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MICROFIBER GLASS DISCS, A NEW SUPPORT FOR SKIN ORGAN CULTURES

by

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13. ABSTRACT

A model system has been developed for studies of the direct effects of high pressure oxygen on skin growth and viability. The data demonstrate the feasibility of the system in studies of the effects of hyperoxygenation by exhibiting the toxicity of 100% oxygen under the conditions employed.

14. KEYWORDS

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FOREWORD

The work described in this report was authorized under Project 1T061101A91A, Studies on the Acceleration of Wound Healing. This work was started in October 1969 and completed in March 1971. The data are recorded in TSD notebooks MN-2300 and MN-2372.

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

DIGEST

A model system has been developed for studies of the direct effects of high pressure oxygen on skin growth and viability. The data demonstrate the feasibility of the system in studies of the effects of hyperoxygenation by exhibiting the toxicity of 100% oxygen under the conditions employed.

CONTENTS

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I. INTRODUCTION.

For several years, this laboratory has studied the effects of high pressure oxygen on the survival of traumatized tissue.[1,2,3] As an extension of a study of the influence of high pressure oxygen on skin pedicle flaps in rabbits, an *in vitro* model system has been established to determine the direct effect of high pressure oxygen on the viability of rabbit skin. The viability of the skin in organ culture is assessed by the uptake of DNA-precursor tritiated thymidine from an aqueous culture medium. The *in vitro* system to be described is designed to allow direct exposure of the epidermis to the gaseous environment while the dermis rests on an absorbant support matrix. The unique support matrix in this system consists of a microfiber glass prefilter. This matrix provides an inert stationary support for the skin organ culture but permits rapid and quantitative removal of the skin for bioassay.

II. MATERIALS AND METHODS.

An adult New Zealand albino rabbit was sacrificed, clipped, and shaved. The abdominal skin surface was cleansed with pHisoHex surgical detergent and zephiran chloride (1:1000) antiseptic (both from Winthrop Laboratories, New York, New York. Skin preparation was completed with three rinses of sterile 0.9% saline solution. Full thickness abdominal skin was removed and cut into 2- by 2-mm squares.

Individual pieces of skin were placed in growth medium with four times the usual concentration of antibiotics for 30 to 45 minutes prior to culture preparation. The standard growth medium was 90% Eagle's minimal essential medium with Earle's balanced salt solution and 10% calf serum. Additives included 2 μmoles/ml L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Media and supplements were obtained from Microbiological Associates, Bethesda, Maryland.

Organ cultures having four pieces of skin each were established in standard 60-mm glass Petri dishes. The growth surface consisted of a Millipore (Millipore Corporation, Boston, Massachusetts) microfiber glass prefilter (No. AP 25-042-00) placed with the smooth side up. The prefilters were washed in 0.9% saline and sterilized in the Petri dishes by autoclaving. Four

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[1] Ackerman, N. B., and Brinkley, F. B. Comparison of Effects on Tissue Oxygenation of Hyperbaric Oxygen and Intravascular Hydrogen Peroxide. Surgery *63*, 285 (1968).

[2] Gruber, R. P., Brinkley, F. B., Amato, J. J., and Mendelson, J. A. Hyperbaric Oxygen and Pedicle Flaps, Skin Grafts, and Burns. Plast. Reconstr. Surg. *45*, 24 (1970).

[3] Gruber, R. P., Billy, L. J., Heitkamp, D. H., and Amato, J. J. Hyperbaric Oxygenation of Pedicle Flaps Without Oxygen Toxicity. Ibid. *46*, 477 (1970).

milliliters of growth medium was added to each dish to saturate and thinly cover the surface of the prefilter. Cultures were incubated in a hyperbaric oxygen chamber (Bethlehem Corporation, Bethlehem, Pennsylvania) at 37°C in an atmosphere containing 5% carbon dioxide and 95% air or oxygen at 60% to 65% relative humidity. The two atmospheric conditions employed above a. referred to as 20% oxygen and 100% oxygen [both at 1 atmosphere absolute (atm abs)].

Two cultures were labeled each day with 2.0 microcuries each of thymidine-methyl-H^3 (New England Nuclear Corporation, Boston, Massachusetts) in 0.2 ml of growth medium. The two cultures were harvested after 24 hours and the tissue was removed. All tissues were washed three times in 0.9% saline solution, blotted on filter paper, weighed, and transferred to plastic liquid scintillation vials. Digestion was carried out in 0.5 ml of NCS solubilizer (Nuclear-Chicago Corporation, Des Plaines, Illinois) for 24 hours at 50°C. At the end of this period, 9.5 ml of scintillation fluid[4] was added to each vial. Samples were counted at 4°C in a Nuclear-Chicago liquid scintillation spectrometer at a counting efficiency of approximately 48%. The count rate in all samples was at least five times the background rate. Specific radioactivity of each sample was calculated from the resulting counts and the known tissue weights.

III. RESULTS.

Tissue viability and growth under culture conditions in 20% oxygen was ascertained by visual inspection as well as by determination of thymidine uptake (figure). The curve obtained demonstrated an initial "growth phase" characterized by a rapidly increasing rate of thymidine uptake and a subsequent "stationary phase," wherein the rate of thymidine uptake increased very slowly. In the presence of 100% oxygen, thymidine uptake was minimal and no growth or stationary phase was evident (figure). For comparison, when tissue was killed by heating in boiling water for 3 minutes (figure), the level of thymidine uptake was approximately the same as in those exposed to 100% oxygen.

IV. DISCUSSION.

A number of previous studies in which skin organ cultures[5,6,7] were used have suggested that oxygen may be toxic to skin if given in too high concentration or at hyperbaric pressures. Reaven and Cox[7] have shown that oxygen at concentrations greater than 40% (at 1 atm abs) decreases mitotic activity and thymidine-methyl-H^3 uptake. In their study, however, small pieces of skin were used (3 to 5 mm in diameter). With larger skin fragments of up to 1.5 cm in diameter, other studies[8] have shown hyperbaric 100% oxygen to prevent central necrosis and maintain survival.

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[4] Patterson, M. S., and Greene, R. C. Measurement of Low Energy Beta-Emitters in Aqueous Solution by Liquid Scintillation Counting of Emulsions. Anal. Chem. *37*, 854 (1965).

[5] Hoppleston, A. E., and Simnet, J. D. Tissue Reaction to Hyperbaric Oxygen. Lancet *7*, 1135 (1964).

[6] McFarlane, R. M., and Wermuth, R. E. The Use of Hyperbaric Oxygen to Prevent Necrosis in Experimental Pedicle Flaps and Composite Skin Grafts. Plast. Reconstr. Surg. *37*, 422 (1966).

[7] Reaven, E. P., and Cox, A. J. Behavior of Adult Human Skin in Organ Culture. J. Invest. Dermat. *50*, 118 (1968).

[8] Stier, H. A., and Halasz, N. A. Organ Culture and Tumor Culture in Hyperbaric Oxygen. Am. J. Med. Sci. *252*, 391 (1966).

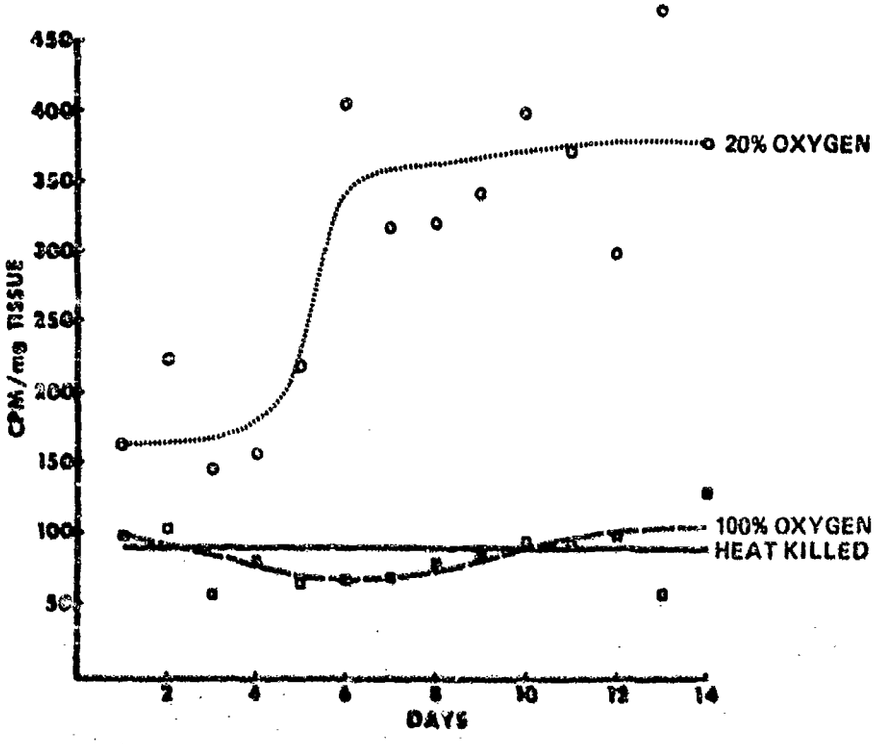

Figure. Uptake of Thymidine-methyl-H^3 by 2- by 2-mm Rabbit Skin Fragments in Organ Culture Using 20% Oxygen (1 atm abs) and 100% Oxygen (1 atm abs). Each point indicates mean uptake of four individual fragments. Solid line shows uptake by heat-killed fragments.

The contradictory nature of the foregoing reports and a requirement for direct assessment of the effect of high pressure oxygen on rabbit skin viability prompted the development of the model system described in this report. The preliminary data obtained demonstrate that 100% oxygen (1 atm abs) is toxic to 2- by 2-mm rabbit skin fragments whereas 20% oxygen (1 atm abs) promotes normal growth. The solution of the problem of skin size versus optimal oxygen concentration and pressure will require additional experimentation. Simplification of the problem, however, may be achieved by use of the microfiber glass disc culture technique employed in this investigation.

V. SUMMARY.

A model system has been developed for studies of the direct effects of high pressure oxygen on skin growth and viability. The data demonstrate the feasibility of this system in studies on the effects of hyperoxygenation by exhibiting the toxicity of 100% oxygen under the conditions employed.

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1. Ackerman, N. B., and Brinkley, F. B. Comparison of Effects on Tissue Oxygenation of Hyperbaric Oxygen and Intravascular Hydrogen Peroxide. Surgery *63*, 285 (1968).

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3. Gruber, R. P., Billy, L. I., Heitkamp, D. H., and Amato, J. J. Hyperbaric Oxygenation of Pedicle Flaps Without Oxygen Toxicity. Ibid. *46*, 477 (1970).

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