological saline solution. The harvesting of pinch grafts was repeated until at least fifty pinch grafts had been obtained (Figure 5). The donor area was then swabed with cotton gauze and sprayed with Terramycin spray.

b) Preparation of fresh pinch-skin grafts recipient wounds:

An area 80mm x 80mm on the recipient site was prepared for aseptic surgery by shaving then thoroughly washing it with soap and eventually disinfecting with 70% ethyl alcohol. An area 60mm x 60mm on the prepared recipient site was measured with a sterilized ruler and demarcated by pricking with the

Fig. 6: The pinch skin grafts (arrows) after harvesting and being placed on a saline soaked gauze in a petri-dish.

tip of a scalpel blade. Full-thickness skin incisions were made along the points of demarcation to outline a square piece of skin 60mm x 60mm. One edge of the incisions was picked up with thumb forceps and the piece of skin undermined by blunt dissection and subsequently removed. Any bleeding from the prepared wound was controlled by application of gentle pressure with cotton gauze swabs.

c) Grafting technique :

The pinch-grafts were picked one at a time with the tip of the thumb forceps and placed on the recipient wound surface with the hair growing side facing upwards. The grafts were placed at intervals of approximately ten millimetres apart, until the whole wound surface was covered. A thin film of Strypen was applied on the wound surface and then covered with a non-adherent dressing. A layer of cotton gauze swabs was placed over the non-adherent dressing followed by another layer of cotton gauze bandage. The procedure was then completed by an outer layer of water-proof adhesive elastic tape. The calf was then given an intramuscular injection of 15 ml of Combiotic.

Pinch skin grafts in granulating wounds.

a) Preparation of granulating recipient wounds:

An area 80mm x 80mm on the recipient site was prepared by shaving using a scalpel blade, soap and water. The area was thoroughly washed with soap and then disinfected with 70% ethyl alcohol. An area 60mm x 60mm of the prepared site was measured with a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the points of demarcation to outline a square piece of skin 60mm x 60mm. By use of thumb forceps, one edge of the incisions was picked up and the piece of skin undermined by blunt dissection and removed. Capillary bleeding was controlled and a thin film of strypen was then applied on the wound surface. Non-adherent dressing was then put onto the wound surface and covered with a layer of cotton gauze swabs. Cotton gauze dressing was then passed over the swabs and an outer layer of waterproof adhesive elastic tape applied. The calf was given an intramuscular injection of 15 ml of combiotic. From then on, the dressings were changed every four days.

On the thirteenth day after surgery, dressings were removed and the wound was washed with physiological

saline solution. The granulation tissue which had formed on the wound was trimmed to a level just below the wound edges, and new dressings applied. On the following day (day 14) the calf was tranquilized with Rompun given by intramuscular injection. The calf was cast on lateral recumbency with the wound bearing limb uppermost. The limbs were restrained by tying with ropes and the head kept down using a head halter tied to the operating table. The dressings on the granulating wound were removed and the wound surface covered with a cotton gauze swab soaked in physiological saline. The edges of the wound were cleaned with physiological saline. The swab on the wound surface was removed. The wound surface was then smoothened by scraping it with a scalpel blade and then flushing with sterile physiological saline solution. A wet cotton gauze swab was applied on the wound while the pinch skin grafts were being prepared.

b) Preparation of pinch-skin grafts:

The pinch-grafts were obtained from the neck of the same animal. A square area measuring approximately 120mm x 120mm on the corresponding side of the neck was shaved with blade then washed with soap and water, and disinfected with 70% ethyl-alcohol by an assistant. In the meantime, the surgeon and the other assistant

prepared for aseptic surgery.

The pinch grafts were harvested from the prepared area by lifting the skin with a double curved suture needle and cutting the tissue at right angles to the treated portion of the skin. This resulted in a cone-shaped piece of tissue approximately 3 millimetres in diameter. The pinch graft was then put in a petri-dish containing 50 ml sterile physiological saline solution. The harvesting procedure was repeated until at least 50 grafts had been obtained. The pinch grafts remained in the petri-dish until they were required for grafting. The donor site was swabbed with cotton gauze and sprayed with Terramycin spray.

c) The grafting technique:

With the tip of sharp pointed forceps individual pinch-skin grafts were picked one at a time and thrust into the granulation tissue of the recipient wound to a depth of 3-5 mm. The tension on the forceps was released and the forceps withdrawn leaving the pinch graft within the granulation tissue. No care was taken to insert the grafts such that the hair growing side faced any particular direction. The process was repeated, placing the grafts approximately 10mm apart until the entire wound surface was covered

with the pinch grafts.

A thin film of the antibiotic cream strypen was applied on the wound surface and the wound dressed with non-adherent dressing, cotton gauze pads, cotton gauze dressing and an outer layer of Leukoplast. The animal was then given an intramuscular injection of 15 ml combiotic.

The control wounds.

Four control wounds measuring 60 mm x 60 mm were made on the metatarsi/metacarpi of the alternate leg of two different calves. All four control wounds were made and treated in the same manner without any grafting being applied until they healed.

An area approximately 80 mm x 80 mm on the metacarpus or metatarsus was prepared for aseptic surgery by shaving with a blade, washing with soap and water and disinfecting with 70% ethyl alcohol. An area 60 mm x 60 mm of the prepared site was measured using a sterilized ruler and demarcated by pricking with the tip of a scalpel blade. Full-thickness skin incisions were made along the demarcation points to outline a square area of skin 60mm x 60 mm. Then using a thumb forceps one edge of the incisions was picked up and the piece of skin

undermined by blunt dissection with the blunt side of a scalpel blade and removed. Any capillary bleeding from the wound so created was controlled by application of gentle pressure with cotton gauze swabs. A thin film of the antibiotic cream strypen was applied on the wound surface. The wound was then dressed with an inner layer of non-adherent dressing, cotton gauze pads, bandage and an outer layer of Leukoplast. The animal was then given an intramuscular injection of 15 mls of Combiotic.

Post operative care on the Experimental wounds.

The control calves were given an intramuscular injection of 15 ml combiotic daily for six days. The dressings were changed every four days until healing was completed. Before applying new dressing, the wounds were examined and rinsed with physiological saline and then a thin layer of strypen applied on the surface.

The animals with the grafted wounds were also each given intramuscular injections of combiotic for six days following the skin grafting. Dressings were changed every four days until wound healing was observed. The sutures, in the full-thickness skin graft cases were removed on the 8th day. Assessment of the 'take' of grafts in each case was made every

four days during the changing of the dressings. The assessments were based on the following parameters:-

a) Healing time:

Grossly visible progressive epithelial migration from the wound edges and from the grafts were evaluated visually at four day intervals from the day of grafting until the grafted surfaces and control wounds had been completely covered by epithelium. The number of days, taken for each wound to be completely covered by epithelium was defined as the healing time. Healing times for all wounds were tabulated when observations were made. Statistical comparisons were then carried out on the results at 5% significance level using the paired student's t-test

b) Graft - acceptance:

Any graft that, was pink in colour, blushed on application of digital pressure, and adhered well to its recipient bed four days after grafting was considered as having 'taken'. Any part of a graft or a whole graft that took and healed in place upto 60 days after grafting was considered as having been 'accepted'. The area of full-thickness grafts that was accepted and the number of pinch-skin grafts

that were 'accepted' were tabulated. The acceptance of any full-thickness graft was calculated by multiplying the length and the width of the 'accepted' skin and expressing the figure 'area of full-thickness skin'. The 'acceptance' of pinch skin grafts was determined by counting the number of pinch grafts that were accepted in any one wound and expressing the figure as 'number of pinch-skin grafts'. The results obtained for graft acceptance were compared statistically at 5% significance level.

c) Wound contraction :

On the 60th day after the grafting, the area of each wound was determined by measuring the wound length and width (using calipers) and then multiplying the two figures to obtain the area of the wound. The amount of wound contraction was determined by subtracting the area of the wound 60 days after grafting from the original area of the wound before grafting. The rate of wound contraction was calculated by dividing the amount of wound contraction 60 days after grafting by 60 days and expressed it in square millimetres contraction/day. The results were then compared statistically at 5% significance level.

d) Macroscopic appearance:

The hair growth on the wound was evaluated visually every four days from the day of grafting until sixty days after grafting. The values obtained were tabulated.

e) Microscopic appearance:

Histological specimens were taken from the experimental wounds in the areas where grafts had 'taken' and been 'accepted'. Then sections measuring approximately 5mm x30mm were taken and trimmed to 1mm x 10mm. The trimmed sections were fixed in 10% formalin for at least 72 hours. The sections were then dehydrated, double embedded in 1% Colloidin in methylbenzoate and then in paraffin wax. Histological sections of 8 µm thickness were prepared and then stained with Hematoxylin and Eosin. The stained sections were observed under the microscope for the presence of hair follicles, sebacious glands and sweat glands at x 10. The number of hair follicles, sebacious glands and sweat glands counted per ten objective fields were tabulated and their means calculated and recorded. Comparison of the results (t-tests) were then carried out at 5% significance level.

RESULTS

General Observations.

Xylazine hydrochloride (Rompun) at the given dosage produced enough analgesia and sedation for the purposes of the experiments. Regurgitation of ruminal ingesta was observed in 7 out of the 14 animals so sedated. The regurgitation started at about 15 minutes after the injection of Rompun and lasted about 2 hours. On healing itching was observed in many wounds and the animals tried to ease the itching by bitting on the bandages and rubbing them against the walls. To prevent the removal of the bandages by the animals, the animals' heads were restrained with a halter tied to the side of the stall nearer the water and feed.

Healing times, Graft acceptance and the rate of wound contraction:

Table I shows the means and standard errors of the results of all 4 experiments. The healing time of the control wounds (group A) was 50.00 ± 8.07 days. Fresh-wounds treated with full-thickness skin grafts (group B) healed in 20 ± 6.41 days. The mean healing time of granulating wounds treated with full-thickness skin grafts (group C) was 62.5 ± 4.58 days. Fresh-

Table 1: The mean values (±SE) for the healing times, wound contraction and graft acceptance in wounds of cattle when treated by the different techniques

	Group A (controls)	Group B	Group C	Group D	Group E
Healing time (days)	50.00±8.07	20 ±6.41	62.5 ±4.58	31.50±1.92	38.5 ±1.05
Rate of wound contraction (mm^2/day)	40.41±2.69	47.25±2.01	34.93±4.40	49.01±4.02	57.43±0.32
Graft acceptance: (area/grafts)	-	2803.5 ± 404.72	0	41.50±2.19	31.00±1.85
(Percentage)	-	(75)	(0)		

Key:
- Group A - controls - wounds not treated with grafts
- Group B - full-thickness skin grafted fresh wounds.
- Group C - full-thickness skin grafted granulating wounds
- Group D - pinch skin grafted fresh wounds
- Group D - pinch skin grafted granulating wounds.

pinch skin grafted wounds (group D) healed in 31.50 ± 1.92 days while the pinch-skin grafted granulating wounds healed in 38.5 ± 1.05 days.

The mean graft acceptance in group B was 2803.50 ± 404.72 mm^2. There were no graft accepted in all the group C experiments where full thickness skin was grafted on the granulating wounds. Group D wounds had a mean graft acceptance of 41.5 ± 2.19 pinch grafts out of the 50 transplanted grafts. The mean graft acceptance of group E wounds was 31.00 ± 1.85 pinch grafts out of the 50 transplanted pinch grafts.

Group B wounds contracted at the rate of 47.25 ± 2.01 mm^2/day while group C wounds contracted at the rate of 34.93 ± 4.40 mm^2/day. Group D wound had a mean rate of contraction of 49.01 ± 4.02 mm^2/day. The mean rate of contraction in group C was 57.43 ± 0.32 mm^2/day.

Table 2 shows the comparative figures between the 5 groups. Significant differences in the healing times were found between groups B and C, B and D and groups B and E. No significant differences in the healing times were found between groups B and A and groups D and E. Insignificant differences in healing times were also found between groups A and B, A and C, A and D and groups

Table 2 Comparison of the healing time, wound contraction and graft acceptance in wounds of cattle treated by the various methods

	Significance ($P < 0.05$)									
	A/B	A/C	A/D	A/E	B/C	B/D	B/E	C/D	C/E	D/E
Healing time	NS	NS	S	NS	S	S	S	S	S	NS
Wound contraction	NS	NS	NS	S	NS	NS	NS	S		NS
Graft acceptance	-	-	-	-	S	-	-	-	S	S

KEY: A/B etc Comparison of two groups A and B

S - Significant

NS - Not significant

A and E.

The difference in graft acceptance between group D and group E wounds was significant.

Significant differences in the rate of wound contraction were found between groups, A and E, C and D and groups C and E. In-significant differences in the contraction rates were found between wounds in group A and B, A and C, A and D and between groups A and E.

The hair growth and the appearance of the healed wounds.

There was no hair growth observed in group A wounds (controls). Group B wounds showed hair growth that varied from slight to good hair cover (Figure 7). Of the eight wounds in group B, four wounds had good hair growth and one wound had moderate hair growth while two wounds showed slight hair growth. Hair growth on group C wounds was not observed. Group D wounds grew hair that ranged between a moderate hair cover to good hair cover (Figure 8). Seven wounds in group D had a moderate hair growth and one wound had a good hair growth on its surface. All the eight wounds in Group A and

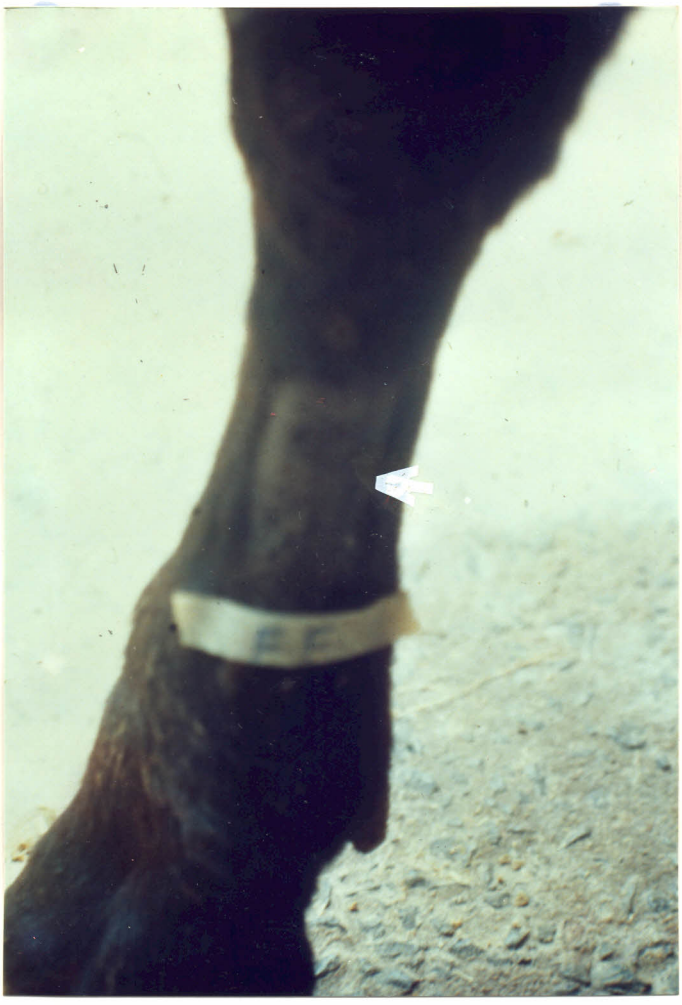

Fig. 7: The gross apperance of a healed fresh full-thickness skin grafted wound (arrow) 60 days after grafting.

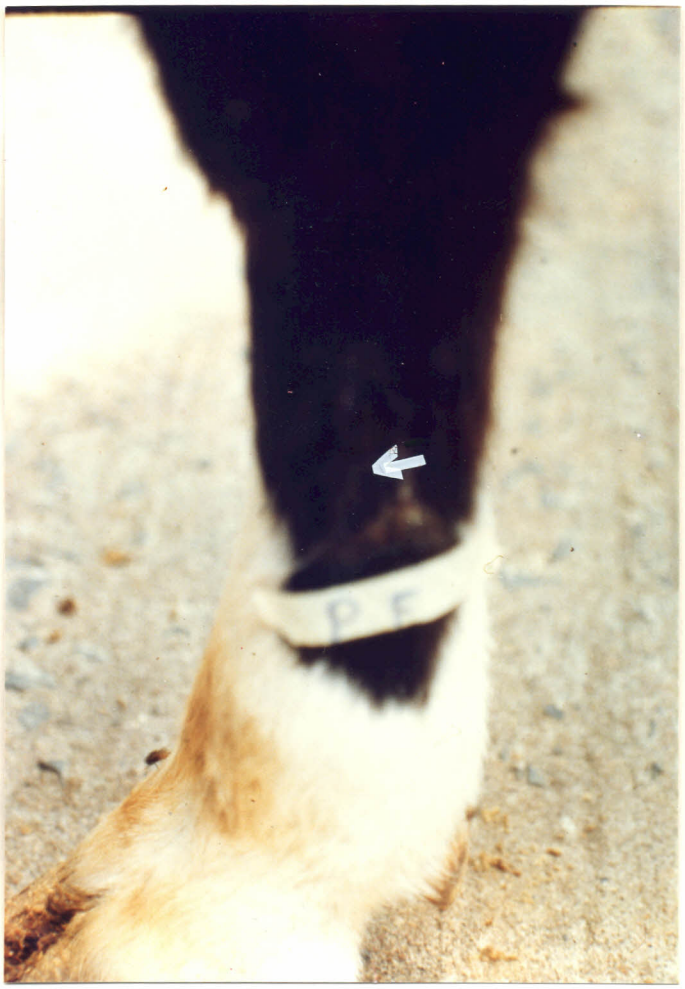

Fig. 8: A pinch skin grafred fresh wound (arrow) 60 days after grafting.

group C wounds were covered by a thin friable epithelial scar. Grafted sites in group B wounds appeared clinically normal in cases where whole grafts had been accepted. Epithelial scar, however, covered those wound areas where the grafts had not been accepted. Two wounds in group D appeared clinically normal. The other six wounds in group D showed the hair growth in tufts. There was epithelial scar between these hair tufts. All eight wounds in group E grew hair in tufts and left only thin strips of epithelium between the tufts. The appearance of the wounds after healing are summarized in Table 3.

Any hair that grew on the grafted sites retained the color of the graft.

The density of the hair follicles, sebacious glands and sweat glands in histological sections taken from grafted wounds after healing.

The mean number of hair follicles seen in sections taken from fresh full-thickness skin grafted sites (group B) was 21.38±1.07 (Figure 9). There were no hair follicles observed in sections taken from granulating wounds grafted with full-thickness skin (Figure 10). The sections taken from fresh wounds grafted with pinch skin (group D) had a value of 11.13 ± 1.14 hair follicles (Figure 11).

Table 3: The appearance of healed wounds in cattle treated by different techniques.

	Wound number							
	1	2	3	4	5	6	7	8
Group A	ES	ES	ES	ES	-	-	-	-
Group B	CN	CES	PES	ES	CN	CN	CN	PES
Group C	ES	ES	ES	ES	ES	ES	ES	ES
Group D	CN	ESH	CN	ESH	ESH	ESH	ESH	CN
Group E	ESH	ESH	ESH	ESH	ESH	ESH	ESH	ESH

KEY:

Group A - Controls - wounds not treated with grafts
Group B - Full-thickness skin grafted fresh wounds
Group C - Full-thickness skin grafted granulating wounds
Group D - Pinch skin grafted fresh wounds
Group E - Pinch skin grafted granulating wounds

CN - Clinically normal
ES - Epithelial scar
PES - Peripheral epithelial scar
CES - Central epithelial scar
ESH - Epithelial scar between hair tufts.

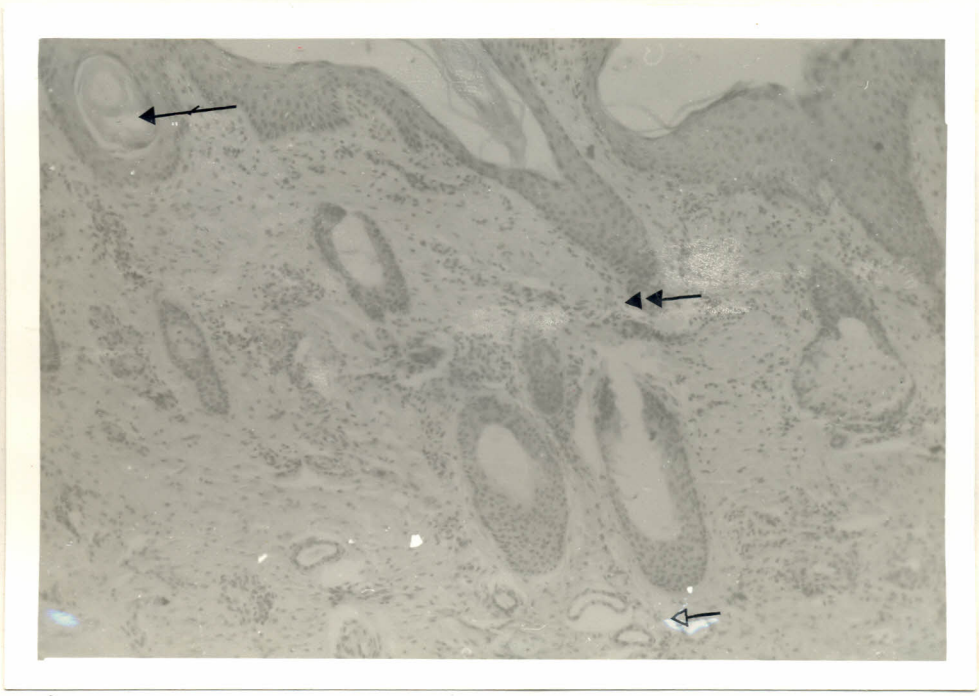

Fig. 9: Histological section (x 100) obtained from a healed fresh wound that had been treated with a full-thickness skin graft.

Hair follicle

Sweat gland.

Sebacious gland.

Fig. 10. Histological section (x 100) of a healed granulated wound after treatment with a full-thickness skin graft, showing complete epithelialization (Arrow).

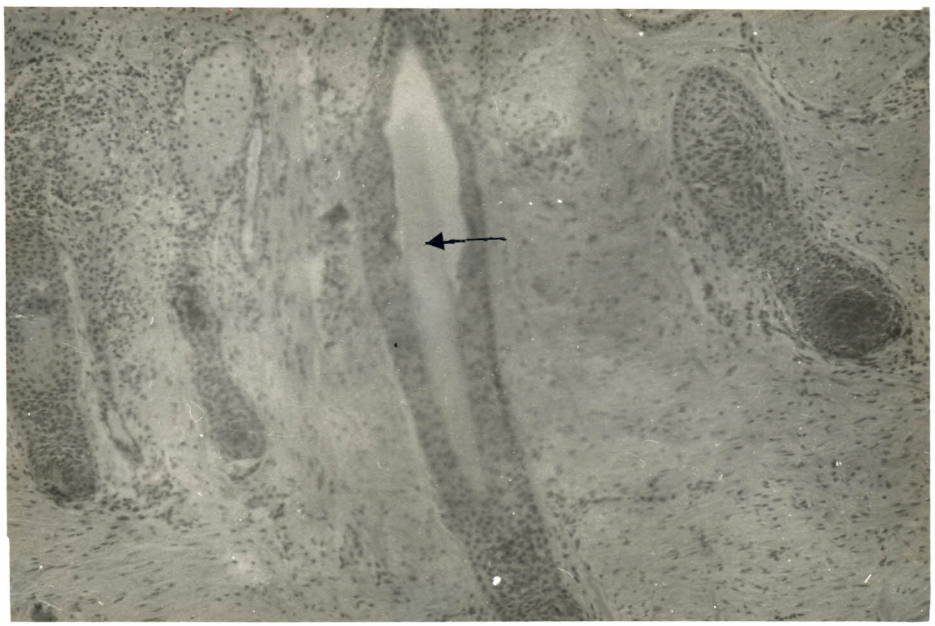

Fig. 11: Histological section (x 100) of a healed pinch skin grafted fresh wound.

→ Hair follicle.

The sections taken from granulating wounds grafted with pinch skin had a value of 12.63 ± 1.03 hair follicles (Figure 12).

The regeneration of sebacious glands in group B wounds was good with a value of 8.5 ± 1.00 . There were no sebacious glands observed in the histological sections obtained from healed wounds in group C. The group D wounds had a mean value of 2.5 ± 0.65 sebacious glands per observation field. A mean figure of 1.63 ± 0.56 sebacious glands were found in group E healed wound sections.

The group B wounds had a mean value of 5.13 ± 0.64 sweat glands per observation area. There were no sweat glands seen in the histological sections obtained from wounds in group C. A mean value of 1.00 ± 0.42 sweat glands was recorded for histological sections of wounds in group D while group E wounds had a mean value of 1.2[illegible] ± 0.48 sweat glands per observation field.

There was no significant difference found in the number of hair follicles between group D and group E wounds.

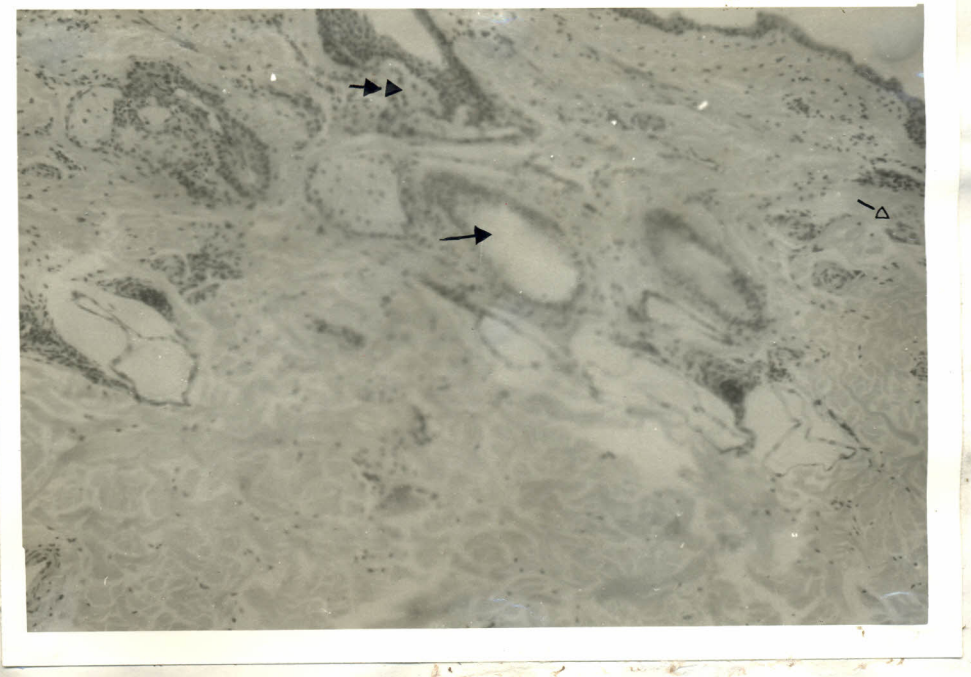

Fig. 12: Histological section (x 100) of a healed pinch skin grafted granulating wound.

→ Hair follicle

→▸ Sebacious gland

→▷ Sweat gland

A significant difference was found between the number of sebacious glands in groups B and C. The difference in the number of sebacious glands in group D and group E wounds was not significant.

There was no significant difference found between the number of sweat glands in group D and group E wounds. A significant difference in the number of sweat glands was found when group B was compared with group C. The comparison of the values between the various groups of wounds are summarized in Table 4.

Full-thickness and pinch skin grafts donor wounds.

All the donor wounds were healed fourteen days after the harvesting of the grafts. The donor sites had grown hair and looked clinically normal twenty eight days after the removal of the grafts.

Table 4: The comparison of values of histological sections of healed wounds of cattle treated by the various techniques.

	Group A	Group B	Group C	Group D	Group E	Significance (P<0.05) D/E	B/C
Number of hair follicles	-	21.37±1.07	NIL	11.12±1.14	12.63±1.03	NS	S
Number of Sebacious glands	-	8.50±1.00	NIL	2.00±0.65	1.63±0.56	NS	S
Number of sweat glands	-	5.12±0.64	NIL	1.00±0.42	1.13±0.48	NS	S

KEY: Group A - Controls - wounds not treated with grafts
Group B - Full-thickness skin grafted fresh wounds
Group C - Full-thickness skin grafted granulating wounds
Group D - Pinch-skin grafted fresh wounds
Group E - Pinch-skin grafted granulating wounds

NS - Not significant
S - Significant

DISCUSSIONS

The healing time of the control wounds was closely near that reported by Frankland (1979) for similar sized wounds in the horse. Since these wounds were not grafted, the epithelial cover and hence the healing time of the wounds depended on the extension of epithelium from the wound margins over the raw surfaces and also on wound contraction.

Fresh wounds treated with full-thickness skin grafts healed faster than the control wounds. This finding supported the observations of Woodruff (1960) who reported similar findings in the human being and also those of Wallace et al. (1962) in the dog.

Granulating wounds that were grafted with full-thickness skin took a longer time to heal compared to the control wounds. This was probably due to bacterial infection of the granulating wounds. The granulating wounds that were treated with full-thickness skin grafts also took a longer time to heal than did fresh wounds when grafted with full-thickness skin. This was because no grafts were accepted in any of the granulating wounds and therefore the epithelial cover which defined the healing time was dependent only on the migration of epithelium

from the wound edges and wound contraction. For the fresh wounds treated with full-thickness skin grafts the epithelial cover over the wounds was dependent however, on the graft acceptance, extension of epithelium from the grafts and the wound edges over any remaining raw surface and wound contraction. Thus the fresh wounds healed faster than the granulating ones.

Healing was faster in fresh wounds treated with pinch skin grafts than in the control wounds. This finding is in agreement with those of Pavletic (1983) who reported that in dogs and horses fresh pinch grafted wounds healed in 21 days. The shorter healing time was thought to be due to the fact that the control wounds were not grafted and the accepted grafts in the fresh pinch skin grafted wounds contributed to the faster epithelialization of the wounds.

The healing time for granulating wounds that were treated with pinch skin grafts was shorter than that obtained for the control wounds. Similar findings have been reported in horses by Woolsey and Schaffer (1952) and Joanne et al (1980). The healing time in these wounds was similar to that obtained in the fresh wounds which were treated with pinch-skin grafts. This was expected because the graft acceptance

in both groups was also similar.

The acceptance of full-thickness skin grafts in fresh wounds agrees with the observations in cattle by Rumawaz and Ressang (1979). The non-acceptance of full-thickness grafts in granulating wounds supports the observations made in man by Woodruff (1960) and in the horse by Frankland (1979). Peacock (1976) attributed the non-acceptance of full-thickness skin grafts in granulating wounds to bacterial infection. In this experiments there were no infections observed in the wounds but the skin grafts were still rejected in the granulating wounds.

Pinch-skin grafts were well accepted in the fresh wounds. Similar observations were made in human skin wounds by Converse (1970). Pavletic (1983) found that pinch skin grafts were readily accepted in fresh wounds of dogs and horses.

The acceptance of pinch-skin grafts in granulating wounds was significantly different from that obtained for the fresh wounds. These results do not agree with the findings in man by Dobson (1980) and Peacock and Van Winkle Jr (1976)

In these experiments, all the wounds decreased in size showing that wound contraction occurred during the healing of the wounds. The results do not agree with the observations made ealier by Frank (1964) who stated that wound contraction did not occur in the lower limbs. The wounds that were treated with full-thickness skin grafts contracted more than the control wounds. This finding also does not agree with the observations of Peacock (1976) that full-thickness skin grafts inhibit wound contraction. The results obtained in the current investigation seem to suggest that skin grafting enhances wound contraction.

The healed control wounds were covered by a thin friable epithelial scar. This supports the observations of Frank (1964) that large skin wounds in the lower limbs healed by second intention. This type of healing produced a thin epithelial scar over the healed wounds (Heinze, 1976).

Full thickness skin grafting in fresh wounds produced good hair cover and cosmetic appearance on the healed wounds. This finding agrees with the report on man by Peacock (1976) that full-thickness skin grafts produced a pliable and strong area that was functionally and cosmetically acceptable and

had good hair. Wallace et al. (1962) observed that full-thickness skin grafts produced good functional and cosmetic effects when used to treat fresh wounds in the dog. Similar observations were also made in horses by Frankland (1979).

The results obtained for hair growth and appearance of healed wounds that had been treated with full thickness grafts while granulating were similar to those of the healed control wounds. It was assumed that after the grafts fell off the granulating wounds continued to heal by second intention like the control wounds.

Hair growth and appearance of the healed wounds as observed in group the fresh pinch grafted wounds agreed with the reports by Woodruff (1960) who had worked in human patients. Similar observations were also reported in the horse by Hoffer and Alexander (1976). Hoffer and Alexander (1976) reported that pinch skin grafts grew hair in tufts which gave a poor appearance to the recipient sites. Although this aspect of the poor appearance may be of significant importance in the human being, it is generally considered unimportant in cattle.

The hair growth and appearance of healed wounds that had been grafted with pinch skin grafts while granulating was similar to that obtained for the

fresh wounds treated with pinch grafts. The appearance of the healed wounds was considered to be acceptable in the lower limbs in cattle.

These findings showed that the accepted grafts grew hair like the donor sites. This observation agreed with earlier observations that successful skin grafts remained true to type and that a full-thickness skin graft from a hair bearing area continued to grow hair after attaching to the recipient site.

There were no hair follicle, sebacious glands and sweat glands in the histological sections taken from healed control wounds and granulating wounds treated with full-thickness grafts. This finding showed that although epithelialization was complete these specialized skin appendages like hair follicles and sweat glands had not been regenerated. Billingham and Medawar (1955) reported that the hair follicles and the sebacious glands were regenerated by the migrated epithelium during the healing of full-thickness skin defects in rabbits. Peacock and Van Winkle Jr (1976) contended that the process of regeneration of these appendages started late in the healing process and usually after epithelialization was completed. The density of hair follicles,sebacious glands and sweat gland in histological section of the healed

wounds, was similar in the fresh and granulating pinch skin grafted wounds.

Healed wounds that had been treated with full-thickness skin grafts while fresh had a greater density of hair follicles sebacious and sweat glands than the granulating wounds treated with full-thickness skin grafts because more full-thickness skin grafts together with their hair follicles and glands were accepted in the fresh wounds.

In the pinch skin grafted wounds full thickness skin occured only in those points where the grafts had been placed. The areas between the grafts were only covered by epithelium of migratory origin.

CONCLUSIONS

From the findings in this investigation, some conclusions may be drawn:-

1. That full-thickness skin grafts are well accepted in, and enhance the healing of, fresh wounds in cattle.

2. That pinch skin grafts are readily accepted in, and enhance the healing of both fresh and granulating wounds in cattle.

3. That when successfully used full-thickness and pinch skin grafts produce good hair cover and a fair cosmetic appearance on the grafted sites after healing.

4. That wound contraction does occur in the healing of ungrafted and grafted lower limb skin wounds in cattle.

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A P P E N D I C E S

APPENDIX 1 to APPENDIX 8

Group A - control-wounds not treated with grafts

Group B - full-thickness skin grafted fresh wounds

Group C - full-thickness skin grafted granulating wounds

Group D - Pinch-skin grafted fresh wounds

Group E - Pinch-skin grafted granulating wounds.

APPENDIX I

Healing times (days) of wounds of cattle after treatment by various techniques

Group	Wound number 1	2	3	4	5	6	7	8
A	36	52	60	52	-	-	-	-
B	16	8	8	8	16	8	40	56
C	60	72	56	50	80	80	52	50
D	32	36	28	28	40	36	28	24
E	36	40	36	36	44	40	40	36

APPENDIX 2 Graft acceptance in cattle wounds treated by the various techniques.

Group	Wound number 1	2	3	4	5	6	7	8
A	-	-	-	-	-	-	-	-
B(mm^2)	3200	3600	3600	3600	2820	3600	1080	928
C	0	0	0	0	0	0	0	0
D(pg)	42	42	49	44	36	33	36	50
E(pg)	30	33	31	35	36	28	35	20

KEY : mm^2 = area in square millimetres (full-thickness graft)

pg = pinch grafts.

APPENDIX 3. The contraction (mm^2/day) of cattle wounds treated by the different methods

Group	Wound number 1	2	3	4	5	6	7	8
A	38.67	34.67	47.50	40.80	-	-	-	-
B	53.50	47.50	50.00	41.50	50.00	50.00	49.58	35.93
C	39.58	40.00	46.00	31.33	22.50	35.00	13.33	51.67
D	59.17	57.83	37.33	30.00	50.00	41.50	57.08	59.17
E	57.50	58.17	58/17	56.33	56.33	56.67	57.50	58.75

APPENDIX 4. The area of healed wounds (mm^2) in cattle treated by different techniques

	Wound number							
Group	1	2	3	4	5	6	7	8
A (mm^2)	1280	1520	750	1152	-	-	-	-
B (mm^2)	390	750	600	1110	600	600	625	1444
C (mm^2)	1225	1200	840	1720	2250	1500	2800	500
D (mm^2)	50	130	1360	1800	300	1100	175	50
E (mm^2)	150	110	220	220	200	450	150	75

APPENDIX 5 The degree of contraction of wounds in cattle treated by the various techniques

Group	Wound number 1	2	3	4	5	6	7	8
A	2320	2080	2850	2448	-	-	-	-
B	2310	2850	3000	2490	3000	3000	2975	2156
C	2375	2400	2760	1800	1350	2100	800	3100
D	3550	3470	2240	1800	3300	2490	3425	3550
E	3450	3490	3380	3380	3380	3400	3450	3525

APPENDIX 6 The number of hair follicles counted in histological sections of cattle wounds treated by different techniques.

	Wound number							
Group	1	2	3	4	5	6	7	8
A	-	-	-	-	-	-	-	-
B	26	18	22	18	21	19	25	22
C	0	0	0	0	0	0	0	0
D	14	16	12	8	8	11	7	13
E	10	16	13	11	8	22	14	16

APPENDIX 7 The number of Sebacious glands counted in histological sections of cattle wounds treated by the various methods

	Wound number							
Group	1	2	3	4	5	6	7	8
A	-	-	-	-	-	-	-	-
B	12	12	6	9	4	8	10	7
C	0	0	0	0	0	0	0	0
D	6	2	3	2	2	0	1	4
E	2	0	1	3	0	3	0	4

APPENDIX 8 The number of sweat glands counted in histological sections of cattle wounds treated by the various techniques.

	Wound number							
Group	1	2	3	4	5	6	7	8
A	-	-	-	-	-	-	-	-
B	3	6	8	3	5	5	7	4
C	0	0	0	0	0	0	0	0
D	2	3	0	2	0	0	1	0
E	2	0	0	0	1	3	0	3

APPENDIX 9 TO APPENDIX 24

Observations made at 4 day intervals on gragted wounds

KEY: a) = colour of the graft
- BC = Black
- B = Brown
- BL = Blue
- W = White
- P = Pink

b) = Blushing of grafts on application of digital pressure
- \+ = Yes
- \- = No

c) = Adherence of grafts to graft beds
- F = Firm
- LC = loose centrally
- LP = loose peripherally

d) = Presence of exudate between grafts and graft beds
- \+ = Present
- \- = Absent

e) = epidermal slough
- \+ = Yes
- \- = No

f) = Hair growth
- \- = No
- \+ = Yes

g) = Graft lost
- \+ = Yes
- \- = No

APPENDIX 9 Full-thickness skin grafts in fresh wounds: Observations at 4 day intervals on Wounds I and II

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	P	B	B	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
I	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e)	-	+													
	f)	-	-	-	+											
	a)	P	P	BL	B	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	LC	LC	LC	LC	Loose Central portion fell off.							
II	d)	-	-	-	+											
	e)	-	-	-	-	+										
	f)	-	-	-	-	-	-	-	+							

APPENDIX 10. Full-thickness skin grafting in fresh wounds. Observations at 4 day intervals on wounds III and IV

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	P	BL	BL	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	LP	LP	LP	LP	Loose edge fell off.							
III	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e)	-	-	-	+											
	f)	-	-	-	-	+										
	a)	P	P	BL	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
IV	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e)	-	-	+												
	f)	-	-	+												

APPENDIX 11

Full-thickness skin grafting in fresh wounds.

Observations at 4 day intervals on Wounds V and VI

		DAYS													
		4	8	16	20	24	28	32	36	40	44	48	52	56	60
WOUND V	a)	P	P	BL	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	d)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	e)	–	+												
	f)	–	–	+											
WOUND VI	a)	P	P	B	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	d)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	e)	–	+												
	f)	–	+												

APPENDIX 12. Full-thickness skin grafting in fresh wounds.
Observations at 4 day intervals on wounds VII and VIII

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VII	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e)	-	+													
	f)	-	+													
	a)	P	P	B	B	B	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VIII	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e)	-	+													
	f)	-	+													

APPENDIX 13. Pinch-skin grafting in fresh wounds. Observations made at 4 day intervals on Wounds I and II

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
WOUND I	a)	P	P	W	B	B	B	B	B	B	B	B	B	B	B	B
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
WOUND II	a)	P	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

KEY

S = Slight

N = None

APPENDIX 14

Pinch-skin grafting in fresh wounds observations at 4 day intervals on wounds III and IV

		DAYS													
		4	8	12	16	20	24	28	32	36	40	44	48	52	60
WOUND III	a)	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	+	+	+	+	+	+
WOUND IV	a)	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	N	N	W	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	+	+	+	+	+

APPENDIX 15. Pinch skin grafting in fresh wounds.

Observations at 4 day intervals on wounds V and VI

		DAYS													
		4	8	12	16	20	24	28	32	36	40	44	48	52	60
WOUND V	a)	P	P	W	B	B	B	B	B	B	B	B	B	B	B
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	+	+	+	+	+	+
WOUND VI	a)	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	+	+	+	+

APPENDIX 16. Pinch skin grafting in fresh wounds.

Observations made at 4 day intervals on wounds VII and VIII

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	P	W	B	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VII	c)	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
	a)	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VIII	c)	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+

APPENDIX 17. Pinch skin grafting in granulating wounds.

Observations made at 4 day intervals on wounds I & II

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	P	W	W	B	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
I	c)	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	a)	P	P	W	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
II	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

APPENDIX 18 Pinch skin grafting in granulating wounds observations at 4 day intervals on wounds III and IV

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	W	W	B	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
III	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	a)	P	P	P	P	W	B	B	B	B	B	B	B	B	B	B
	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	c)	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

APPENDIX 19. Pinch-skin grafting in granulating wounds. Observations at 4 day intervals on wounds V and VI

		DAYS														
		4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
	a)	P	P	P	W	B	B	B	B	B	B	B	B	B	B	B
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
V	c)	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	a)	P	P	P	P	W	B	B	B	B	B	B	B	B	B	B
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VI	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

APPENDIX 20

Pinch-skin grafting in granulating wounds. Observations at 4 day intervals on wounds VII and VIII

		D A Y S													
		4	8	12	16	24	28	32	36	40	44	48	52	56	60
	a)	P	P	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VII	c)	S	S	N	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	+	+	+	+	+	+
	a)	P	P	P	W	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
WOUND	b)	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VIII	c)	S	S	S	N	N	N	N	N	N	N	N	N	N	N
	d)	-	-	-	-	-	-	-	-	-	-	-	-	+	+

APPENDIX 21 Full-thickness skin grafting in granulating wounds. Observations at 4 day intervals.

		DAYS									
		4	8	12	16	20	24	28	32	36	40
	a)	P	P	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	
WOUND	c)	F	F	F	F	F	LP	LP	LP	LP	
I	d)	+	+	+	+	+	+	+	+	+	
	e)	-	-	-	-	+					
	f)	-	+								
	g)	-	-	-	-	-	-	-	-	-	+
	a)	P	B	B	B	B	B	B	B	B	
	b)	+	-	-	-	-	-	-	-	-	
WOUND	c)	F	F	F	F	LP	LP	LP	LP		
II	d)	-	+	+	+	+	+	+	+		
	e)	-	-	-	-	+					
	f)	+									
	g)	-	-	-	-	-	-	-	-	+	

APPENDIX 22. Full-thickness skin grafting in granulating wounds. Observations made at 4' day intervals.

		DAYS						
		4	8	12	16	20	24	28
	a)	P	B	B	B	B	B	B
	b)	+	-	-	-	-	-	
WOUND	c)	F	F	F	LP	LP	LP	
III	d)	-	+	+	+	+	+	
	e)	-	-	+	+	+	+	
	f)	-	+	+	+	+	+	
	g)	-	-	-	-	-	-	+
	a)	P	B	B	B	B	B	B
	b)	+	-	-	-	-	-	-
WOUND	c)	F	F	F	LP	LP	LP	
IV	d)	-	+	+	+	+	+	
	e)	-	-	-	+			
	f)	-	+					
	g)	-	-	-	-	-	-	+

APPENDIX 23. Full-thickness skin grafting in granulating wounds.
Observations at 4 day intervals.

		D A Y S											
		4	8	12	16	20	24	28	32	36	40	44	48
	a)	P	P	B	B	B	B	B	B	B	B	B	B
	b)	+	+	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	F	LP	LP	LP	LP	LP	LP	
V	d)	-	-	-	+	+	+	+	+	+	+	+	
	e)	-	-	-	-	-	+	+					
	f)	-	-	+									
	g)	-	-	-	-	-	-	-	-	-	-	-	+
	a)	P	P	B	BC	BC	BC	BC	BC	BC	BC	BC	BC
	b)	+	+	-	-	-	-	-	-	-	-	-	-
WOUND	c)	F	F	F	F	LP	LP	LP	LP	LP	LP		
VI	d)	-	-	+	+	+	+	+	+	+	+		
	e)	-	-	-	-	-	+	+					
	f)	-	-	+									
	g)	-	-	-	-	-	-	-	-	-	-	-	+

APPENDIX 24. Full-thickness skin grafting in granulating wound. Observations at 4 day intervals.

D A Y S

		4	8	12	16	20	24	28	32
	a)	P	P	BC	BC	BC	BC	BC	BC
	b)	+	+	-	-	-	-	-	-
WOUND	c)	F	F	F	F	LP	LP	LP	
VII	d)	-	-	+	+	+	+	+	
	e)	-	-	-	+				
	f)	-	+						
	g)	-	-	-	-	-	-	-	+
	a)	P	P	BC	BC	BC	BC	BC	
	b)	+	+	-	-	-	-	-	
WOUND	c)	F	F	LP	LP	LP	LP		
VIII	d)	-	+	+	+	+	+		
	e)	-	-	-	+				
	f)	-	+						
	g)	-	-	-	-	-	-	+	