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WOUND HEALING AND CELLULAR MICROENVIRONMENT

Final Technical Report

by

I. A. Silver

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13. ABSTRACT

Investigations of oxygen supply to dermal and epidermal elements in healing tissue have been made with oxygen electrodes in rabbit ear window preparations and in human subjects. Effects of mild stress and hemorrhagic and endotoxic shock on wound environment have been measured with oxygen, pH, and carbon dioxide electrodes; a new micro carbon dioxide electrode is described. Development of a laser flash technique for measuring diffusion of oxygen in tissue is described. Studies have been initiated on the effects of anticollagen antibodies on fibroblasts in order to examine their possible role in the slow healing of burns. Oxygen applied directly to healing surfaces is likely to speed epidermal healing, and wound dressings that exclude atmospheric oxygen are likely to slow epidermal healing. The development of hemorrhagic or endotoxic shock delays wound healing because vessels in a damaged area are especially liable to perfusion failure and to leakage. Treatment of shock by intravenous infusion of fluids of low colloid osmotic pressure may well worsen the local environment of a wound.

Key Words: Wound Healing: Tissue oxygen tension; Fibroblasts; Microcirculation; Shock; Skin permeability to oxygen; Carbon dioxide electrode; Oxygen diffusion rate in tissue; Anticollagen; Burns: slow healing.

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Summary

Investigations of oxygen supply to dermal and epidermal elements in healing tissue have been made with oxygen electrodes. The effects of mild stress and of haemorrhagic and endotoxic shock on wound environment have been measured with microelectrodes sensitive to oxygen, pH and P_{CO2}. The development of a method for measuring diffusion of oxygen in tissue based on a laser flash technique is described together with a new, micro P_{CO2} electrode. Studies have been initiated on the effects of anticollagen antibodies on fibroblasts with a view to examining their possible role in the slow healing of burns.

It is concluded that increased rates of healing of superficial wounds are likely to be obtained if the oxygen concentration of atmospheres above the wound is increased. Wound dressings which exclude access of atmospheric oxygen seem likely to slow epidermal healing.

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General Introduction

The projects reported here have been carried out principally at the Universities of Cambridge and Bristol, and at the Bristol Royal Infirmary, while collaborative activities have been possible with Dr. Hunt in the San Francisco Medical Center, Ca., with Dr. Juha Niinikoski of the Department of Surgery, University Hospital of Turku, Finland, with Dr. A. Glinos of WRAIR, Washington D.C. Joint studies have been made with Dr. Britton Chance and Dr. Leena Mela of the University of Pennsylvania at Philadelphia.

Work performed during the contract period has formed the basis of a number of scientific communications. Three publications relevant to various aspects of the projects are now in Press and will appear shortly (1,2,3). One of these forms the basis of the section on 'Epithelialisation in relation to oxygen tension'.

This report is divided into sections.

The first deals with technical developments, experimental models and materials and methods.

The second is concerned with measurements made in healing connective tissue.

The third is an account of the effects of oxygen on epithelial tissue growing across open wounds.

The purpose of the investigations has been to study the effects of various conditions which are likely to occur in the clinical situation, on the local cellular environment in wounds, and to discover where possible, how these changes in the environment may promote or discourage healing. Experimental and clinical trials have been instituted to see if information gained from these basic studies can be applied usefully to encourage more rapid healing.

SECTION 1

Technical developments

Since the methods of investigation used in this contract are largely extensions of or developments from those already published in the Final Technical Report of Contract DAJA37-69-C-1169 (4) new aspects only will be described.

1) Rabbit ear chambers

The modifications of the Sumner Wood (5) chamber previously described (6) which allowed measurements of tissue microenvironment in a thin layer of healing tissue have continued to give adequate access for electrode and fluorescence studies. A problem was encountered during the course of the present investigation when uniformly poor growth of tissue in some batches of chambers occurred. This interfered with assessment of other factors as possible causes of reduced healing rate. The cause of the inhibition of tissue growth was found to be the variable quantity of plasticiser present in different batches of the acrylic sheet being used for the manufacture of the chambers. Apparently the plasticisers slowly leaked out into the tissue fluid and eventually reached toxic levels. This toxic effect was identified by placing the various components of the ear chambers in separate rabbit fibro-blast cultures and observing the responses of the cultured cells. Many samples of acrylic induced zones of dead cells around themselves in a manner reminiscent of the death of bacterial colonies around antibiotic test discs.

Attempts to remove the plasticisers were unsuccessful so other materials were investigated for suitability for ear chambers. It was found that one material, polymethylpentene, possessed almost all the qualities that were desirable for long term implants in tissue and appeared to be quite free of toxic effects.

Polymethylpentene (TPX, Imperial Chemical Indutries Ltd., U.K.) is a light (S.G.< 1) slightly opalescent, transparent, somewhat flexible plastic with good machining properties. It contains no plasticiser and can be bonded with the appropriate cement (Chemlok 305, Durham Raw Materials Ltd., London, U.K.). It is resistant to distortion at normal autoclaving temperatures (120°C) and ear chambers made from it can therefore be heat sterilised, which eliminates the danger of toxic gas retention, a feature of ethylene oxide sterilisation of acrylic chamb ..

TPX chambers have been modified from acrylic chambers by machining them from rod in two pieces only (acrylic chambers were made in three separate parts). The 'table' and 'plug' of the chamber are turned as a single unit, with a solid plug (hollow in the acrylic pattern). The table and free end of the plug are then polished, the ring and the 'skirt' attached with adhesive, and the whole assembly is then baked at 70°C until the adhesive is hard (2-3 days). A solid plug is acceptable because the material is so light. This construction adds strength to the chamber and enables the plug surface to be cleaned easily if it should become covered with blood or other exudate. The TPX chambers are somewhat more easily scratched than the acrylic chambers but are less fragile and out of the first 150, only two have been broken in use. The comparable breakage rate for acrylic chambers would be about 20.

2) Operation

The mode of insertion of ear chambers has been modified slightly from that previously described, in order to render damage to the ear cartilage less likely. It has been found preferable to raise the skin flaps and to make the skin pouch for the accommodation of the chamber before punching a hole in the ear cartilage. The hole is now punched just before the chamber is inserted and this has considerably reduced the number of chambers that had to be rejected, or which grew unsatisfactorily, because of tearing of the ear cartilage at the edges of the hole.

3) Electrode systems

a) oxygen micro-electrodes of the types already described (6) have continued to be used in this investigation, both for surface measurements and for insertion into tissues, and also for measuring the O_2 environment under occlusive dressings (see Section 3).

b) multiwire, surface oxygen electrodes have been constructed similar to those described by Huch (7).

These were made by inserting a number of 5 or 10μ platinum wires into individual glass capillary tubes which were subsequently fused together. The end of the platinum-in-glass assembly was ground flat and the free wires at the back of the capillaries were twisted carefully together. The electrode assembly was placed inside a Perspex housing 5mm in diameter and fixed with adhesive so that the electrode tips protruded slightly below the casing. A silver wire was included in the adhesive and extended into the space between the electrodes and the casing. Contact with the backs of the electrode wires was made with a mercury seal. Electrolyte was introduced between electrodes and the casing, and the open end of the case was sealed with a 12 or 25μ Teflon membrane secured by an 'O' ring.

Both macro and micro-electrodes were polarised with a D.C. potential of between -600 and -800 mV., which was determined by the individual electrode characteristics. The current output from the electrodes, which was directly proportional to the oxygen concentration in the electrolyte between the electrode tip and the membrane, was fed to a high input impedance DC amplifier. Micro-electrodes gave a current of about 10^{-13}A/mmHg PO_2 whereas multiwire electrodes gave relatively large currents of the order of 10^{-5}A/mmHg PO_2, although this varied according to the size and number of the wires used to make the cathodes.

c) <u>micro-pH electrodes</u>. It has become very desirable to measure local extracellular pH, and, if possible, intracellular pH particularly in respect of changes occurring during shock. The earliest useful electrode of the appropriate size range was that of Caldwell (8) but its dimensions are too large for all but rather crude readings. Hinke (9) described a much smaller electrode for measuring intracellular sodium concentrations and other microelectrodes for measuring ionic concentrations have been produced by Walker (10) and Carter et al. (11). All have some disadvantages, especially for intracellular work, in that a considerable length of ion sensitive glass must be inserted into a cell. This is acceptable for some exceptionally large invertebrate cells but is generally unsatisfactory for most mammalian cells. Recently Thomas (12,13) has developed an ingenious 'recessed' sodium sensitive electrode with a tip of 1.0u or less in which the ion sensitive element is protected by a normal pyrex glass micropipette.

By using Thomas' technique but employing pH glass (Corning 0150) instead of sodium sensitive glass (NAS-11-18) it has been possible to construct a recessed pH electrode small enough to insert into a single mammalian liver cell or macrophage. Such electrodes have a rather slow response (between 0.5-2 min for 95% change). They have a very high impedance and require careful electrical shielding. They are also extremely fragile and can be used only in conjunction with a rigid system.

d) micro-P_{CO2} electrode. As a development from the recessed 'Thomas type' micro-pH probe it has been possible to make an electrode of 1.0μ tip size which responds to local extracellular or intracellular CO_2 concentrations. The electrode is constructed as for a pH probe and then the recess is filled with 0.01 N $NaHCO_3$ and this is sealed into the recess by covering the tip of the electrode with Rhoplex resin AC-35 (Rohm Haas, Philadelphia, Pa.). The electrode is then used as a conventional pH probe, the pH change being linearly related to the $\log_{10}$ of the CO_2 change.

4) <u>Shock models</u>

a) Haemorrhagic shock was induced in anaesthetised rats by cannulating a femoral artery and bleeding the animal into a heparinised reservoir over a period of 30 minutes until the systemic blood pressure was 30 mmHg. This pressure was maintained by withdrawal or injection of blood as necessary.

In rabbits, similar treatment was used except that blood pressure was not lowered beyond 55-50 mmHg because the animals rapidly succumbed if the systemic pressure fell below this level.

b) Endotoxic shock was induced in rabbits and rats by intraperitoneal or intravenous injection of polysaccharides either from Serratia marcescens or Escherichia coli (Difco Laboratories Inc., Surrey, U.K.). The dosage used varied from batch to batch and was based on one LD50.

5) Minor stress

Animals in which granulation tissue was growing were subjected to changes in handling routine or were accommodated in unfamiliar surroundings which were s[illegible] to intermittent changes of noise level.

6 Anticollagen sera

Purified collagen of various origins (rabbit, rat, human) was used to stimulate specific anticollagen immunoglobulins in [illegible] animals. The gamma-globulins were recovered and purified by routine methods and labelled with fluorescein-iso-thiocyanate (FITC) or with high molecular weight peroxidase [illegible] from Horse-radish or with cytochrome oxidase (14,15). Fab fragments of anti-collagen IgG were prepared and similarly labelled.

7) Human fibroblasts, of a strain originally derived from the stroma of a reticulum cell sarcoma, were used for testing the specificity of the antihuman collagen IgG. This strain has a characteristically high production of collagen in culture and the cell walls appear to be incomplete in the areas where protocollagen strands are extruded.

8) A small oxygen chamber designed to accommodate a human arm or leg under slightly increased pressure was kindly lent by Mr. P.M. Lock for the preliminary investigation on the effects of local oxygen therapy to slow healing wounds. This proved to be useful in special circumstances but was somewhat cumbersome, and has been replaced for routine purposes by a simple polyethylene bag, strapped to the limb. Oxygen is fed into the bag as necessary, to maintain a high O_2 concentration over the affected area.

9) Healing in relation to type of wound dressing. Sk[illegible] dressings tested were (a) standard hospital gauze swabs, (b) Nonad-tulle (Allen and Hanbury), (c) 12 and 6[illegible] Teflon Film, (d) Polythene film, and (e) Melinex polyester film (I.C.I.).

Short term alterations in wound oxygen tension were achieved (1) by altering the respiratory gas composition, (2) by enclosing the wounded area in a plastic bag whose gas content could be varied, and (3) by altering both respiratory gas tension and the atmosphere over the wound. With small animals this last was done by enclosing the animal in a case inside a gas filled bag.

The investigation of oxygen permeability of skin and healing processes were carried out on small laboratory animals (rats, mice and rabbits) and also on various human volunteers. A few measurements were also made on pigs.

Skin stripping was carried out with standard commercial cellulose tape.

10) The Laser flash system for investigation of O_2 diffusion rate in tissue (in collaboration with Dr. Britton Chance, Philadelphia). The level of pyridine nucleotide reduction in cells of interest was measured by U.V. fluorescence with a 10μ spot. The exciting wavelength was 366 nM and the emission was at about 450nm. The oxygen concentration in the region under observation was reduced by giving the animal a mixture of 95% N_2 and 5%CO to breathe, or more safely, by giving the animal 100% N_2 to breathe and passing a current of CO gas across the surface of the organ under investigation. In these hypoxic conditions, cytochrome oxidase combines completely with carbon monoxide and the NADH fluorescence becomes maximal. The animal was then ventilated with oxygen and the advent of oxygenated blood to the vicinity of the U.V. spot was detected by a rapidly responding oxygen micro-electrode placed against a capillary. At that moment, ideally, when the vascular oxygen pool was being disseminated by diffusion into the tissue spaces and an oxygen gradient was being established, a liquid dye laser was flashed at wavelength of 585 nm. This ruptured the cytochrome a_3-CO bond. If there was oxygen in the vicinity at the time of the flash the mitochondria responded very rapidly by oxidation of the NADH to NAD with a consequent reduction in fluorescence intensity. Those mitochondria that oxygen had not reached at the time of the flash became oxidised more slowly. It was possible to vary the time of the flash in relation to time of arrival of oxygen in the capillary bed and also to site the U.V. spot at different distances from the capillary and at different points along a capillary.

This made it possible to plot the process of oxygenation in a tissue and to investigate the changes that might occur as a result of pathological processes such as shock, trauma, oedema etc. or inflammation.

The method has not yet been perfected and some technical problems remain to be worked out. Among these are:

1) Tissue in which O_2 diffusion is slow, and this applies in early healing tissue and in traumatised or shocked conditions show a relatively small response to the laser flash if there is any appreciable photo-dissociation of a_3-CO caused by the U.V. light. This can be alleviated to some extent by a device to keep the fluorescence excitation at a low level until a moment before the flash, when full intensity is developed.

2) Cytochrome oxidase is far removed from pyridine nucleotide in the respiratory chain and NADH fluorescence is not therefore an ideal monitoring system for mitochondrial oxidation. Unfortunately flavoprotein fluorescence (which would be more suitable) is seriously interfered with by haemoglobin changes in non perfused systems.

These problems can be overcome and the almost two dimensional ear chamber model healing tissue makes a very simple system for investigation, compared with normal three dimensional preparations.

ii) Oxygen sterilisation.

During the period when acrylic ear chambers were in use, heat sterilisation of the prosthesis was impossible due to distortion of the acrylics at temperatures above 55°C. Cold ethylene oxide gas sterilisation was satisfactory for killing organisms but presented problems when freshly sterilised material was required for immediate use. The ethylene oxide dissolved in the plastic and was released slowly over a period as long as 3 days. The implanting of material into the body, which still contained small amounts of ethylene oxide could lead to local cell death in the vicinity of the implant. [illegible] alternative method of cold sterilisation [illegible] by Mr. P.M. Lock and based on high pressure oxygen [illegible] tested and found to be satisfactory for sterilising acrylic,

polythene and polyvinyl plastics. The system consists of a small pressure tank in which articles to be treated are placed after individual packeting. Oxygen from a standard gas cylinder is passed into the pressure tank until a pressure of 10 atmospheres is achieved and the tank is then closed and left for 30 minutes. It is then depressurised.

Plastic materials, deliberately contaminated with a wide range of pathogenic and other bacteria, including sporulators were found to be bacteriologically sterile after such treatment. No tests on viral contamination have yet been carried out. This system offers a simple and effective means of sterilising both solid and porous articles that are heat sensitive, and leaves no contamination.

12) Polymethacrylate sponge has been used in surgery for tissue replacement but it appears to excite bone formation after it has been in position for some months (16). Dr. G. Winter, of the National Orthopaedic Hospital, Stanmore, kindly provided a sample of material for testing in tissue culture. The characteristics of the material which induced bone formation in the body were that it was hydrophilic, that calcium deposited on its surfaces, that its pore size was between 40 and 60μ and that it was actively invaded by fibroblasts. There seemed to be some doubt as to whether the bone formation seen in tissue implants was derived from altered fibroblastic activity or from invasion of the foam with a new population of cells, possibly of bone marrow origin, which displaced the original fibroblast population.

Polymethacrylate sponges have now been kept for more than one year, in human, pig and other fibroblast cultures. Although calcium has deposited on the surface of the material and cells have invaded all the pores, no activity resembling bone formation has developed.

13) Microblood flow system. A T.V. line selection system was developed to try to quantify local blood flow in relation to local PO_2 measurements in wound tissue. A small blood vessel was identified under an appropriate microscope objective and a Link T.V. camera was connected to one eye piece of the microscope. The T.V. image was displayed on a monitor and a frame line was selected for analysis in the region of the image near to one oxygen microelectrode.

The camera was arranged so that the lines crossed the capillary at right angles. The selected line was displayed on an oscilloscope. Variations in density of the image on different sections of the line appeared in terms of voltage on the screen. The passage of a red cell across the line changed the picture density and therefore the voltage on the line in the vicinity of vessel. This system is still in the development stage but shows some promise. The chief difficulty has been inadequate response time and a tendency for 'persistence of vision' in the videcon. It also has an inherent limitation that it can only be used for measurement in vessels with a slow rate of flow, i.e. where red cells pass in single file with clear separation between them.

14) Macrophage culture. Rabbits were injected intraperitoneally with 5ml sterilised medicinal mineral oil and macrophages were harvested 7 days later after the animals were sacrificed. The cells were then cultured in hypoxic condition for varying periods, and their phagocytic activity tested at intervals by exposing them to carbon particles. It was hoped to test whether macrophages in conditions of hypoxia may produce a substance which could stimulate capillary endothelial growth. In vivo, capillary [illegible] appear to follow a 'lead' from macrophages but to [illegible] we have been unable to show that any specific chemo[illegible] substance is involved.

15) Multipoint oxygen [illegible] on coverslips have been made by [illegible] of [illegible] of platinum on melinex films but it has [illegible] up to the present time to obtain sufficiently good insulation of the gold track connections to make this system reliable.

SECTION II

Measurements in healing connective tissue

The importance of oxygen supply to healing tissue had been established both in terms of rate of repair, the subsequent strength of the scar and biochemical processes (17,18,19,20). Somewhat less attention has been paid to other local factors such as P_{CO2} and pH, chiefly because of the lack of suitable detector devices, although Hunt and co-workers (21) have made measurements of these parameters in wound fluids. The development of suitable microelectrodes as described in Section I has enabled a preliminary assessment of local cellular environment to be made using granulation tissue in the rabbit ear chamber as a test situation.

a) Findings in normal wound repair

1) Extracellular pH

Unlike oxygen gradients, pH gradients appear to be shallow except at the extreme edge of the healing wound. No major differences in extracellular pH were found in regions where capillary flow was established; all readings were within the range pH 7.0-7.3. Within the wound cavity, more than 150μ from the leading capillaries, the situation was much more variable and values as low as pH 6.0 were encountered. The pH level appeared to parallel the cell content. A predominance of polymorphonuclear cells was associated with low pH whereas macrophages were usually the main cell type above pH 6.3.

2) Extracellular P_{CO2}

In normally perfused tissue it was difficult to detect any CO_2 gradients. As in the case of pH measurements, the only obvious differences in CO_2 concentration were those between the wound cavity and the perfused tissue. Even at the wound edge, no gradients could be detected that were clearly associated with any particular structure or cell type. There was a smooth rise in P_{CO2} from the perfused capillary zone to the wound fluid. Unlike the pH, the P_{CO2} in the wound fluid appeared to be correlated only with cell population density and not with cell type, although this might be misleading, since high cell densities were usually found to be associated with polymorphonuclear cells.

CO_2 levels were rather sensitive to respiration rate, and it was found in anaesthetised animals that tissue P_{CO2} tended to rise during the observation period. Typically, in well perfused tissue the P_{CO2} was between 38 and 42 mmHg; at the wound edge, in recent wounds (early healing stage) it was 45-50 and in the wound fluid it might be as high as 60-70 mmHg, if there was a large wound cavity. During the late stages of healing when the cavity was almost eliminated CO_2 tensions in the wound fluid fell to 45-50 mmHg.

b) P_{CO2}, PO_2 and pH during shock

1) Haemorrhagic shock Measurements were made in two models. The first was the rabbit ear chamber and the second was the carrageenin induced granuloma in the rat.

During the bleeding out period, i.e. the phase of acute haemorrhage which precedes the shock state it was noticeable that perfusion of wound tissue slowed and finally stopped before the systolic blood pressure had fallen more than 20 mm Hg. The accompanying changes in local gas tensions are set out in the following table (1). It is apparent that granulation tissue is very susceptible to the effects of lowered systemic pressure.

TABLE 1 PO_2, P_{CO2} and pH values during acute haemorrhage

		Systolic blood pressure (Rat) mm Hg					
		110	100	80	60	40	30
PO_2 mmHg (average)	Wound edge	21	13	3	0	0	0
	Liver	33	28	12	5	<1	<1
	Brain	27	28	25	26	21	17
P_{CO2} mmHg	Wound edge	45	50	65	82	-	>100
	Liver	43	43	48	55	-	68
	Brain	38	38	35	38	-	39
pH	Wound edge	7.0	6.9	-	6.5	6.3	-
	Liver	7.1	7.1	-	6.9	6.7	-
	Brain	6.95	6.95	-	7.0	6.95	-

Each figure is a mean of 50 readings

When true 'shock' developed, i.e. after half an hour at 30 mmHg systolic pressure P_{CO2} levels as high as 120 mmHg were found in wound cavity and the surrounding non perfused tissue and pH levels fell to 5.5 in wound fluid after 6 hours of shock.

It is perhaps of some significance that the liver is evidently very sensitive to changes in systemic circulation but the whole liver is not affected equally at once. Oxygen needle electrodes indicate that the microcirculation fails on a microregional basis and that some areas lose perfusion very early in acute haemorrhage while others are more resistent. The measurements in Table 1 are from an area in which the microcirculation failed early.

A few intracellular measurements of P_{CO2} and pH have been made on liver cells and macrophages during normal conditions and in shock. These indicate that changes of as much as one pH unit may occur during haemorrhagic shock, but as yet the technique is insufficiently worked up to give absolute figures.

2) <u>Endotoxic shock</u> Anaesthetised rabbits and rats, given L.D.50 doses of endotoxins by the intraperitoneal route, showed early changes of PO_2, pH and P_{CO2} in wound tissue, before any change of systemic blood pressure was apparent (Table 2). These changes were preceded by a very short period of increased PO_2 levels which lasted for 1-2 mins and started about 10 min after the injection of the endotoxin.

TABLE 2 Extracellular PO_2, P_{CO2} and pH changes in wound tissue (Rabbit ear chamber) after L.D.50 dose of E.Coli lipopolysaccharide (i.p)

	Time after injection (mins)				
	0	10	15	30	120
PO_2	22	26	12	0	0
P_{CO2}	45	45	48	63	>100
pH	7.1	7.1	7.1	6.8	5.9

Parallel to the microelectrode measurements, routine electron microscopy of samples of liver and granulation tissue during the development of shock states hasbeen carried out in this laboratory and also by Dr. Mela in Philadelphia. The mitochondrial changes which are a marked feature of shock in liver cells and fibroblasts in the growing and synthetic phase, but not in the resting phase, appear to parallel the pH changes (22). Biochemical assays of liver mitochondrial activity in endotoxic shock show that uncoupling of oxidative-phosphorylation occurs during the phase of mitochondrial damage which can be seen at the ultra-structural level.

Attempts to reproduce the local effects of shock in liver and granulation tissue by induction of hypoxia have shown that hypoxia is not the main cause of shock damage.

Table 3 shows the PO_2, P_{CO2} and pH changes in liver and granulation tissue when a normovolaemic animal was respired with an O_2 level which reproduced the tissue PO_2 seen in haemorrhagic shock.

TABLE 3 Extracellular PO_2, P_{CO2} and pH in liver and granulation tissue in a normovolaemic animal respired on10% O_2 90% N_2

		Time Mins.				
		0	10	30	60	120
PO_2	Liver	31	10	4	6	3
	Wound	20	3	<1	0	0
PCO_2	Liver	43	41	45	43	42
	Wound	45	40	47	47	48
pH	Liver	7.1	7.1	7.0	7.1	7.0
	Wound	7.0	7.0	7.0	7.0	6.95

No obvious changes in mitochondrial structure of liver or wound fibroblasts took place after this treatment and it is noticeable that local pH changes are minimal. Blood flow patterns varied during the hypoxic episode but at no time did microcirculation stop completely.

c) Long term effects of stress and shock on healing rate and wound environment

1) Minor stress The growth of granulation tissue in rabbit ear chambers was followed in two groups of rabbits. These animals all had one chamber in the right ear and were divided randomly. One group was examined once every 2 days and the other was examined twice each day. Examination consisted of removal of the animal from its cage, carrying it in a basket to a bench, and looking at the ear chamber through a binocular microscope. The whole procedure lasted about 5 minutes. Each group consisted of twelve animals. At the end of the test period, when all the chambers were completely healed, a second chamber was inserted into the left ear of each rabbit and the experiment was repeated with the handling procedure for the two groups reversed.

It will be apparent to the reader that even the minor stress of routine handling can materially affect healing rates in somewhat timid animals such as rabbits. (Minor stress can also cause [illegible] changes in oxygen supply to human wounds - see section [illegible]).

2) Haemorrhagic shock Rabbits with established but incompletely healed wounds were subjected to haemorrhage followed by a period [illegible] to render them normovolaemic. The rate of healing of the chambers after varying periods of low blood pressure are shown in Table [illegible] and the effects on local PO_2 in the wound bed are shown in Figures [illegible] and [illegible].

It is clear that in the rabbit, an animal that is rather sensitive to blood loss, a period of severe haemorrhage lasting as short a time as [illegible] min. has a prolonged effect on wound blood flow, and an even more profound effect on healing rates.

TABLE 4 Effects of various periods of haemorrhage to a systolic pressure of 50 mmHg on wound healing in rabbit ear chambers (n = 6 for each group)

	Period of hypovolaemia (mins)				
	10	20	30	60	120
%Prolongation of healing time	3±1	5±3	28±10	31±7	30±10

(Normal healing time with this type of chamber was 23 days ±4)

(The effect of 10 and 20 min hypovolaemia is not statistically significant)

It appears that the increase in healing time closely follows the point at which prolonged recovery of the local microcirculation becomes evident.

Results of wound healing times from survivors of endotoxic shock were very variable. Repair times were prolonged but no clear picture emerged as to the relationship between the degree of inhibition of healing and the local microenvironment in the wound.

d) Effect of artificial environment

In an attempt to determine the general conditions most conducive to healing, rabbits with established but unhealed ear chambers were exposed to various atmospheric O_2 contents during the healing period. The percentages of oxygen supplied were 100, 40, 21 (air) and 15. The animal cages were enclosed in large plastic bags and the gas was supplied from a cylinder containing the appropriate mixture.

The results of this experiment are shown in Fig.4 which indicates that the most rapid healing can be expected to occur in an atmosphere of about 40% O_2, but a pure oxygen atmosphere retards connective tissue healing as do O_2 concentrations below those of air. These results will be discussed later but they accord with those of Niinikoski on [illegible] healing in rats [illegible].

e) Effects of foreign bodies

Macrophages around foreign bodies in wounds rapidly reduce the oxygen content of the foreign material [illegible]. No particular changes of pH and P_{CO_2} appear to be associated with inert foreign bodies, although many, so called biocompatible materials appear to exert a long term effect on tissue behaviour. Foreign bodies of biological origin stimulate a lymphocytic response, and, if bacterial contamination is present, major changes of local environment may occur.

Blood clots in wounds act as degradable foreign bodies to some extent and exert a somewhat equivocal influence on healing. Fig.5 shows the characteristic delaying effect of the presence of a clot on the healing process in an ear chamber. The work of the macrophages in removing the clot appears to hamper the advance of fibroblasts and blood vessels. Electrode studies in such clots indicate that they are capable of acting as oxygen stores, and prevent the development of the normally low PO_2 in the wound cavity unless infection supervenes.

However, in certain non healing situations, the release of blood into the wound cavity is sometimes followed by renewed healing activity - possibly this is the result of macrophage attack on the clot.

f) Mechanical stress

Chambers incorporating inert porous threads were implanted and allowed partly to heal so that granulation tissue invested the threads. The threads were then subjected to tension and fixed under load, by adhesive, to the outer side of the chamber. The behaviour of the cells around the threads was then studied, together with the oxygen environment.

The immediate effect of the strain was distortion of the tissue and alteration of the microcirculation and a fall in local PO_2. This persisted for 24 to 36 hours and was followed by hyperaemia in the stretched zone. No statistically significant changes took place in the overall healing times of stretched and unstretched tissue, but alignment of fibres and cells along lines of stress, was an obvious feature of some chambers.

g) Anticollagen antibodies have been described in the blood of burned patients, together with several other less specific immunological responses (24,25). It seemed appropriate to test the possibility that one factor in the slow healing of burns might be interference with new collagen formation at a healing surface by circulating anticollagen.

FITC labelled antihuman collagen was tested against a culture of human fibroblasts that produced large amounts of collagen. It was found that the collagen rapidly became fluorescently labelled and could be demonstrated under U.V. illumination. In high concentrations the anticollagen caused lysis of the fibroblasts. Similar results were obtained with antirabbit collagen on rabbit fibroblasts.

Application of anti-rabbit collagen to the growing ear chamber situation produced thrombosis in the precapillaries and an Arthus type reaction with polymorphonuclear aggregation outside the fully formed blood vessels. So far no conclusive evidence has been found that low concentrations of circulating anticollagen IgG in rabbits has any effect on the growth of granulation tissue.

SECTION III

Oxygen Tension and Epithelialisation

Introduction

There are many widely scattered references to the effects of oxygen on healing phenomena in different tissues (6,17,18,19) but relatively few which refer specifically to epidermis (26,27). Many of these latter are empirical observations on the clinical response of non-healing, open or infected wounds to treatment by various forms of hyperbaric oxygen application (28,29). There are also many reports on the effects of oxygen on the healing of burns (30,31) and others particularly on the beneficial results that may attend hyperbaric oxygen therapy in cases of skin grafting (32).

With regard to the general oxygen environment of the various layers of the skin little has been published beyond measurements of diffusion of oxygen through intact skin during a search for suitable methods of measuring arterial PO_2 without surgical interference to patients (7,33).

No measurements of the relative oxygen permeability of the different layers of intact human skin have been reported except by Evans and Naylor (33) in relation to cellulose-tape stripped skin and by Penneys (34) on isolated human stratum corneum.

The relationship of local wound environment to speed of epithelialisation has been considered by Winter (35) in the pig and by Hinman and Maibach (36) in man through observation on epidermal regeneration under inert films. Although a relationship between oxygen permeability of the films studied and the rate of repair was noted, no measurements of the actual oxygen environment of the cells was made.

A major effort to measure oxygen tension during subepidermal wound healing has been carried out by Hunt and Zederfeldt and their colleagues (19,21) and contributions in this field have also been made by Niinikoski (18,23), Remensnyder and Majno (37) and Silver (2,6). This work has established the value of the oxygen electrode for investigations on healing tissue, and it seemed appropriate to utilize similar well tested methods to establish (a) what is the normal range of oxygen environment of intact skin, (b) what changes in the environment can be expected in normal epidermal healing, (c) if imposed changes in this environment can affect healing rates, (d) what conditions exist in naturally slow healing skin defects such as burns and chronic ulcers, (e) the source of the normal epidermal O_2 supply; is it mostly from the blood or from the air? (f) if the supply differs in damaged epidermis, (g) what is the effect of traditional and more recently developed surgical dressings, and (h) what happens during the development of superficial infections.

Methods

Electrodes were applied to the skin by micromanipulator. Oxygen multiwire macroelectrodes were placed with great care to ensure that they did not cause local pressure and thus affect the microcirculation of the region being measured. The method described by Huch (7) was found to be satisfactory. This involved a thick silicone rubber pad around the electrode which distributed its weight over a large area and minimised oxygen diffusion from the air under the electrode.

Microelectrodes were inserted either directly into the epidermis or through an occlusive dressing, under direct vision.

Observations

1) Oxygen diffusion through intact skin

Measurements were made at the surface of skin with multi-cathode electrodes under different conditions of oxygen breathing, skin temperature and physiological

states. Further observations were carried out with microelectrodes inserted into the upper layers of the dermis and deep layers of epidermis when respiratory gas tensions were kept constant and changes in PO_2 were made in the gas over the skin at the electrode site. The most striking feature of these measurements was the rather low oxygen permeability of normal human skin under resting conditions, a feature shared with the pig, as compared to the more oxygen permeable characteristics of the skin of the small laboratory animals.

A further point of some interest was that oxygen breathing in man did not necessarily elevate the PO_2 in the epidermis although it almost invariably did so in small animals.

In contrast to oxygen breathing, vasodilatation induced by warming the body or limb distant from the measuring site always raised the PO_2 in normal human skin, but warming the skin locally near the electrode site did not have a constant effect. There was an apparent difference in permeability between subjects of about 20 years old and those of 40 years; the younger skin appeared to be more permeable. However, with the small number of subjects examined the difference was not statistically significant. Different skin areas also showed considerable variation in oxygen transmission and this seemed to be correlated with the thickness of the cornified layer.

Figures for surface PO_2 of different species breathing air and 100% O_2 for 15 minutes are given below (Table 5). Each figure is the mean of 30 readings The ambient air temperature was 20°C but the skin temperature was not measured. The human subjects exhibited a mild degree of vasodilatation as judged by skin colour before oxygen breathing.

This indicates the variability of PO_2 of the human skin but does not give a guide to the PO_2 in the basal layers, nor does it show the considerable effects of temperature.

TABLE 5 PO_2 (mm Hg) at the skin surface

Species/site		Air	O_2 for 15 mins	
Man	- Medial forearm	7mmHg ± 4.3	21 ± 10.8	
	Palm	5.2 ± 3.8	12.0 ± 8.2	
	Earlobe	18.1 ± 7.1	32.4 ± 14.4	
Mouse	- Back	28 ± 7.7	123 ± 21.2	Depilated
Rabbit	- Ear	25.3 ± 8.4	125 ± 24.1	Depilated

A series of experiments to show the effect of temperature on medial forearm skin PO_2 in man is summarised in Table 6. The subjects were in a sitting position and had been exposed to the ambient temperature for 15 minutes before measurements were started.

These figures indicate the quantity of oxygen reaching the outer layers of the skin from the dermal capillary bed in varying conditions.

Diffusion of oxygen in the opposite direction was measured with micro-electrodes in the basal layer of the epidermis of the forearm after occlusion of the circulation by tourniquet. Before application of the tourniquet the skin around the electrode was covered with a thick polyester film (Melinex, I.C.I. Ltd.) and as soon as the PO_2 in the basal layer of the skin had fallen to zero after the cessation of circulation (about 2 min) the Melinex was removed to allow access of air to the skin surface near the electrode. In 8 out of 20 observations the basal layer PO_2 remained at zero, and in 2 cases it rose to 8 mm Hg. In the remaining 10 cases the values were between 3 and 5 mm Hg. It seemed likely therefore that although O_2 did penetrate the stratum corneum it was very rapidly used by the deeper living layers of epidermis which therefore kept the PO_2 low. The major source of error in these measurements was the exact placement of the electrode point in relation to the basal layers of the skin. The electrodes were inserted to a standard depth and then withdrawn until there was no dimpling of the skin. Records from electrode tracks that showed bleeding after electrode removal were discarded.

In contrast to the observation above, it was noticed in rat, rabbit and mouse, that microelectrodes in the upper dermis as well as in deep epidermis registered oxygen tensions of up to 25 mm Hg even after the death of the animal, which indicated considerable inward diffusion of oxygen.

Microelectrode measurements in the basal layer of intact human forearm skin at an ambient temperature of 20°C showed very variable oxygen tensions, with a mean value in the region of 20 mm Hg.

TABLE 6 Effect of ambient temperature on PO_2 (mmHg) at the skin surface of man

Ambient Temperature	Air	O_2 for 15 mins.
4°C	2.1 ± 1.3	2.2 ± 1.5
20°C	7.0 ± 4.3	21.0 ± 10.8
37°C	40.3 ± 14.8	97 ± 30.7

An attempt to obtain a more reliable "average" PO_2 for the basal layers was made by examining fluid from suction blisters and also from spontaneous friction blisters. Unfortunately, even the most gently produced suction blister induced local dermal hyperaemia and readings of PO_2 blister fluid were uniformly high; around 40-50 mm Hg. Similar hyperaemia was also seen after cellulose tape stripping of the epidermis.

2) Diffusion through stripped human skin

After stripping the epidermis with cellulose tape to the glistening moist layer, large multipoint electrodes were placed on the exposed surface of the skin and air diffusion was minimised by application of a heavy mineral oil with low oxygen solubility around the electrode, and covering this with a polyester film. The stripped skin had a high PO_2, of the order of 40 mm Hg and showed brisk responses to the breathing of oxygen, presumably because local vasodilatation had been induced b the stripping. The day after stripping, the PO_2 at the kin surface had fallen to around 25 mm Hg, and a day later was only 10 mm. It remained at about this level thereafter.

3) Oxygen environment in superficial wounds

Loss of continuity of epidermis inevitably causes more or less of a vascular response in the dermis as well as destroying the barrier properties of the epidermis, both with respect to water loss and oxygen trapping.

In small incised wounds reaching the dermis in human skin, and to a lesser extent in laboratory animals the first environmental effect was the reversal of the oxygen gradient in the cut skin edge, and the exposure of dermis and basal layers of epidermis to atmospheric oxygen tension and also to drying by evaporation. This loss of the oxygen diffusion barrier was very short lived, even if there was no gross haemorrhage. Within a few minutes of incision the cut edges accumulated debris, either from clotting blood or plasma oozing into the wound cavity. As soon as a clot was established the oxygen tension at the bottom of the wound began to fall. Within a few hours of wounding the PO_2 at the dermo-epidermal junction was often less than 10 mm Hg. This appeared to be due

partly to the relatively low O_2 permeability of the scab that had formed over the incision and partly to the changes in the dermal blood flow that occurred during the inflammatory response. The small amounts of fluids that leaked from inflamed capillaries were quite sufficient to increase diffusion distances significantly and to cause drastic alterations in dermal tissue PO_2 (2,3). Added to this was the accumulation of polymorphonuclear cells which had a high O_2 uptake. Polymorphs were subsequently replaced by macrophages which acted as an oxygen "sink" (4) between the capillaries and the epidermal cells. Nevertheless, the basal cells of the epidermis started to move across the wound under the base of the scab, through the upper, desiccated layers of the dermis in what seemed to be a relatively hostile environment. These migrating cells were presumably capable of using oxygen although little seemed to be available from the capillary bed. Oxygen breathing at this time, 12 to 24 hours after wounding, had little or no effect on the epidermal PO_2 at the wound edge. Diffusion of oxygen from the air was also severely limited by the scab, and again only a very slow change in wound PO_2 could be observed from increasing the O_2 concentration in the air above the wound. Oxygen supply to the basal layers of epidermis did not appear to increase until about 4 days after wounding, when fibroblast and endothelial proliferation in the dermis was well established. By this time the epidermis was several cells thick and was rapidly re-establishing its own normal structure.

In thin skinned animals the scab was less permeable to oxygen than the normal skin.

Effect of Shock

A limited number of measurements of wound PO_2 were performed on 20 anaesthetized rabbits during acute haemorrhage and early haemorrhagic shock to determine the effects of cardiovascular changes on the oxygen supply to superficial wounds. Atmospheric air was excluded by a melinex film. It appeared that one of the first responses to bleeding was a reduction in blood flow to and PO_2 in, the wound area. During the acute phase of haemorrhage to a mean arterial pressure of 55 mm Hg the PO_2 fell to zero in all wounds studied (see also Table 1). Reinfusion of

blood within 15-20 mins resulted in re-establishment of normal tension during a half hour period (Fig.3). If blood was withheld for more than three quarters of an hour, no short term recovery of wound PO_2 occurred during reinfusion, although normal blood pressure was re-established. Previous observations of deeper wounds in rabbits indicated that these remained hypoxic for at least 48 hours after 45 mins of lowered blood pressure (Fig.2).

The observations are summarized in Table 7.

Measurements under occlusive films and wound dressings

Some occlusive films have been reported as markedly affecting epithelial migration rates in pig and man (35,36) Measurements were made on superficial abrasions on human skin covered by Teflon, Polythene or polyester films and also on abrasions covered with gauze swabs, by means of microelectrodes inserted through the covering into the surface across which epithelial migration was occurring. Similar observations were carried out on depilated rabbit skin bearing superficial incisions reaching the upper layers of the dermis. The measurements obtained are summarized in Table 8.

It was possible in human skin to manipulate a microelectrode under direct vision to measure first the PO_2 above epithelium and below the dressing, and then to advance the electrode tip through the newly migrated epithelium near the wound edge. This was feasible in the case of the transparent plastic coverings but was impracticable for the swabs or paraffin tulle. It can be seen from the table that oxygen permeable films allowed the development of a completely different type of epithelial environment than is present in naturally healing wounds under a scab, whereas films of low oxygen permeability such as polyester allow the development of an oxygen environment very similar to that under a scab. The situation under a gauze dressing may be of some significance. Under a dry swab there is normal scab formation and the oxygen environment is similar to that in an uncovered wound with a scab. In wounds where there has been fluid loss into a swab, whether or not a scab was present, the PO_2 in the wound surface was so low as to be difficult to measure with the techniques that were used. Exudate soaked swabs can therefore form a considerable barrier to oxygen diffusion from the air.

TABLE 7 Effects of Haemorrhage on Epidermal Wound PO_2 (mm Hg) in Rabbits when O_2 Diffusion from Air is excluded, measured with a Surface Electrode

State	Number of animals	Air breathing	Oxygen breathing for 15 min
Normal Anaesthetized	20	20.0± 7.4	83.7±24.6
15 min at blood pressure 55 mm Hg	10	0.0	1.3± 0.5
15 min at blood pressure 55 mm Hg + reinfusion to B.P. >90 mm Hg	10	5.6	23.2± 7.8
45 min at blood pressure 55 mm Hg	10	0.0	0.0
45 min at blood pressure 55 mm Hg + reinfusion to B.P. >90 mm Hg	10	0.0	0.0

TABLE 8 Oxygen Tension under Wound Dressings (mm Hg)

Species	Site	Wound covering					
		Teflon	Poly-ethylene	Poly-ester	Gauze dry (under scab)	Swab wet	Non-ad tulle
Man	Above epithelium	135	123	21	5	2	0
	Below epithelium	108	89	4			
Rabbit	Wound cavity	128	113	18	20	7	8

If the PO_2 of the gas above a wound covered with a dressing was changed, the expected alterations were found below the dressing according to its properties. Thus, pure oxygen directed onto teflon covered wounds raised the epidermal PO_2 to nearly 700 mm Hg while similar treatment of polyester or wet swab-covered wounds resulted in very much smaller changes (see Table 9).

When epidermal continuity was re-established the oxygen gradient within the epithelium began to change so that by 4 days after incision or abrasion a contribution to the basal epidermal layers of oxygen from the vasculature of the dermis could be detected by covering the wound surface with an oxygen barrier and then having the subject breathe pure O_2 for 5 minutes. By 8 days the wound PO_2 gradients had been reversed due to the relatively impermeable nature of the newly established cornified layer, and achieved a situation similar to that found in normal skin.

Effects of Minor Stress on Skin Wound PO_2

Skin circulation in man is notoriously responsive to emotional stress and vasoconstriction of healing dermal wounds in rabbits is a feature commonly associated with the retardation of wound healing which may occur if an animal is placed in unfamiliar surroundings or is exposed to noise or physical disturbance (Fig.1). A few measurements were therefore made on PO_2 of stripped epidermis, using a surface electrode, in man and in rabbits, to test the effect of very minor stress on oxygen supply from the dermis. Measurements were obtained from 6 people, three of whom were familiar with the investigation and quite relaxed, and three who were new to the situation and mildly apprehensive. In the case of the rabbits, measurements were made in an unfamiliar room with a high intermittent noise level which had previously been shown to be associated with contraction of blood vessels in healing connective tissue. The results, which form only a very small group, are shown in Table 10.

Each figure represents a mean of 6 readings on each subject, thus the figures for the rabbits are a mean of 18 readings.

TABLE 9 Effect of 5 min exposure of wound area to pure O_2 atmosphere, on PO_2 of wound surface below various dressings

Dressing	PO_2 (mm Hg)
Teflon	685
Polythene	628
Polyester	173
Nonad-tulle	151
Dry swab (measured under scab)	48
Wet swab	35

TABLE 10 PO_2 (mm Hg) in stripped skin during mild stress

	Subjects	PO_2 breathing air	PO_2 breathing O_2 for 5 min
Experienced Group	A	28	98
	B	42	163
	C	36	148
Naive Group	D	15	43
	E	7	16
	F	11	14
Familiar room	Rabbits(3)	45 ± 4.0	189 ± 17.3
Noisy room	Rabbits(3)	12.6 ± 4.7	40 ± 6.7

Effects of Infection

Occlusive plastic skin dressings in man are frequently associated with superficial wound infections and destruction of epidermis. Similar infections may develop on depilated rabbit skin where a superficial abrasion is covered by plastic film. Measurements of PO_2 under occlusive dressings on rabbit skin wounds indicated that even when oxygen permeable films were used, developing bacteria were able to lower skin surface oxygen concentrations very considerably. An interesting feature of such measurements was that the fall in PO_2 considerably preceded any visual indication of infection. The bacteria encountered under the films were Proteus, Pseudomonas, and Staphylococci spp. Epidermal cell detachment from the underlying tissue was a feature of infection under inert films and occurred soon after the rapid fall of PO_2 due to the bacteria was detected. When infiltration of polymorphonuclear cells in the wound appeared, the PO_2 was further reduced and approximated to zero even under Teflon films. Spontaneous infections were most commonly seen under polyester film.

Measurements in Superficial Burns

Blisters were raised on human skin by mild thermal burns. Examination of the fluid in such blisters showed an oxygen environment somewhat different from that in suction or friction blisters. PO_2 in blister fluid was low initially although it rose after a few hours, apparently due to slow diffusion of O_2 into the fluid from the air. The damaged tissue under the blister exhibited a PO_2 of zero and no change could be elicited when oxygen was breathed for 15 minutes. Microelectrodes were also inserted into the reddened skin at the edge of the blister and again very low oxygen tensions were recorded which showed little or no alteration during oxygen breathing. The major difference in oxygen environment between incised or abraided wounds on the one hand and minor burns on the other, was that very low tensions in burns persisted for five or six days after injury, whereas in the other injuries re-establishment of normal PO_2 gradients started after about 3 days, provided no infection developed.

Clinical trial

A clinical trial study has been set up in collaboration with Mr. K. Lucas, Consultant Orthopaedic Surgeon to the United Bristol Hospitals to evaluate the use of local and systemic oxygen at atmospheric pressure, in the treatment of severely damaged skin over compound fracture sites. This trial has been instigated to try to improve healing especially in those areas where blood supply to the skin may be minimal following trauma. It is also including cases where skin grafting has been used to repair deficits.

The results so far, on a small number of cases are encouraging and approaches have been made to Mr. Lucas from the hospital accident service, for extension of the trial to cover non orthopaedic cases.

Discussion

The development of the various systems outlined in this report give useful tools for investigation of tissue conditions associated with healing and non-healing situations. The fundamental studies of oxygen diffusion and the measurement of pH and P_{CO2} in shock should help in developing the rational approach to the treatment of trauma cases.

Results confirmed that increased oxygen supply to wounds will accelerate the healing process, both as regards connective tissue and, more particularly, epidermis. There is, however, some discrepancy between deep and superficial healing in regard to the response to very high levels of oxygen tension. Connective tissue repair appears to reach a maximum rate when inspired air contains 40% O_2, but it declines if the ambient O_2 concentration rises above this level. On the other hand epithelial repair rates improve when direct access of 100% O_2 to the healing surface is allowed. This apparent anomaly is probably due to vascular factors which lead to vasoconstriction in small vessels at high oxygen tensions and which therefore tend to lower, rather than increase O_2 supplies in wound areas when very high concentrations of O_2 are breathed. Of course pulmonary changes also occur during pure oxygen breathing (38) which lead to eventual lowering of the PO_2 and which reinforce the failure of supply to the wound.

Thus it seems a rational approach to encourage maximum healing rates could well be to enrich the oxygen content of the inspired air to a level of 30 or 40% O_2 and at the same time to expose the surface of the wound to pure O_2 to encourage epidermal migration. In clinical terms the sealing of the defect by the epithelium is usually the first priority.

The observations on shock and haemorrhage merely confirm clinical experience with reference to the effects on healing processes. The prolonged recovery from shock appears to be due in part to leakage of fluid from vessels which increases diffusion distances in extravascular spaces and thus renders the tissue environment hypoxic and hostile

to cellular activity. The practice of resuscitating shocked patients with massive infusions of fluid with low colloid osmotic pressure increases this leakage and might actually retard recovery.

Damaged Skin

Minor superficial damage such as cellulose tape stripping of normal human skin produces marked changes in the oxygen permeability. Not only is the skin permeability increased, but the normal oxygen gradients may be reversed, especially where the dermal capillaries are not dilated. It might be reasonable to postulate that the control of epidermal growth and replacement could to some extent be dependant on the direction and steepness of oxygen gradients within the epidermis.

When more severe damage is considered and the movement of epidermal cells across a wound surface is examined, it appears that under a scab the conditions of oxygen supply are far from good. Epidermal cells before starting migration accumulate glycogen and it would seem that much of their energy requirements during migration under a scab must be derived from glycolytic activity. Nevertheless, epidermal cells in the migratory phase do have a considerable capacity for oxygen uptake as can be seen from Table 8 where a single layer of migratory cells is shown to modify considerably the amount of oxygen reaching the deeper tissue from the air.

Winter's observation on the healing rates of epidermal wounds in pig under different types of occlusive films and the effects of different oxygen atmospheres on such healing (39) strongly suggests that oxygen supply can be a major factor in determining the rates both of mitotic activity and of epithelial movement. The more limited data on movement of regenerating epidermis under inert films reported here support Winter's studies and also indicate that occlusive plastic skin dressings should probably be evaluated in terms of their oxygen permeability as well as water vapour and CO_2 permeability. Other factors which must also be considered are those of the heat retaining character of the film (40).

The few measurements that have been made on superficial burns suggest that at least one aspect of the delayed healing characteristic of this type of injury, may be the lack of oxygen availability to the damaged tissue. Clearly however, a great many more measurements of burn environment must be carried out before any firm conclusions can be reached, but the diffuse nature of burn injuries presents tissue with a special problem in that there is no clear, undamaged region from which regeneration can start. This unsatisfactory state is further complicated by the lack of early development of new blood vessels and a persistently low tissue PO_2. The divergent results from the use of oxygen in burn therapy show that more carefully controlled observations are necessary before the role of oxygen in burn healing can be properly evaluated.

Reports on the use of oxygen therapy for prolonging the survival of skin grafts have been generally encouraging. It may at first sight seem surprising that intermittent exposure of epidermis to hyperbaric oxygen for short periods at long intervals could have any lasting beneficial effect on cell renewal. However, if one considers the very fluctuating behaviour of the natural environment of the deeper layers of the epidermis in terms of oxygen supply and the drastic reversals of O_2 gradients that occur during epithelial damage and repair, it may well be that external, artificially applied changes in gradient could provide a necessary stimulus to proliferation, as well as supplying some extra oxygen temporarily for metabolic usage.

The observation reported here on the relative ineffectiveness of <u>short-term</u> oxygen breathing in changing wound PO_2 has also been noted in regard to dermal wounds. It seems that externally applied oxygen direct to the wound surface, for instance by enclosing the treatment area in a plastic bag full of O_2, is a more certain, if rather slow, way of altering the wound environment. This is particularly true of even minor burns. Such locally applied oxygen will not have a great effect unless the wound is free of either natural or surgically applied oxygen diffusion barriers. Eschars and exudate-clogged gauze dressing are particularly good barriers whereas plastic films which allow water vapour and oxygen diffusion, and yet keep the wound surface moist and suitable for

epidermal migration seem to provide almost ideal conditions. The problem of infection under such films still remains to be dealt with.

The information presented in this paper partially answers the questions posed in the introduction to Section III but considerable scope is left for speculation on the question of whether or not the level of oxygen supply is a vital or merely secondary factor in epidermal regeneration.

Conclusions and Recommendations

Oxygen applied directly to healing surfaces is likely to encourage and speed epidermal healing, particularly if the damaged area can be kept moist.

The development of haemorrhagic or endotoxic shock delays wound healing because vessels in a damaged area are especially liable to perfusion failure and to leakage. Treatment of shock by intravenous infusion of fluids of low colloid osmotic pressure may well worsen the local environment of a wound.

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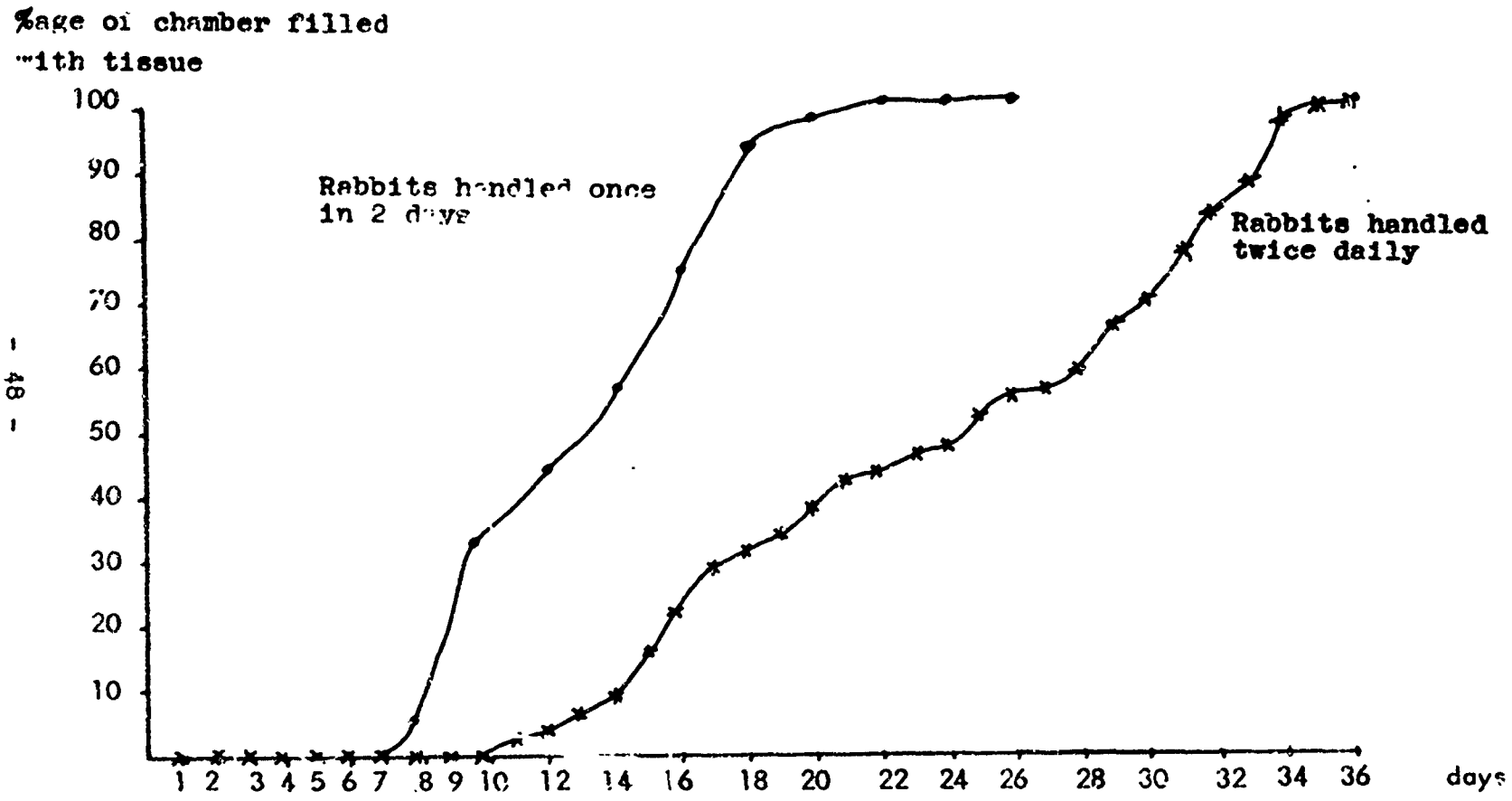
Fig. 1

12 μ thick healing pattern
percentage table covered in respect of time



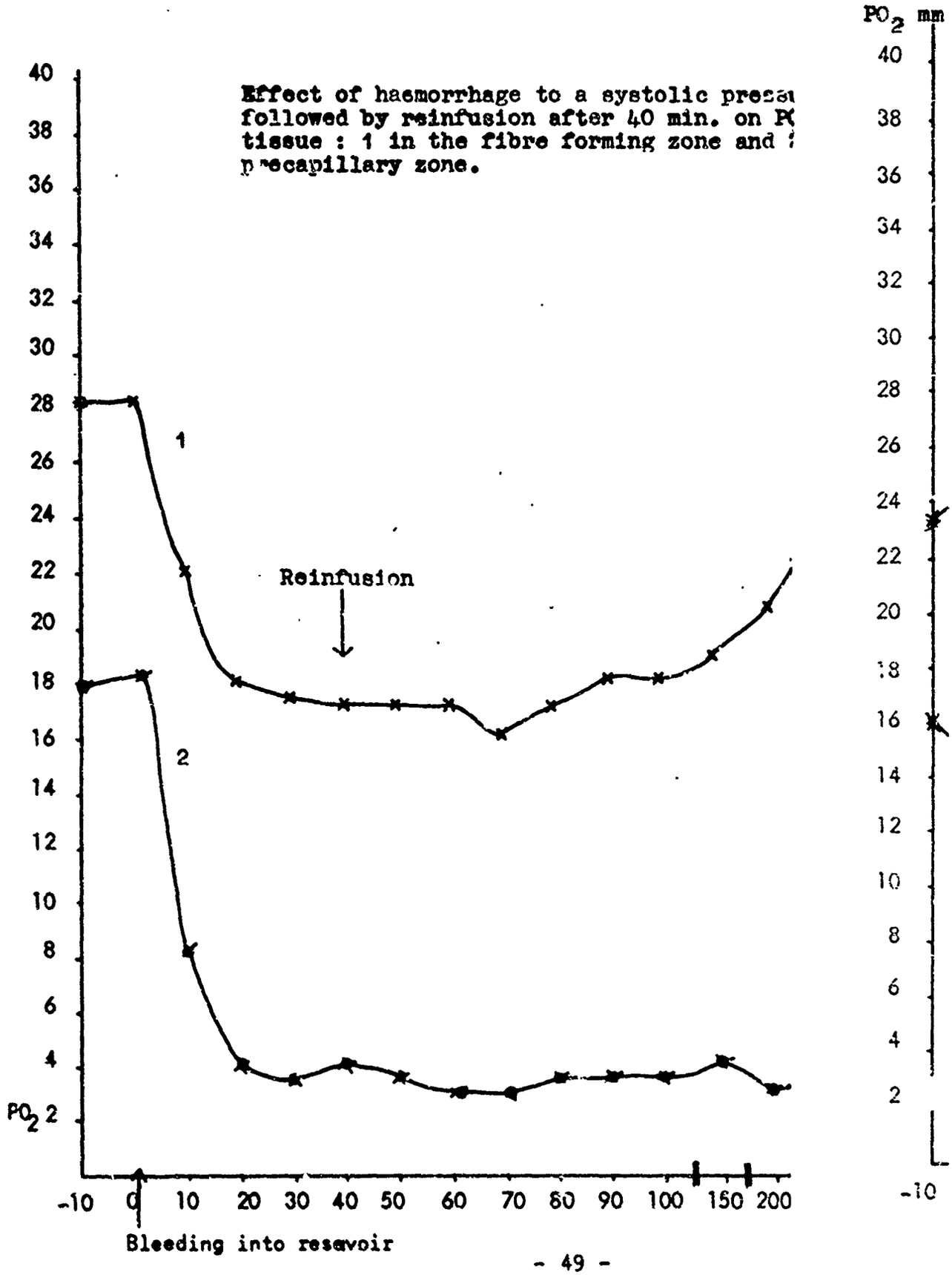

Effect of haemorrhage to a systolic pressu
followed by reinfusion after 40 min. on P
tissue : 1 in the fibre forming zone and
precapillary zone.
PO2 mm
PO2
Reinfusion
Bleeding into reservoir
1
2
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
-10
0
10
20
30
40
50
60
70
80
90
100
150
200

Fig. 3

Hg

Effect of haemorrhage to a systolic pressure of 50 mm Hg followed by reinfusion after 20 min. on PO_2 of wound tissue: 1 in the fibre forming zone and 2, in the precapillary zone.

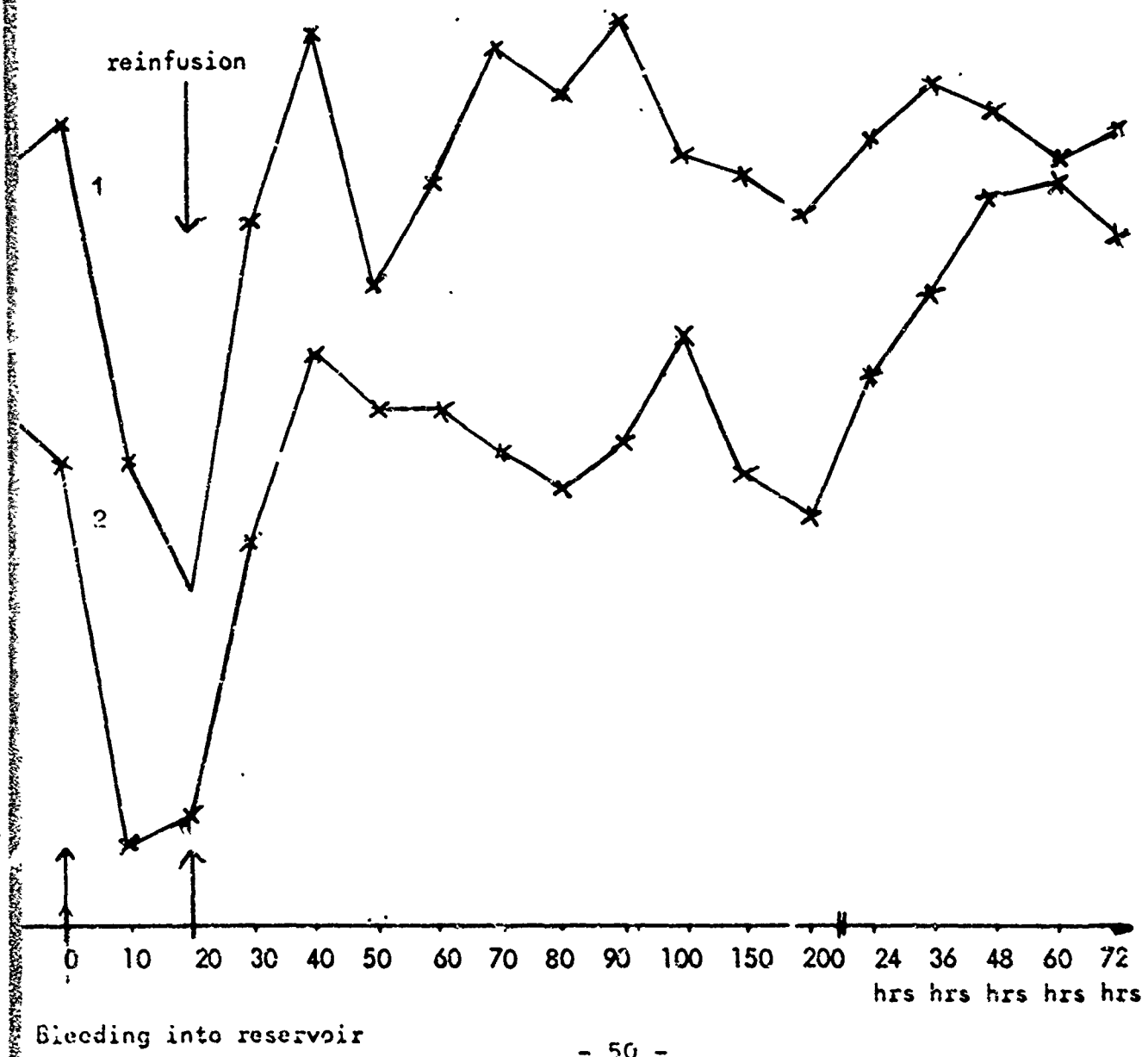
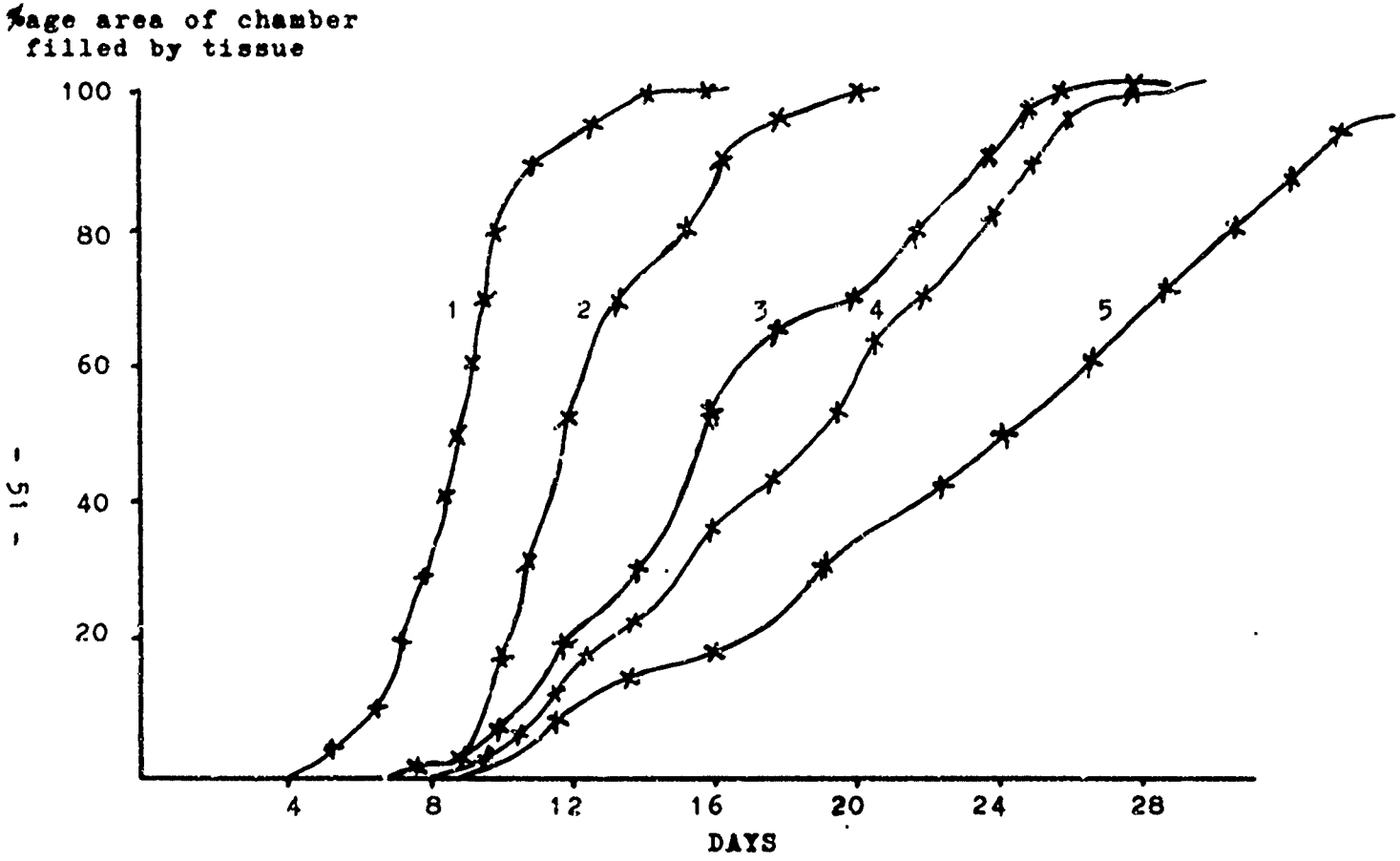


Fig.4

Long term effects of atmospheric PO_2 on healing rates in rabbit ear chambers



1. Rabbits living in 40% O_2
2. " " " 21% O_2 (air)
3. " " " 15% O_2
4. " " " 60% O_2
5. " " " 100% O_2