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A CYTOGENETIC STUDY OF NEW ZEALAND NUCLEAR TEST VETERANS: THE COMET ASSAY

A thesis presented in partial fulfilment of
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New Zealand

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- ABSTRACT -

Between 1952 and 1958, forty thousand troops witnessed or assisted in the detonation of nuclear weapons in and around Australia and Christmas Island. Of these forty thousand troops there were 550 sailors from New Zealand; the remainder were mainly from Australia or Britain, together with a Fijian contingent. Since the end of this test series, the participants have maintained that they were exposed to radiation that has affected their health. The New Zealand test veterans say that their lifespan has been reduced by at least 10 years and there have been an unusually high number of genetic disorders among them and their children. The possible genetic effects of this radiation exposure have never been fully investigated.

One of the most popular techniques for detecting DNA damage is the single-cell gel electrophoresis assay (SCGE), also known as the COMET assay. The COMET assay was used throughout this study to determine if veterans of the Operation Grapple tests have long-term genetic effects as a result of their participation. The COMET assay measured three factors to determine the overall genetic damage in these veterans: the tail length; the tail moment; and the Olive tail moment. Only the tail length had a significant amount of difference after a comparison with a control group was conducted ($P = 0.046$). However, the mean genetic damage in these veterans was lower than that of the control group. It is unclear if this result is due to an anomaly in the data, or due to some other complex factor. An epidemiological analysis revealed a possible link between the mortality of these veterans and the number of weapons detonated.

The collection of these one hundred samples, not including re-collections, from several areas of New Zealand became a logistical nightmare. To minimise this problem a pilot study was also incorporated into this research to determine if blood samples could be cryopreserved for extended periods of time without an accumulation of genetic damage due to the freezing process. The COMET assay was also used to determine this damage. The cryopreservation of these samples induced extensive genetic damage. Only 7 from the total of 60 frozen samples were retrieved with a level of damage that was not significantly different from the original, unfrozen sample ($P = > 0.050$). It appears that the routine use of cryopreserved blood samples for cytogenetic testing is not possible at this time and further study is required.

- ACKNOWLEDGEMENTS -

An application was submitted to the human ethics committee for permission to use human subjects in this study. The application was considered and approved before research commenced. Copies of the letters of permission are included in Appendix One.

First and foremost, I would like to thank my research supervisor Dr. Al Rowland, for his tireless efforts in getting my project off the ground, and also for his moral support throughout. Without your input, humour, and help I would never have gotten as far as I have. Thank you.

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- ABBREVIATIONS USED IN THIS REPORT -

α - Alpha

β – Beta

BrdU - 5-bromo-2-deoxy-uridine

Bq - Becquerel

C - Control group

Ca - Calcium

CA - Chromosome Aberration

CASP - COMET Assay Software Project

CDC - Centre for Disease Control

Ci - Curie

Cm - Centimetres

^{60}Co - Cobolt-60

^{137}Cs - Cesium-137

CT - Comet Threshold

DAPI - 4', 6-Diamidino-2-phenylindole

DMSO - Dimethylsulfoxide

DNA - Deoxyribose Nucleic Acid

DPS - Disintegrations Per Second

DSB - Double-Strand Breakages

E - Experimental (nuclear test veteran) group

EDTA - Ethylenediamine Tetraacetic Acid

EMF - Electromagnetic Field

EPA - U.S Environmental Protection Agency

Et al. - *Latin,* and others

FCS - Foetal Calf Serum

FISH - Fluorescence *In Situ* Hybridisation

γ - Gamma

G - Grams

GHz - Gigahertz

Gy - Gray

H^+ - Proton

^{2}H - Deuterium

^{3}H - Tritium

H_2O - Water

H_2O_2 - Hydrogen Peroxide

HCT - Head Centre Threshold

HT - Head Threshold

^{131}I - Iodine-131

IDDM - Insulin Dependent Diabetes Mellitus

^{192}Ir - Iridium-192

KT - Kilotons

L - Litres

LET - Low Linear Energy Transfer

Leuk - Leukocyte

LMAgarose - Low-Melting-point Agarose

MCi - Microcurie

µL - Microlitres

µm - Micrometers

µM - Molar concentration in micromoles/litre

M - Molar concentration in moles/litre

MA - Milliamperes

Mb - Megabytes

Mci - Millicurie

Mg - Milligrams

Ml - Millilitres

Mm - Millimetres

MN assay- Micronucleus assay

MqH_20 - Milli-Q water

Ms - Milliseconds

MSv - Millisieverts

MT - Megatons

NaCl – Sodium Chloride

NaOH - Sodium Hydroxide

Neutr - Neutrophil

NIDDM - Non-insulin Dependent Diabetes Mellitus

Nm - Nanometres

NMAgarose - Normal Melting-point Agarose

N or No. - Number

NTV - Nuclear Test Veterans

NZNTV - New Zealand Nuclear Test Veterans

NZNTVA - New Zealand Nuclear Test Veterans Association

OTM - Olive Tail Moment

P - HMNZS Pukaki

PBMC - Peripheral Blood Mononucleate Cells

PBS - Phosphate Buffered Saline

PHA - Phytohaemagglutinin

R - HMNZS Rotoiti

R.N.Z.N - Royal New Zealand Navy

ROS - Reactive Oxygen Species

RPMI - Roswell Park Memorial Institute

SCE - Sister Chromatid Exchange

SCGE - Single Cell Gel Electrophoresis

SD - Standard Deviation

SE - Standard Error

SSB - Single-Strand Breakage

Ssp. - Sub-species

^{85}Sr - Strontium-85

^{89}Sr - Strontium-89

^{90}Sr - Strontium-90

Std. Dev - Standard Deviation

TL - Tail Length

TM - Tail Moment

^{235}U - Uranium-235

V/v - Volume per volume

WU - U excitation (wide band) filter

x g - Gravities

^{90}Y - Yttrium-90

^{90}Z - Zirconium-90

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