RADIOBIOLOGY SYMPOSIUM
1954
SYMPOSIUM DE RADIOBIOLOGIE

BACQ & ALEXANDER

RADIOBIOLOGY SYMPOSIUM
1954
SYMPOSIUM DE RADIOBIOLOGIE

RADIOBIOLOGY SYMPOSIUM
1954
SYMPOSIUM DE RADIOBIOLOGIE

Proceedings of the
Symposium held at
Liège
August-September,
1954

Comptes-Rendus
du
Symposium de Liège
Août-Septembre,
1954

Z. M. BACQ

Professeur à l'Université de Liège
Membre Correspondant de l'Académie Royale
de Médecine de Belgique

and

PETER ALEXANDER

Chester Beatty Research Institute
Institute of Cancer Research
Royal Cancer Hospital
London

NEW YORK
ACADEMIC PRESS INC., PUBLISHERS
LONDON
BUTTERWORTHS SCIENTIFIC PUBLICATIONS
1955

BUTTERWORTHS PUBLICATIONS LTD.
88 KINGSWAY, LONDON, W.C.2

U.S.A. Edition published by
ACADEMIC PRESS INC., PUBLISHERS

125 EAST 23RD STREET
NEW YORK 10 NEW YORK

Set in Monotype Baskerville type
Printed in Great Britain by Spottiswoode, Ballantyne & Co. Ltd.
London and Colchester

FOREWORD

In December 1952 an informal discussion was held under the auspices of the Faraday Society in Cambridge on the chemistry of biological after-effects of ultraviolet and ionizing radiations. At this well-attended meeting it became apparent that the study of radiobiology, in which many scientists were now engaged, would be advanced by having informal and friendly symposia so that the workers from different fields and disciplines engaged in studying the effects of ionizing radiation could meet and discuss. For this purpose a small committee composed of Bacq, Bonet-Maury, Hevesy, Hollaender and Mitchell was set up and the first such meeting was organized in Aarhus (Denmark) in July 1953. The great success of this meeting showed that the need existed and that the general scheme of informality limited numbers, and emphasis on discussion was sound. The second symposium on Radiobiology was held in Liège and these meetings are now planned to take place annually. The great International Congress cannot cater satisfactorily for radiobiology since this subject cuts right across the boundaries of the different sciences and it is necessary that physicists, chemists, biochemists, physiologists, cytologists, geneticists and radiotherapists should look together at the problem involved.

To ensure that the different members of the symposia can make their full contribution we felt that it would be very useful to circulate preprints of the paper or at least to provide detailed abstracts. This is particularly necessary when scientists meet who research in different subjects, since the specialist reading a paper can often not be followed easily by those not working in the same field. Yet, it is the fusion of the different disciplines which these symposia are intended to promote. At the Liège meeting papers or abstracts were pre-circulated; the high level of the discussion and the large programme covered show the value of the measure.

In this volume we have collected the papers which were presented and the discussion except in a few cases where authors had made arrangements for publication elsewhere. A few participants were invited to contribute reviews but the majority of the papers consist of new experimental work. We felt that it was of the utmost importance that publication should be quick in this rapidly growing field. The appearance of this volume four months after the deadline for contributions was made possible only by the authors who adhered strictly to the dates given them and above all by Messrs. Butterworth who gave us every assistance and who dealt so expeditiously with the production.

Z. M. Bacq
P. Alexander

Liège and London
December 1954

CONTENTS

THE LIÈGE MEETING

The Radiobiology Meeting in Liège was held from August 29th to Sept. 1st, 1954, in the Anatomy Building of the University and was attended by scientists from twelve countries. The names of participants are given on page xiv.

The Members were entertained by the Mayor to a banquet and to a reception by the Provincial Governor; the Congress was officially opened by the Rector of the University, Prof. M. Dubuisson. To ensure that the heavy programme could be carried out, a tight time schedule had to be adhered to; this was made possible by providing lunch and afternoon tea, served by the Ecole d'Hôtellerie of the City of Liège, in a room adjacent to the lecture theatre. As a result the cost of the Meeting was great and the catering and circulation of reprints was made possible only by generous grants of the Belgian Government, of the Foundation Francqui (Brussels) and of the Centre Anticancéreux of the Liège University (Chairman, Prof. J. Firket).

I owe a great debt to my secretary, Mrs. M. Goutier, for her very efficient collaboration; considerable help was also given by Dr. J. Frederic and by Prof. Chevremont's staff.

Z. M. B.

PARTICIPANTS

Belgium

Prof. Bacq, Z. M.	Laboratoire de Pathologie, 1, rue des Bonnes Villes, Liège.
Dr. Betz, H.	1, rue des Bonnes Villes, Liège.
Prof. Desaive, P.	Institut de Radiothérapie, Hôpital de Bavière, Liège.
Dr. Duchesne, J.	1, rue Professeur Mahaim, Cointe-Sclessin.
Dr. Dor, L.	93, rue des Wallons, Liège.
Dr. Errera, M.	1850, Chaussée de Wavre, Bruxelles.
Dr. Fischer, P.	rue de Visé, Jupille.
Prof. Firket, J.	Institut de Pathologie, 1, rue des Bonnes Villes, Liège.
Dr. Fredericq, J.	Institut d'Anatomie, rue de Pitteurs, Liège.
Dr. Fredericq, P.	1, rue des Bonnes Villes, Liège.
Dr. Garsou, J.	Institut de Radiologie, Hôpital de Bavière, Liège.
Dr. Gerebtzoff, M.	Institut d'Anatomie, rue de Pitteurs, Liège.
Dr. Goutier, R.	Institut de Pathologie, 1, rue des Bonnes Villes, Liège.
Mme Goutier-Pirotte, M.	Institut de Physiologie, Place Delcour, Liège.
Dr. Herve, A.	Institute de Radiothérapie, Hôpital de Bavière, Liège.
Dr. Henry, J.	Institut Jules Bordet, 1, rue Héger Bordet, Bruxelles.
Dr. Heusghem, C.	81, avenue de l'Observatoire, Liège.
Prof. Maisin, J.	Voer des Capucins, Louvain.
Dr. Maisin, H.	Voer des Capucins, Louvain.
Dr. Nizet, A.	Hôpital de Bavière, Clinique Médicale B, Liège.
Dr. Ramioul H.	8, rue du Batty, Cointe-Sclessin.
Dr. Simon (Mlle)	Institut Jules Bordet, 1, rue Héger Bordet, Bruxelles.
Dr. Verly, W.	26, rue Jonruelle, Liège.
Prof. Welsch	Centre de recherches pour antibiotiques, Boulevard de la Constitution, Liège.
Prof. Gueben	50, rue des Wallons, Liège.
Dr. Govaerts, J.	Institut de Physique Nucléaire, Place du XX, Août, Liège.
Dr. Lecomte, J.	11, rue Blès, Liège.
Dr. Vanremoortere, E.	11, rue G. Truffaut, Liège.
Dr. Beaumariage, M. (Mlle)	563, rue de Herve, Bois de Breux.
Dr. Closon, J.	Centre Anticancéreux de l'Université de Liège, Hôpital de Bavière.

Canada

Dr. Howard, B.	Atomic Energy of Canada, Ltd., Chalk River, Ontario.
Dr. Newcombe, H. B.	Atomic Energy of Canada, Ltd., Chalk River, Ontario.

Denmark

Dr. Arley, N.	Institute of Theoretical Physics, University of Copenhague.
Dr. Christensen, B.	Finsen Memorial Hospital, Strandboulevarden 49, Copenhague.
Dr. Mogens, F.	Fiseninstitutet og Radiumstationen, Strandboulevarde, Copenhague.
Dr. Johansen, C.	Finsen Laboratory, Strandboulevarden 49, Copenhague.

France

Dr. Bonet-Maury	Institut du Radium, 26, rue d'Ulm, Paris 5e.
Dr. Burg, C.	Institut de Physique Biologique, Faculté de Médecine, Strasbourg.
Prof. Chevallier, A.	Faculté de Médecine, Université de Strasbourg.
Dr. Davydoff, S. (Mlle)	Fort de Châtillon, Fontenay-aux-Roses, Paris.
Dr. Duplan, J. F.	Collège de France, 3, rue d'Ulm, Paris 5e.
Dr. Giaux	Centre Oscar Lambret, 17, rue du Croquet, Lille, France.
Dr. Latarjet, R.	Institut du Radium, 26, rue d'Ulm, Paris 5e.
Dr. Lefort, M. (Mme)	8, rue Pastourelle, Paris 3e.
Dr. Lourau-Pitres (Mme)	8, rue Ceusier, Paris 5e.
Dr. Magat, M.	Laboratoire de Chimie-Physique, 11, rue Pierre Curie, Paris 5e.
Dr. Chapiro	Laboratoire de Chimie-Physique, 11, rue Pierre Curie, Paris 5e.
Dr. Mandel, P.	18, rue Lamey, Strasbourg.
Dr. Swyngedauw	140, Boulevard de la Liberté, Lille.

Germany

Prof. Rajewsky, B.	Forsthausstrasse, 70 Frankfurt a.M.Süd.
Dr. Pauli	Forsthausstrasse, 70 Frankfurt a.M.Süd.
Dr. Hug	Forsthausstrasse, 70 Frankfurt a.M.Süd.
Dr. Koch, R.	Institut de Radiobiologie, Université de Freiburg, i.Br.

Great Britain

Dr. Abbatt, J. D.	Radiotherapeutics Research Unit, Hammersmith Hospital, Ducane Road, London, W.12.
Dr. Alexander, P.	The Chester Beatty Research Institute, Fulham Road, London, S.W.3.

Dr. Alper, T. (Miss)	Experimental Radiopathology Research Unit, Hammersmith Hospital, Ducane Road London, W.12.
Dr. Belcher, E. H.	Physics Department, Royal Marsden Hospital, Fulham Road, London, S.W.3.
Dr. Boag, J. W.	20 Hollycroft Avenue, London, N.W.3.
Dr. Britton, R. H. W.	Director of Research, Horlicks Ltd., Slough, Buckinghamshire.
Dr. Butler, J. A. V.	Chester Beatty Institute, Fulham Road, London, S.W.3.
Dr. Conway, B. E.	Chester Beatty Institute, Fulham Road, London, S.W.3.
Dr. Court Brown, W. M.	Radiotherapeutics Research Unit, Hammersmith Hospital, Ducane Road, London, W.12.
Dr. Dale, W. M.	Christie Hospital, Wilmslow Road, Manchester 20.
Dr. Ebert, M.	Experimental Radiopathology Research Unit, Hammersmith Hospital, Ducane Road, London, W.12.
Dr. Elson, L. A.	Chester Beatty Research Institute, Fulham Road, London, S.W.3.
Dr. Gray, L.H.	Radiobiological Research Dept., Mount Vernon Hospital and The Radium Institute, Northwood, Middlesex.
Dr. Harriss, E. B. (Miss)	Royal Marsden Hospital, Fulham Road, London, S.W.3.
Dr. Holmes, B. E. (Mrs.)	Department of Radiotherapeutics, University of Cambridge, Downing Street, Cambridge.
Dr. Hornsey, S. (Miss)	Hammersmith Hospital, Ducane Road, London, S.W.12.
Dr. Howard, A. (Mrs.)	Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.12.
Dr. Howard-Flanders, P.	Hammersmith Hospital, Ducane Road, London, W.12.
Dr. Jolles, B.	Department of Radiotherapy, General Hospital, Northampton.
Prof. Koller, P. C.	Chester Beatty Research Institute, Fulham Road, London, S.W.3.
Dr. Lane, G. Roy	University of Southampton.
Dr. Lajtha, L. G.	Department of Radiotherapy, Churchill Hospital, Oxford.
Dr. Lamerton, L. F.	Physics Department, Royal Marsden Hospital, Fulham Road, London, S.W.3.
Dr. Laser, H.	Molteno Institute, University of Cambridge.
Dr. Lasnitzki, I. (Mrs.)	Strangeways Research Laboratory, Cambridge.
Dr. Laws, J. O.	Dept. of Experimental Pathology, Medical School, Leeds, 2.

Dr. Loutit, J.	M.R.C. Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berkshire.
Dr. Mee, L. K. (Mrs.)	Department of Radiotherapeutics, University of Cambridge, Downing Street, Cambridge.
Prof. Mitchell, J. S.	Department of Radiotherapeutics, Downing Street, Cambridge.
Dr. Mole, R. H.	Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berkshire.
Dr. Oster, I.	Institute of Animal Genetics, West Mains Road, Edinburgh.
Dr. Oliver, R.	Churchill Hospital, Oxford.
Dr. Paterson, E. (Miss)	Christie Hospital and Holt Radium Institute, Withington, Manchester 20.
Dr. Paton, J. P. J.	87 Stewarton Drive, Cambuslang, Lanarkshire.
Dr. Popjak, G.	Hammersmith Hospital, Ducane Road, London, W.12.
Dr. Philpot, J.	M.R.C. Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berkshire.
Dr. Revell, S. H.	Chester Beatty Research Institute, Fulham Road, London, S.W.3.
Prof. Rotblat, J.	St. Bartholomew's Hospital, London, E.C.1.
Dr. Scott, O.C.	Radiobiological Research Dept., Mount Vernon Hospital and The Radium Institute, Northwood, Middlesex.
Dr. Suit, H. D.	Radiobiological Laboratory, Dept. of Radiotherapy, Churchill Hospital, Oxford.
Dr. Smith, C. H.	Dept. of Radiotherapeutics, University of Cambridge.
Dr. Stocken, L. H.	Biochemical Laboratory, University Museum, Oxford.
Dr. Trowell, O. A.	M.R.C. Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berkshire.
Dr. Haigh, M. V. (Mrs.)	Christie Hospital, Manchester 20.
Dr. Wright, E. A.	Experimental Radiobiology Research Unit, Hammersmith Hospital, Ducane Road, London, W.12.
India	
Dr. Gopal Ayengar, A. R.	Chief Research Cytologist, Biology Division, Indian Cancer Research Centre, Parel, Bombay 12.
Japan	
Dr. Nishiwaki, Y.	Dept. of Biophysics, Osaka City University, School of Medicine, Osaka.

Norway

Dr. Devik, F.	Instituut for Patologi, Rikshospitalet, Oslo.
Dr. Eldjarn, L.	Biochemical Department, Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo.
Dr. Michaelsen, K.	Institute of Genetics and Plant Breeding, Agricultural College of Norway.
Dr. Mossige, J. (Mrs.)	The Norwegian Radium Hospital, Oslo.
Dr. Oftedal, P. (Miss)	Radiumhospitalet, Bestum-Oslo.

Sweden

Dr. Anderson, G.	The Gustaf Werner Inst. for Nuclear Chemistry, Uppsala.
Dr. Ehrenberg, L.	Inst. of Organic Chemistry, Sandasgat, 2, Stockholm.
Dr. Forssberg, A.	Institut de Radiophysics, Stockholm.
Dr. Gelin, O. E.	Plant Breeding Institution, Weibullsholm, Landskrona.
Prof. Gustafsson, A.	Statens Skogoforskningsinstitut Experimentalfältet.
Dr. Nybom, N.	The Gustaf Werner Institute for Nuclear Chemistry, University of Uppsala.
Dr. Ostergen, G.	Institute of Genetics, Lund.
Dr. Sherman, R. S., Jr.	Karolinska Sjukhuset, Radiulhemment c/o Radiotherapy, Stockholm.

Switzerland

Prof. Neukomm, S.	Centre Anticancéreux romand, Hôpital cantonal, Lausanne.
Dr. Mouton, R. F.	Battelle Memorial Institute, 7, route de Drize, Genève.
Dr. Lerch, M.	Centre Anticancéreux romand, Hôpital cantonal, Lausanne.
Dr. Peguiron	Centre Anticancéreux romand, Hôpital cantonal, Lausanne.

The Netherlands

Prof. Cohen, J. A.	Lange Kleiweg, 139 Risjwijk, Z.H.
Dr. Davids, J. A. G.	Lange Kleiweg, 139 Risjwijk, Z.H.
Dr. Sangster, I.	Steffenskamp 15, Laren, N.H.
Dr. Sobels	Genetisch Instituut, Stationsstraat 9, Utrecht
Dr. van Bekkum, D. W.	Lange Kleiweg, 139 Risjwijk, Z.H.
Dr. Vos, O.	Lange Kleiweg, 139 Risjwijk, Z.H.

United States of America

Dr. Behnke, A. R.	U.S. Naval Radiobiological Defense Laboratory, San Francisco Naval Ship Yard (Hunter Point), San Francisco, 24, California.
Colonel Blount, R. H.	U.S.A.F., Assistant Air Attaché, American Embassy, Paris.

Dr. Carlson, G. J.	Dept. of Zoology and Entomology, University of Tennessee, Oak Ridge National Laboratory.
Dr. Cole, L. J.	U.S. Naval Radiobiological Defense Laboratory, San Francisco, California
Dr. Deschner, E. E. (Miss)	Brookhaven National Laboratory, Upton, Long Island, N.Y.
Dr. Gey, O. G.	Jolius Hoffe University, Baltimore 5.
Dr. Hollaender, A.	Oak Ridge National Laboratory, Biology Division, Oak Ridge, Tennessee.
Dr. Hutchens, J. O.	Scientific Liaison Officer, Embassy of the U.S.A. Office of Naval Research, Keysign House, 429 Oxford Street, London, W.1.
Dr. Jacobson, L. O.	University of Chicago, Argonne Cancer Research Hospital, Chicago 37, Illinois.
Dr. Kirby-Smith, J. S.	Oak Ridge National Laboratory, Oak Ridge, Tennessee.
Dr. Kellner, A.	Brandeis University, Waltham, Mass.
Dr. Patt, H. M.	Argonne National Laboratory, P.O. Box 299, Lemont, Illinois.
Dr. Pollard, E.	Sloane Physics Laboratory, Yale University, New Haven 11, Connecticut.
Dr. Reimer, C. H.	Jolius Hoffe University, Baltimore 5.
Dr. Swanson, C. P.	Dept. of Biology, The Johns Hopkins University, Baltimore 18, Maryland.
Yugoslavia	
Dr. Hajdukovic, S.	Laboratoire de Biologie, Institut Boris Kidric Vintcho-Belgrade, P.O.B. 522.

ACTION DES RADIATIONS IONISANTES SUR LES LIPIDES

André Chevallier et Constant Burg

Institut de Physique biologique, Faculté de Médecine de Strasbourg (France)

Si les progrès de la radiochimie dans le domaine des milieux aqueux et des substances hydrosolubles ont été considérables au cours des dix dernières années, par contre, les connaissances au sujet de l'action des radiations sur les substances liposolubles sont encore très sommaires.

Une mise au point de la question peut paraître actuellement prématurée, et, de fait, les travaux portant sur ce problème précis sont très peu nombreux et les études systématiques font défaut.

La difficulté s'accroît encore si l'on veut se limiter aux substances lipidiques d'ordre biologique, c'est-à-dire aux acides gras et leurs esters, aux phospholipides, aux stérols, aux vitamines et hormones liposolubles.

C'est ainsi que pour essayer d'englober tous les aspects du problème et, en nous référant, en premier lieu, à des préoccupations d'ordre biologique, nous examinerons non seulement les résultats physico-chimiques se rapportant à une expérimentation *in vitro*, où le rayonnement a été appliqué directement à la substance liposoluble, mais également les modifications biologiques qui ont été obtenues, comme conséquences de l'irradiation d'un animal vivant. Dans ce dernier cas, il est certain que si quelques faits peuvent paraître clairement en rapport avec une action du rayonnement sur les lipides organiques eux-mêmes, la plupart ne seront que l'effet indirect d'une intervention portant sur des systèmes enzymatiques complexes ou sur des éléments hydrosolubles présents dans les tissus vivants. Mais, dans l'incapacité où nous nous trouvons de faire le départ entre les premiers et les seconds, nous tiendrons compte des uns et des autres.

Les considérations que l'on peut envisager sur le mécanisme d'action des radiations ionisantes sur un milieu quelconque, se classent naturellement en effets primaires à conséquences immédiates d'un caractère plus spécialement physique et en effets secondaires, en général, d'ordre chimique.

Il ne semble pas, au premier abord tout au moins, que l'on doive faire une place particulière aux milieux lipidiques en ce qui concerne les effets primaires. Ceux-ci sont en effet pratiquement représentés par les phénomènes d'ionisation d'une part, et d'excitation, d'autre part, et sont en relation avec les lois d'absorption de l'énergie reçue par le milieu. Mais il ne faut pas oublier qu'une même molécule peut donner naissance à un grand nombre de types d'ions différents pour des conditions d'irradiation donnée.

Dans le cas de l'excitation, comme dans le cas de l'ionisation, il faut s'attendre à voir intervenir la qualité du rayonnement utilisé dans les lipides comme dans les milieux aqueux. Une même quantité d'énergie administrée sous forme de rayonnement γ, d'électrons ou de particules α, aura des effets

différents en raison des différences considérables de densité des paires d'ions formés le long de la trajectoire de ces diverses particules (photons, β, α)[1, 2]. De ce fait, le rendement ionique, soit le rapport entre le nombre de molécules ayant réagi et le nombre des paires d'ions formés dans le milieu, sera très différent suivant le type du rayonnement et l'énergie qu'il transporte. L'accroissement de la densité des paires d'ions par unité de longueur favorisant la recombinaison ionique, ce dernier phénomène perdra progressivement de son importance au cours de l'utilisation d'un rayonnement X plus pénétrant ou d'un rayonnement γ. Ce fait qui explique en partie le faible rendement obtenu dans certaines réactions radiochimiques en milieu aqueux avec le rayonnement α comparé au rendement obtenu avec le rayonnement γ intervient probablement de la même manière lorsqu'on se trouve en milieu lipidique.

A partir des réactions primaires ainsi déclenchées, on considère que les molécules excitées ou ionisées subissent des fragmentations ou des modifications structurales avec apparition de formes fugaces (radicaux libres) ou de composés stables. Ces molécules excitées peuvent également subir un processus de conversion interne, c'est-à-dire un transfert de l'énergie d'excitation en énergie d'oscillation des atomes constituant la molécule. La molécule ainsi excitée pourra alors provoquer des réactions qu'elle ne serait normalement susceptible d'induire qu'à des températures plus élevées[3]. Ainsi certains des produits d'irradiation pourront avoir la possibilité de se combiner entre eux ou avec d'autres fragments moléculaires pour donner des combinaisons stables. D'autres, au contraire, pourront être le point de départ de réactions en chaîne de différents types. Ces réactions seront caractérisées par un rendement ionique très élevé et elles pourront être profondément modifiées par la présence dans le milieu de certaines substances en quantités très faibles, substances qui ont la possibilité de réagir avec les radicaux libres présents pour donner, soit des composés stables arrêtant ainsi la réaction, soit au contraire former de nouveaux radicaux libres qui pourront donner naissance à un type de chaîne différent du précédent.

Quoiqu'il en soit, et sans entrer dans l'étude générale du mécanisme intime de la réaction qui a été faite par divers auteurs[4-6], il est évident que toutes les reactions secondaires sont étroitement liées à la constitution des molécules irradiées et à la composition du milieu dans lequel elles se trouvent. Dans le cas des substances liposolubles la même considération s'applique intégralement et chaque milieu constitue, en somme, un cas d'espèce.

Cependant nous pouvons déjà présumer que dans les milieux lipidiques la présence d'oxygène dissout sera particulièrement importante. On peut penser que la quantité d'oxygène présente soit trop faible pour que les modifications de sa propre molécule par le rayonnement tiennent une place importante. Par contre, les molécules d'oxygène pourront réagir avec les radicaux libres formés à partir des substances lipidiques. Cette réaction peut amener la constitution d'une réaction en chaîne dont certains éléments peroxydiques seront susceptibles d'être décelés à l'analyse. Cependant une telle réaction est susceptible d'être modifiée soit dans le sens de l'accélération, soit dans le sens du ralentissement par la présence dans le milieu de substances qui, à l'état de traces, ont la possibilité d'intervenir dans la réaction et amener ainsi la réalisation d'un état assimilable à un effet pro ou antioxygène.

L'intervention de l'α-tocophérol, comme nous le verrons plus loin, illustre ce point de vue.

Un second aspect important de la radiochimie des substances lipidiques se rapporte à la présence dans le milieu irradié, soit d'un solvant lipidique, soit d'une autre phase liquide, aqueuse en principe. Dans ce dernier cas, le 'cage effect' de FRANCK-RABINOVITCH prend peut-être une importance plus grande que dans le cas d'une phase lipidique continue. Mais, d'autre part, l'intervention du rayonnement ionisant pourra provoquer, à partir du solvant, des radicaux libres qui ne se seraient pas formés au dépens de la substance liposoluble. Le fait est tout à fait remarquable en ce qui concerne les émulsions de lipides dans l'eau et au niveau desquelles se produisent des radicaux OH formés au dépens de la phase aqueuse. Bien entendu, on peut toujours penser à une intervention de ces radicaux sur les lipides présents.

Les phénomènes peuvent encore se compliquer si dans la phase aqueuse se trouvent des substances hydrosolubles à grand pouvoir réactionnel. Celles-ci, en réagissant avec les radicaux libres formés, soit dans la phase aqueuse, soit dans la phase lipidique, seront susceptibles de modifier le cours des réactions en chaîne qui tendent à prendre naissance dans cette dernière.

On arrive ainsi à des systèmes réactionnels extrêmement compliqués, mais malgré leur complexité et leur incertitude, de telles éventualités méritent d'être étudiées de près, car elles réalisent précisément les conditions qui se rencontrent au niveau des tissus vivants.

Pour tenir compte des considérations précédentes, nous sommes conduits à présenter les résultats expérimentaux qui ont été obtenus à propos de l'action des radiations ionisantes sur les lipides de la manière suivante.

Dans la première partie, consacrée à l'expérimentation *in vitro*, nous envisagerons successivement l'action des radiations : (*1*) sur des lipides purs en l'absence de solvant et de phase aqueuse, en examinant successivement les réactions en l'absence d'oxygène dissout puis en présence d'oxygène, (*2*) sur des lipides en présence d'oxygène dissout et d'une phase aqueuse pure ou contenant des substances hydrosolubles, et (*3*) sur des graisses complexes.

Dans la seconde partie, nous distinguerons, à la suite de l'irradiation d'un animal ou d'un tissu vivant, les modifications qui ont été constatées : (*1*) d'une manière immédiate sur les graisses du tissu adipeux, (*2*) de façon indirecte sur les constituants lipidiques de différents organes ou tissus : intestin, sang, foie, cerveau.

EXPERIMENTATION 'IN VITRO'

ACTION DES RADIATIONS IONISANTES SUR DES LIPIDES EN L'ABSENCE D'OXYGÈNE DISSOUT ET EN L'ABSENCE D'EAU ET DE SOLVANTS LIPIDIQUES.

Très peu de travaux ont été consacrés à l'étude de la radiochimie des acides gras supérieurs en l'absence d'oxygène. Cependant on peut, à partir de résultats obtenus sur des hydrocarbures en phase vapeur[7], ou en phase liquide, conclure à la possibilité d'isomérisation et de fragmentation de la molécule. A ce titre, la fragilité de la liaison carbone–carbone entre la fonction

carboxyle et le carbone adjacent, permet de supposer que la réaction de décarboxylation doit être assez aisée. Les résultats des expériences de SHEPPARD et BURTON[8] montrent ces différentes possibilités.

Sheppard et Burton ont irradié avec des particules α de l'acide acétique à 130° C sous forme vapeur et les acides caprylique, laurique et palmitique sous forme solide à la température ambiante.

Ces auteurs ont de plus observé la présence de pentadécane dans les produits d'irradiation de l'acide palmitique et de undécane dans les produits d'irradiation de l'acide laurique, c'est-à-dire des hydrocarbures, respectivement en C_{15} et en C_{11}, mettant bien en évidence l'importance des réactions de décarboxylation. Il faut également noter la formation de petites quantités de monoxyde de carbone due, soit à une action directe du rayonnement, soit par action sur le dioxyde de carbone et l'hydrogène moléculaire H_2 formé au cours de la réaction.

On peut, en outre, supposer que la formation de radicaux libres et des atomes libres d'hydrogène détermine des réactions de condensation ou de polymérisation. L'étude de l'action des radiations ionisantes sur les phospholipides et sur les différentes vitamines et hormones liposolubles en l'absence d'oxygène n'a pas été faite, soit à l'état cristallisé, soit en solution. Il faut cependant noter une observation[9] qui relèvent une destruction du carotène et de la vitamine A en solution dans l'hexane, en l'absence d'oxygène, sous azote.

ACTION DES RADIATIONS IONISANTES SUR LES LIPIDES EN PRÉSENCE D'OXYGÈNE DISSOUT ET EN L'ABSENCE D'EAU ET DE SOLVANTS LIPIDIQUES. (*1*) *Esters d'acides gras purs.* En présence d'oxygène dissout, le problème des modifications chimiques provoquées par l'irradiation des esters d'acides gras prend un aspect un peu différent. On doit avoir, dans ce cas particulier, les mêmes réactions qu'en l'absence d'oxygène, c'est-à-dire formation d'atomes d'hydrogène, de radicaux hydrocarbonés de longueurs diverses et de molécules stables d'hydrocarbures, à nombre d'atomes de carbone variable, dûes à des réactions de décarboxylation ou de fragmentation de chaîne. Il serait intéressant de rechercher systématiquement la présence de telles substances dans les produits d'irradiation en présence d'oxygène dans la limite où techniquement leur mise en évidence est possible.

A côté de ces réactions il faut envisager la possibilité de formation d'ions et de radicaux libres à partir de l'oxygène moléculaire dissout. La probabilité de cette réaction par action directe du rayonnement est cependant très faible si l'on tient compte du fait que le nombre d'atomes ionisés de chaque élément, dans un milieu donné, est grossièrement proportionnel au rapport en poids de cet élément dans le milieu. Les radicaux libres formés directement par action des radiations ionisantes sur l'oxygène moléculaire dissout seraient donc proportionnellement très peu nombreux et leur intervention ultérieure dans des réactions en chaîne pourrait être considérée, en principe, comme négligeable.

Une autre réaction, par contre, prend une importance des plus grandes. En effet, on peut envisager l'existence de réactions entre les radicaux libres formés au dépens des molécules d'acides gras et l'oxygène moléculaire dissout entraînant la formation de réaction en chaîne.

Une de ces réactions, entre autres, entraîne la formation de peroxydes suivant le schéma :

$$RH \rightsquigarrow R^{\bullet} + H^{\bullet} \quad R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$
$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

Ces peroxydes donnant des réactions analytiques relativement très sensibles ont été recherchés par de nombreux auteurs.

HANNAN et BOAG[10] en utilisant l'oxydation de l'ion Fe^{2} en Fe^{3} en présence de thiocyanate, ont observé la présence de peroxydes dans des échantillons de stéarate, oléate et linoléate de méthyle, ainsi que dans de la tristéarine irradiée. Les auteurs administrent des doses de 2.000.000 de roentgen équivalent physical (rep) sous forme d'électrons provenant d'un générateur de van de Graaff. Ils ont relevé des quantités de peroxydes de

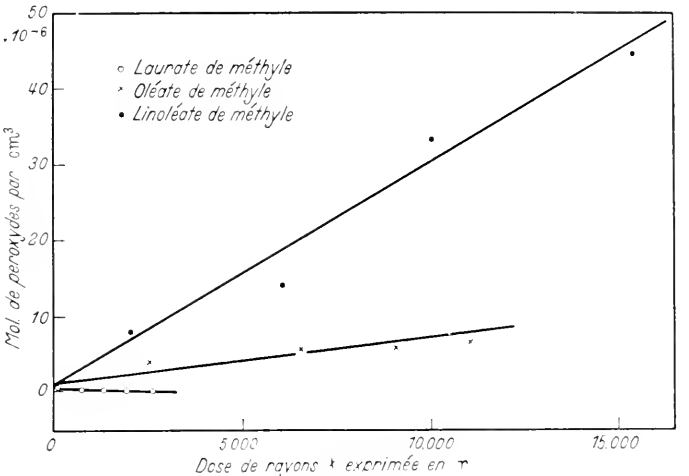


Figure 1. Variation du taux de peroxydes formés au cours de l'irradiation par les rayons X en fonction de la dose

l'ordre de 2 à 3 micromolécules par gramme d'esters irradiés. Ces mêmes auteurs ont obtenu des résultats analogues par irradiation d'hexane et de *cyclo*hexane.

Il est intéressant de relever qu'à très basse température — 70° C, le taux de peroxydes obtenu dans ces expériences a été très faible par rapport à celui obtenu à la température ambiante. Ce fait est, peut être, lié au ralentissement de la vitesse de diffusion de l'oxygène.

Des résultats analogues ont été obtenus par CHEVALLIER et BURG[11] en utilisant la méthode de dosage de HARTMAN et GLAVIND[12], c'est-à-dire l'oxydation de dichlorodihydroxyphénylènediamine en 2 : 6-dichlorophénol indophénol qui donne dans le xylène additionné d'acide acétique une couleur rouge intense dont l'intensité peut être déterminée par spectrophotométrie.

Les expériences ont consisté dans l'administration à des esters méthyliques, des acides laurique, oléique et linolénique de doses de rayons X variant entre 2.500 r et 15.300 r sous 80 kV, soit à un acide saturé et à des acides contenant une et deux doubles liaisons (*Figure 1*).

On peut constater que pour 2.500 r le laurate de méthyle ne présente pas de peroxydes décelables par la méthode utilisée. Mais, par contre, leur production est très nette dans le cas de l'oléate et encore plus marquée dans le cas du linoléate de méthyle.

Hannan et Boag[10] avaient obtenu des peroxydes à partir du stéarate de méthyle qui est également un acide gras totalement saturé. Mais il faut observer que dans l'expérience de Hannan et Boag les doses de rayonnement utilisées sont entre 100 et 1.000 fois supérieures à celles que nous avons employées dans notre expérience personnelle. Du fait que pour des doses identiques, le linoléate de méthyle présente un taux de peroxydes beaucoup plus élevé que l'oléate de méthyle qui n'a qu'une seule double liaison. On peut conclure que la présence des doubles liaisons facilite considérablement la production des peroxydes.

Il serait intéressant d'étudier de plus près la cinétique de formation de ces peroxydes et de vérifier s'ils ont la même structure que les hydroperoxydes formés au cours de l'auto-oxydation des acides désaturés. Il y aurait également intérêt à savoir si les peroxydes formés peuvent être détruits par le rayonnement lui-même.

On peut d'autre part, penser que cette production de peroxydes par réaction en chaîne doit s'accompagner de la formation de produits de polymérisation due à l'interruption des chaînes.

La possibilité des réactions suivantes est en effet à envisager :

$$R^{\bullet} + R^{\bullet} \rightarrow R{-}R \qquad R^{\bullet} + ROO^{\bullet} \rightarrow R{-}O{-}O{-}R$$
$$ROO^{\bullet} + ROO^{\bullet} \rightarrow R{-}O{-}O{-}R + O_2$$

La présence de polymères, difficile à mettre en évidence, n'a pas été recherchée. Cependant il faut relever une observation[10] qui, par irradiation d'ester de l'acide linolénique à $-70°$ C par des électrons, a obtenu une gélification de l'échantillon, traduisant sans doute un phénomène de polymérisation ou de condensation.

Cas particulier des esters d'acides polyéthyléniques (isomérisation). Dans le cas particulier des acides polyéthyléniques on pouvait se demander si la formation de peroxydes s'accompagnait d'une isomérisation des doubles liaisons qui passent en position conjuguée, isomérisation que l'on constate lors de la formation d'hydroperoxydes lors de l'auto-oxydation de ces acides à l'air atmosphérique. La présence de ces doubles liaisons est très facile à déceler. Elles entraînent en effet l'apparition d'une bande d'absorption extrêmement importante à 2.340 A. L'expérience confirme ce point de vue : la présence de cette bande d'absorption a été en effet relevée lors de l'irradiation d'esters de l'acide linolénique par Mead[13] en utilisant des rayons X obtenus sous 250 kV et par Chevallier et Burg[11] en utilisant des rayons X sous 80 kV.

La variation de l'absorption à 2.340 A est proportionnelle à la variation de la concentration en peroxydes du milieu pour des conditions expérimentales données, et la sensibilité de la détermination de l'absorption est tellement grande qu'on peut l'utiliser pour suivre la variation du taux de peroxydes du milieu. Il ne faut cependant pas oublier que le rapport entre le coefficient d'absorption à 2.340 A et le taux de peroxydes peut

varier suivant les conditions expérimentales. Il ne faudra donc utiliser cette méthode qu'avec beaucoup de prudence.

La présence de peroxydes dans les graisses irradiées entraîne également une diminution considérable de la stabilité de ces graisses en présence de l'oxygène atmosphérique, surtout en ce qui concerne les acides désaturés.

En effet les peroxydes peuvent se décomposer en donnant naissance à de nouvelles réactions en chaînes, par exemple suivant le schéma proposé par Bolland et Ten Haven[14] dans le cas des hydroperoxydes de l'acide linoléique.

$$ROOH \rightarrow R^{\bullet} \qquad \text{réaction d'initiation}$$
$$ROOH \rightarrow ROO^{\bullet}$$
$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet} \qquad \text{réaction de propagation}$$
$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$
$$R^{\bullet} + R^{\bullet} \rightarrow R{-}R \qquad \text{réaction de fin de chaîne}$$
$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$
$$RO_2^{\bullet} + RO_2^{\bullet} \rightarrow ROOR + O_2$$

Ces réactions secondaires peuvent se produire sous l'action du rayonnement ou spontanément longtemps après la fin de l'irradiation.

Sur le plan pratique, elles posent des problèmes importants pour la conservation des aliments stérilisés par les radiations ionisantes et peuvent peut être intervenir en radiobiologie et expliquer certains effets tardifs du rayonnement.

(2) *Esters gras en présence de traces de substances réactionnelles.* Une autre conséquence des réactions en chaînes provoquée par les réactions ionisantes au niveau des esters gras est la possibilité d'interaction entre les radicaux libres propagateurs de la réaction et les substances présentes à l'état de traces dans le milieu. Ces réactions peuvent entraîner, soit des fins de chaînes, soit au contraire, donner naissance à de nouvelles chaînes de réaction d'une nature différente.

Un exemple d'un tel phénomène nous est donné par les anti-oxygènes dans le domaine de l'auto-oxydation des esters gras désaturés. Ainsi, par exemple, l'auto-oxydation de l'ester éthylique de l'acide linoléïque qui se propage par les deux radicaux $ROO^{\bullet}$ et $R^{\bullet}$ peut être freinée par l'hydroquinone qui réagit avec le radical $ROO^{\bullet}$ pour donner un produit stable, provoquant par là une interruption de la chaîne[14].

Un point particulièrement important est l'étude de l'intervention sur les réactions radiochimiques des vitamines et hormones liposolubles dont certaines, comme les tocophérols, ont des propriétés anti-oxygènes bien connues. Mead et Polister ont examiné l'action d'un très grand nombre de ces substances sur du linoléate de méthyle au cours de l'irradiation par les rayons X, mais les expériences ont été faites en présence d'eau, ce qui entraîne des conséquences particulières et nous reviendrons sur ces travaux dans un chapitre suivant.

L'action de la plupart des vitamines et hormones liposolubles sur les esters acides gras purs, en l'absence de solvant, n'a pas fait l'objet d'étude systématique. Cependant Chevallier et Burg[11] ont relevé que la présence de α-tocophérol, en très faible quantité, freine d'une façon importante la formation de peroxydes par les rayons X dans le linoléate de méthyle. Le

tocophérol aurait donc, dans ces conditions, des propriétés analogues aux propriétés anti-oxygènes dont il fait preuve au cours de l'auto-oxydation des acides gras à l'air. Une étude cinétique approfondie du phénomène présenterait un grand intérêt (*Figure 2*).

(*3*) *Vitamines liposolubles.* A côté de ces travaux sur la radiochimie des esters gras, il faut citer un certain nombre d'études qui ont été faites sur les vitamines liposolubles. Comme la plupart de ces vitamines sont cristallisées à la température ambiante, la plupart des travaux ont été effectués sur des solutions dans l'hexane. La structure de ce solvant se rapprochant de celle de la chaîne hydrocarbonée, on peut penser que les radicaux libres formés par les radiations à partir de l'hexane sont de même nature que ceux formés à partir des acides gras. Ainsi si on est obligé de tenir compte d'une action

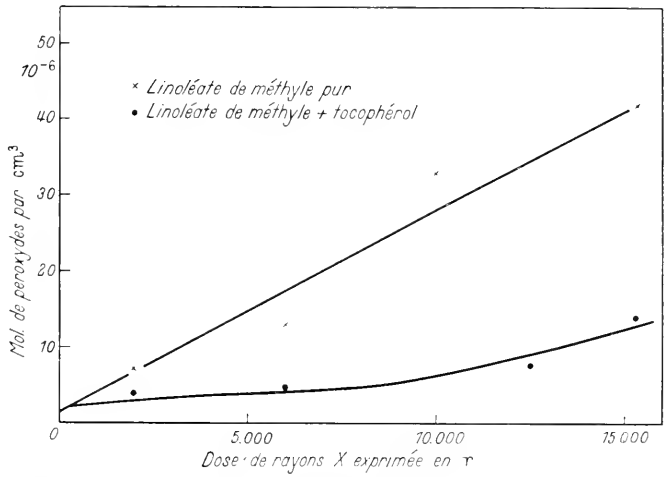


Figure 2. Variations du taux de peroxydes formés au cours de l'irradiation du linoléate de méthyle en fonction de la dose, en présence et en l'absence de tocophérol

indirecte du rayonnement sur les vitamines dissoutes par l'intermédiaire du solvant, on peut penser au moins que qualitativement les réactions seront les mêmes. Il faudra cependant rester très prudent dans l'interprétation des rendements ioniques obtenus.

La destruction de l'acétate de vitamine A et du β-carotène en solution dans l'hexane sous l'action de rayons X ou d'électrons, a été étudiée[9, 15].

Il est important de noter que la vitamine A, en solution dans des esters d'acides gras, semble beaucoup plus rapidement détruite par le rayonnement que lorsqu'elle est seule.

Cette fragilité particulière de la vitamine A en solution lipidique peut avoir son importance dans le problème de la stérilisation des produits alimentaires par le rayonnement.

L'étude radiochimique des autres vitamines liposolubles, D, K et même E, soit à l'état pur, soit en solution, ne semble pas avoir été faite jusqu'à présent.

Action des Radiations Ionisantes sur les Lipides en Présence d'Oxygène Dissout et d'Eau. L'étude de l'action des radiations ionisantes sur les lipides en présence d'eau présente un grand intérêt en ce sens que l'on se rapproche davantage des conditions qui existent normalement dans l'organisme. Malheureusement le problème se complique d'une façon considérable. Il faut, en effet, tenir compte des possibilités des réactions de la substance lipidique elle-même et en présence d'oxygène tout en envisageant la possibilité de réactions indirectes dues aux radicaux libres formés dans la phase aqueuse au dépens de l'eau. Il faut envisager ici la possibilité d'actions indirectes analogues à celles que Dale[16] a observées dans le domaine hydrosoluble.

On peut admettre, avec Weiss[17], que l'irradiation de l'eau forme essentiellement des radicaux H et OH, par exemple, suivant le mécanisme proposé par Lea[18].

L'électron peut également réagir avec un accepteur d'électrons[19], par exemple :

$$H^+ + e \rightarrow H^\bullet \quad H^\bullet + H^+ \rightarrow H_2^+$$

l'ion H_2^+ pouvant à son tour fixer un électron et donner naissance à de l'hydrogène moléculaire. Si cette hypothèse est exacte, l'hydrogène qui est formé en très faible quantité pendant l'irradiation d'eau pure[20] doit être augmenté d'une façon importante en solution acide[19].

En présence d'oxygène, on peut également avoir des interactions entre les radicaux formés dans l'eau et l'oxygène moléculaire dissout. On peut en particulier avoir la réaction

$$O_2 + H^\bullet \rightarrow HO_2^\bullet$$

En présence d'oxygène, l'irradiation de l'eau entraîne également la formation de petites quantités d'eau oxygénée qui ont été décelées pour la première fois par Bonet-Maury[21]. Cet auteur a obtenu, en faisant agir les corpuscules α du radon sur de l'eau, 0,3 γ de peroxyde d'hydrogène par microcuries détruit, ce qui correspond à 0,54 molécules de peroxyde d'hydrogène par paire d'ions formés dans la solution. Le taux d'eau oxygénée était beaucoup plus abondant en présence d'oxygène dissout qu'en l'absence d'oxygène[22]. Loiseleur[23] a également montré que le rendement en eau oxygénée était fonction du pH, le rendement étant plus élevé aux pH acides. On conçoit aisément que ces produits (radicaux : $H^\bullet$, $OH^\bullet$, $HO_2^\bullet$, eau oxygénée) formés au dépens de l'eau peuvent réagir sur les acides gras présents au niveau de l'interface eau/lipide et modifier profondément l'action des radiations ionisantes sur le substrat lipidique.

(1) Acides gras purs. (a) Etude en l'absence d'oxygène. Mead[13] a irradié de l'acide linoléïque dans l'eau à pH 8,5–9 en l'absence d'oxygène et en présence d'azote avec des rayons X à 250 kV et a constaté une conjugaison des doubles liaisons, conjugaison qui accompagne généralement la formation de peroxydes. Il est à noter cependant que le rendement ionique était faible, voisin de 9. Dans ces conditions, si les peroxydes ne se sont pas formés au dépens d'oxygène résiduel, il faut admettre leur formation à partir de radicaux libres formés au dépens de la phase aqueuse, fait qui présente le plus grand intérêt.

(*b*) *En présence d'oxygène.* Mead[13] a étudié le comportement de l'acide linoléique dans l'eau à pH 8,5–9 en présence d'oxygène. Il a relevé une conjugaison très importante des doubles liaisons avec un rendement ionique assez élevé de l'ordre de 100 environ, ce qui semble bien impliquer l'existence d'une réaction en chaînes. Il est à noter cependant que le rendement ionique varie d'une façon importante suivant la dose de rayons X et suivant la vitesse à laquelle cette dose est administrée. En collaboration avec le même auteur, Polister[24] a également étudié le cas d'émulsions de linoléate de méthyle, irradiées avec des rayons X à 250 kV. Ils ont retrouvé, en présence d'oxygène, le même rendement ionique élevé en doubles liaisons conjuguées et en peroxydes, que lors de l'irradiation d'acide linoléique dans l'eau. Ces peroxydes ont été dosés suivant la méthode de Lea[18].

Polister et Mead[24] se sont également adressés à des émulsions d'esters de l'acide β-éléostéarique qui présente trois doubles liaisons conjuguées sous l'influence du rayonnement X. Ils ont constaté la disparition d'une des

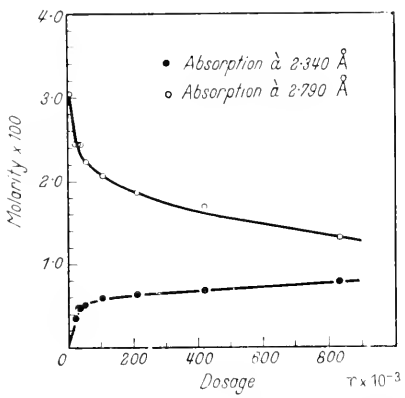


Figure 3. Variations de l'absorption ultraviolette de l'acide β-éléostéarique au cours de l'irradiation par les rayons γ du cobalt (communiqué par J. F. Mead)

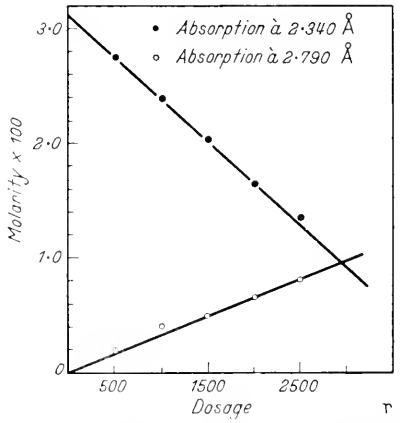


Figure 4. Variations de l'absorption ultraviolette de l'acide β-éléostéarique au cours de l'irradiation par les rayons X à 250 kV (communiqué par J. F. Mead)

trois doubles liaisons conjuguées. Cette étude est assez commode à faire, une triple liaison conjuguée donnant une bande d'absorption intense à 2.790 A, il suffit de suivre l'absorption à cette longueur d'onde et de mesurer l'absorption à 2.300–2.350 A qui indique la formation de deux doubles liaisons conjuguées due à la disparition d'une des trois doubles liaisons conjuguées qui existait précédemment. Il est à noter que si qualitativement la réaction est la même avec des rayons X à 250 kV et le rayonnement γ du cobalt, quantitativement les deux réactions se déroulent d'une façon très différente. Il faut remarquer que dans toutes les expériences de Mead[13], il y avait dans l'eau de petites quantités d'alcool éthylique.

Il y aurait intérêt à étudier la possibilité de formation d'alcool secondaire ou de glycol par action du radical OH sur une double liaison, par un mécanisme analogue à celui mis en évidence par Keller et Weiss[25] au cours

de l'irradiation du cholestérol et du 3-β-hydroxypregn-5-en-20-one en solution aqueuse en présence d'oxygène dissout.

(2) *Vitamines liposolubles pures en émulsion aqueuse.* Mead a également entrepris des travaux sur le comportement des émulsions aqueuses de plusieurs vitamines liposolubles en présence d'oxygène sous l'action, soit de rayons X, soit de rayons γ.

La méthylnaphtoquinone n'a pas présenté de modifications de son spectre d'absorption après administration de 40.000 r sous 250 kV. L'acétate de vitamine A, par contre, est rapidement détruit. L'analyse spectrale des produits d'irradiation montre la disparition progressive des cinq doubles liaisons conjuguées avec apparition de fragments de la chaîne primitive, fragment à deux ou trois doubles liaisons conjuguées. Il faut remarquer que

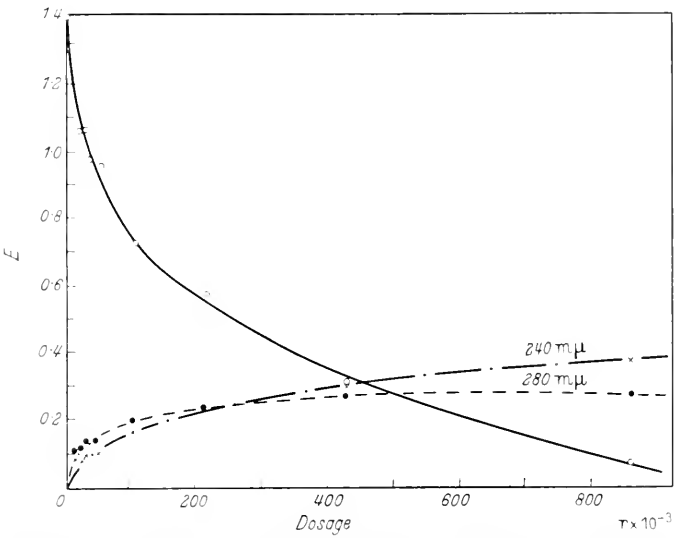


Figure 5. Variations de l'absorption ultraviolette de l'acétate de vitamine A au cours de l'irradiation (communiqué par J. F. Mead)

l'absorption ultraviolette des produits de destruction est tout à fait semblable à celle qui a été observée autrefois par Chevallier et Dubouloz[26] dans le cas de l'oxydation photochimique de cette même substance.

L'ergostérol, la provitamine D, est également détruite par l'irradiation. Les bandes d'absorption aux environs de 2.800 Å disparaissent progressivement pour donner naissance à une nouvelle bande d'absorption à 2.340 Å (*Figure 6*). Une étude plus approfondie de la radiochimie de ces deux vitamines est actuellement entreprise dans le laboratoire de Mead[13]. Par contre il ne semble pas que nous possédions actuellement de données précises sur la vitamine E, malgré l'importance qu'elle joue dans les phénomènes d'oxydation.

(3) *Acides gras en présence de traces de substances vitaminiques liposolubles.* La formation de réaction en chaînes au cours de l'irradiation d'acides gras purs en présence d'oxygène, nous permet de penser que la présence de certaines vitamines à l'état de trace, peut modifier profondément l'aspect des réactions

en réagissant sur les radicaux formés par le rayonnement. C'est effectivement ce qui a été observé[24]. Ces auteurs ont administré à des émulsions de linoléate de méthyle dans l'eau des doses de rayons de 1.000 r sous 250 kV, ceci après avoir ajouté à l'émulsion des quantités très faibles de différentes vitamines liposolubles et ils ont déterminé la vitesse de conjugaison des doubles liaisons. L'acétate de vitamine A, à concentration élevée, inhibe d'une façon importante la conjugaison des doubles liaisons.

Le γ-tocophérol fait également preuve d'un pouvoir inhibiteur considérable, mais à des concentrations beaucoup plus faibles. Une concentration de $2{,}6 \times 10^{-5}$M est encore efficace. Pour des concentrations plus élevées, le pouvoir inhibiteur du tocophérol est total et le rendement ionique tombe

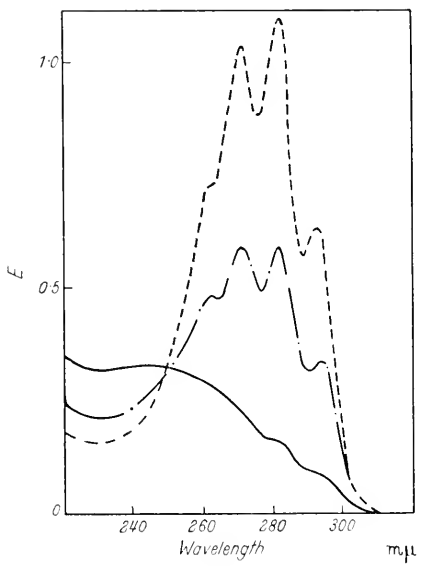


Figure 6. Modifications spectrales de l'ergostérol au cours de l'irradiation (communiqué par J. F. Mead)

a zéro. A la dose de 1.000r les auteurs n'ont pas relevé de destruction appréciable du tocophérol.

La vitamine D a également fait preuve d'un pouvoir inhibiteur, mais beaucoup moins marqué. Pour des doses de 4.000r les auteurs n'ont pu observer de modifications importantes du spectre de la vitamine D. Les trois vitamines A, D et E ont donc toutes trois une action inhibitrice marquée, surtout la vitamine E.

(4) Acides gras en présence de substances hydrosolubles. Polister et Mead[24] ont également examiné la possibilité d'interaction de substances dissoutes dans la phase hydrosoluble sur les réactions en chaînes induites par le rayonnement en phase liposoluble. L'acide ascorbique, la cystéine, le glutathion, suivant leur concentration, inhibent à des degrès divers, la conjugaison des doubles liaisons du linoléate de méthyle. Dans le cas de l'acide ascorbique, à une concentration de 9,5 mole pour cent on a une inhibition de toute conjugaison pour des doses de rayons X de 1.000r. Il faut noter que cet effet protecteur s'accompagne d'une destruction notable de la vitamine.

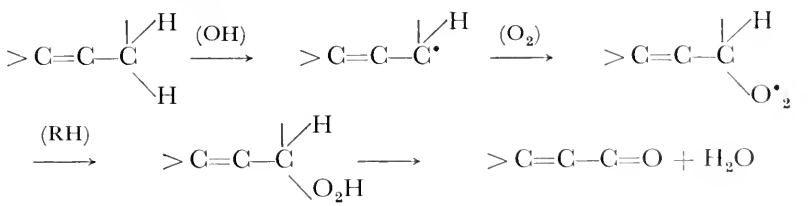
Dans le cas de la cystéine on voit apparaître, au cours de l'irradiation, une bande d'absorption à 2.750 Å qui n'a pas pu encore être interprétée, mais qui indique la complexité des réactions.

Dans les interactions entre les substances hydrosolubles et les processus d'oxydation qui se déroulent dans la phase liposoluble on peut se demander s'il s'agit d'une interaction à l'interface lipide/eau entre les substances hydrosolubles comme l'acide ascorbique et les radicaux intervenant dans les réactions en chaînes en phase liposoluble. Il pourrait s'agir en somme d'un mécanisme analogue à celui de l'action anti-oxygène du tocophérol ou de l'hydroquinone au cours de l'auto-oxydation des acides gras. Mais on peut se demander, au contraire, s'il ne s'agit pas plutôt d'un effet analogue à l'effet de protection observé par Dale. Ces substances protectrices, comme l'acide ascorbique, réagissant cette fois avec les radicaux libres formés par les radiations dans la phase aqueuse et non avec ceux formés en phase lipidique comme dans l'hypothèse précédente.

Mead et Polister ont également étudié l'action de la catalase pour établir si la présence d'eau oxygénée, fournie par l'irradiation dans la phase aqueuse, était susceptible de faciliter la formation d'hydroperoxydes en phase lipidique. Le rendement ionique de la conjugaison des doubles liaisons pour une dose de 1,000r en présence de catalase est de 80,5 et en l'absence de catalase de 72,5. Il semble que dans les conditions où se sont placés ces auteurs, on ne relève pas d'influence appréciable de la catalase.

Sterols et Hormones Steroïdes. Un certain nombre de travaux ont été consacrés à l'étude de la radiochimie des stérols et des hormones stéroïdes en présence d'eau. Nous ne nous étendrons pas sur cette question. Elle a fait l'objet d'un rapport récemment[27] et nous ne rappellerons que les résultats essentiels. Les principales modifications chimiques relevées peuvent être rapportées à la présence dans l'eau soit de radicaux $OH^{\bullet}$, soit de radicaux $HO^{2\bullet}$ dûs à la réaction $H^{\bullet} + O_2 \rightarrow HO^{2\bullet}$, l'atome libre $H^{\bullet}$ étant formé par le rayonnement à partir de l'eau. L'irradiation du cholestérol par les rayons X a été étudiée[25]. On a essentiellement une attaque de la double liaison en 5–6 avec formation de dérivés hydroxylés.

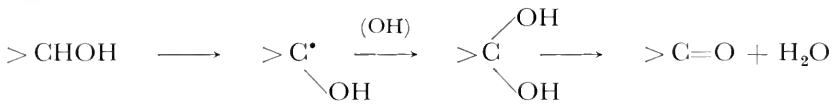
On relève également la formation d'une fonction cétone en position 7 sans doute suivant le schéma



qui fait intervenir l'oxygène dissout.

L'irradiation du 3 β-hydroxypregn-5-en-20-one entraîne également l'apparition de dérivés hydroxylés par un mécanisme analogue à celui relevé à propos de la double liaison du cholestérol. Keller et Weiss[25] ont également irradié des solutions aqueuses d'acide cholique; ils ont obtenu de la formation de l'acide 3 α : 12 α-dihydroxy-7-ketocholanique.

On a à nouveau une attaque de la position 7 sans doute suivant le mécanisme



Les mêmes auteurs ont entrepris une étude de l'oestrone en solution alcaline ou en solution acétique aqueuse. Ils ont obtenu la formation de lactone. On obtient dans ce cas une attaque du noyau pentagonal et non pas du noyau aromatique, ce qui laisserait penser à une sensibilité plus grande du cycle pentagonal aux radicaux OH.

D'autres travaux ont été également entrepris sur la progestérone et la cortisone. Mais les auteurs ont rencontré de grandes difficultés quant à l'isolement des produits d'irradiation qu'il est souvent difficile d'obtenir à l'état cristallisé.

L'intérêt de ces travaux sur la radiochimie des stéroïdes en solution aqueuse est considérable en ce sens qu'elle nous donne un aperçu des phénomènes qui peuvent se passer *in vivo* au niveau de ces molécules. Il faut également observer que les produits obtenus par irradiation sont souvent analogues à ceux obtenus par oxydation biologique *in vivo* et ceci ouvre peut être un champ de recherches intéressant.

Action des Radiations Ionisantes sur des Graisses Complexes. Nous n'avons rapporté jusqu'ici que les travaux qui avaient été effectués sur des substances pures dans des conditions bien définies. D'autres travaux ont été effectués sur des milieux naturels beaucoup plus complexes, généralement dans le but d'étudier le problème de la stérilisation des aliments par les radiations ionisantes.

Hannan avec Boag[10] et Shepherd[28, 29], avaien étudié le comportement du beurre après administration de 2.000.000 de rep sous forme d'électrons produits par un générateur de van der Graaff. Ils ont tout d'abord observé une destruction rapide de la vitamine A et du carotène, observation qui avait déjà été faite par Faila et collaborateurs, et plus tard par Chalmers *et al.*[9] Ils ont constaté également la destruction des anti-oxygènes présents dans le beurre, essentiellement représentés par le tocophérol, destruction qui se traduit par la disparition du temps de latence de la graisse au cours de son auto-oxydation spontanée à l'air.

Ces auteurs ont également observé un fait très curieux. Ils ont procédé, en présence d'air, à l'irradiation d'échantillons de beurre à $-70°$ C, puis ils ont conservé ces différents échantillons à des températures diverses allant de -70 à $+20°$ C, et ont déterminé la variation du taux de peroxydes en fonction du temps dans chaque échantillon. Ils ont constaté que la formation ultérieure de ces peroxydes est beaucoup plus importante pendant la conservation aux environs de $-20°$ C qu'aux autres températures.

D'autre part, si l'irradiation est effectuée en l'absence d'oxygène et la conservation à diverses températures dans les mêmes conditions, la mise en contact avec l'air des divers échantillons montre la formation beaucoup plus rapide des peroxydes pour l'échantillon conservé à $-20°$ C. De plus, un

séjour de 15 minutes à $+20°C$ suffit pour faire disparaître le phénomène. Les auteurs attribuent le phénomène à la formation à $-70°C$ d'un radical relativement stable à $-20°C$ qui se détruirait à des températures plus élevées et dont la formation ne nécessiterait pas la présence d'oxygène.

Il est intéressant de noter que les produits dont la vitesse de formation, après irradiation, est plus grande à $-20°C$ qu'aux autres températures, donnent les réactions des peroxydes, c'est-à-dire oxydent KI en I_2 et Fe^{2+} en Fe^{3+}, mais réagissent également avec le réactif de Schibsed qui est un test de Schiff modifié et dont on se sert en général pour déceler la présence d'aldéhyde de poids moléculaire élevé.

Des travaux ont été également effectués sur le lait et Kung et collaborateurs[30] ont constaté que le carotène et la vitamine A contenues dans ce liquide étaient détruits par le rayonnement γ. Une dose de 10^6 r détruisant 85 pour cent de la vitamine A et 45 pour cent du carotène. Il est à noter que la sensibilité de ces deux substances au rayonnement est beaucoup plus faible qu'à l'état pur en solution dans l'hexane, par exemple. La vitamine A et le carotène en solution dans l'hexane, sont détruits par le rayonnement avec un rendement ionique voisin de l'unité[9]. Dans le sérum humain le rendement ionique tombe à 0,01. La vitamine A et le carotène semblent être protégés contre le rayonnement, aussi bien dans le sang que dans le lait et ceci s'explique assez bien à la lumière des travaux de Polister et Mead[24].

ACTION DES RADIATIONS IONISANTES SUR LES LIPIDES DE L'ORGANISME 'IN VIVO'

Lorsqu'on soumet un organisme vivant à l'action des radiations ionisantes, on relève toute une série de modifications portant sur les lipides des différents organes. Certaines de ces modifications semblent être dues à une action directe du rayonnement sur les molécules lipidiques, action par conséquent voisine de celles qui peuvent être obtenues *in vitro*. C'est ainsi que l'on peut considérer l'apparition de substances à caractères peroxydiques dans le tissu adipeux. D'autres modifications, au contraire, semblent relever d'une action indirecte, c'est-à-dire être la conséquence de perturbations provoquées dans d'autres systèmes, sans doute en phase hydrosoluble. Nous étudierons successivement ces deux aspects de l'action du rayonnement X sur les lipides de l'organisme.

Action des Radiations Ionisantes sur les Lipides du Tissu Adipeux après Irradiation d'un Animal Vivant. Dubouloz *et al*[31] ont mis en évidence la présence de peroxydes dans un extrait acétonique de peau de rats, après avoir administré des doses variables de rayons X allant de 3.500 r à 20.000 r. Les taux de peroxydes obtenus étant de l'ordre de 10^{-8} molécule par centimètre carré de peau. Une partie de ces substances donnant des réactions à caractère peroxydique est soluble dans l'éther de pétrole, ce qui met en évidence leur caractère lipidique. Les auteurs ont également fait une observation curieuse : pour des doses de 3.500 r et de 5.000 r le taux de peroxydes dosés immédiatement après l'irradiation augmente ensuite progressivement pour atteindre un maximum vers le septième jour. La

méthode de dosage utilisée était une méthode basée sur l'utilisation de la thiofluorescéine mise au point par Dubouloz. Cette méthode, extrêmement sensible, permet de doser $0,5 \times 10^{-8}$ molécule de peroxydes.

Bacq *et al*[32], en utilisant la méthode de Hartman et Glavind, ont recherché des peroxydes dans des extraits au xylène de la graisse peritesticulaire de souris et de rat. Cette recherche a été effectuée 30 minutes après administration à l'animal de 1.000r sous 80kV. Ces auteurs ont relevé des titres en peroxydes de l'ordre de $0,3 \times 10^{-6}$ mole par gramme de graisse. Mais comme les résultats obtenus sur les animaux témoins indiquent des valeurs négatives, il faut être assez prudent dans l'interprétation de ces résultats.

Horgan et Philpot[33, 34] ont également entrepris de nombreux travaux sur le dosage des peroxydes organiques dans l'extrait butanolique de souris ayant reçu des doses de rayons X de 950r. Ils ont utilisé tout d'abord une méthode de Glavind et Hartman modifiée qui leur a permis de déceler des quantités de peroxydes correspondant à un rendement ionique de 70, mais la vitesse de réaction étant différente de celle observée avec les hydroperoxydes habituels. Les auteurs ont alors mis au point une nouvelle méthode de dosage de peroxydes faisant intervenir comme réactif le chlorure stanneux en dosant l'excès de chlorure stanneux, n'ayant pas réagi, par réduction soit du 2 : 6-dichloroindophénol, soit du bleu de phénol. Dans ces conditions les auteurs ont obtenu des peroxydes avec un rendement ionique voisin de celui obtenu précédemment. Pour 950r ils ont relevé un taux de peroxydes de $2,18 \times 10^{-7}$ mole par gramme de souris, soit un rendement ionique de 84. Le problème est cependant plus compliqué qu'il n'apparaît à première vue et Philpot vous entretiendra lui-même des travaux qu'il a entrepris entre autres sur la nature de ces peroxydes.

On peut être surpris d'observer un taux de peroxydes aussi faible dans les graisses du tissu adipeux après irradiation. Cependant Chevallier et Burg[11] ont constaté qu'il existait dans les graisses de réserve des substances qui freinaient la formation des peroxydes au cours de l'irradiation. En effet si l'on procède à l'irradiation, soit de fragment de tissu adipeux, soit d'extrait éthéré, on ne relève, après administration de rayons X à dose de 1.500r que des taux de peroxydes négligeables. Par contre, si on fait passer au préalable la solution éthérée sur une colonne d'alumine d'activité convenablement choisie, l'irradiation de la solution lipidique entraîne la formation d'une quantité considérable de peroxydes. Cette expérience montre bien l'existence, dans le tissu adipeux, de substances inhibant la formation de peroxydes, substances qui sont restées sur la colonne d'alumine et qui sont sans doute du tocophérols.

Modifications des Substances Lipidiques de Divers Tissus après Irradiation d'Animaux Vivants. Celles-ci correspondent en principe aux effets, soit quantitatifs, soit qualitatifs constatés au niveau des diverses substances lipidiques appartenant aux organes ou aux tissus d'un animal soumis à l'action des radiations ionisantes, sans que l'on puisse rapporter les effets contrôlés à une action directe des radiations.

(*1*) *Lipides totaux.* Bacq *et al* [52] ont étudié les variations des lipides totaux de l'organisme du rat après administration de doses léthales (1.000r) de rayons X à 200 kV. Pour éviter les difficultés soulevées par l'anorexie due

aux rayons, le travail a été effectué sur des animaux soumis au jeûne. Les graisses étant extraites suivant la méthode de Kumagawa, reprises dans l'éther de pétrole et pesées après évaporation du solvant. Les graisses totales, qui s'établissent en moyenne vers 22 grammes au départ, accusent une diminution très marquée. Chez les témoins vers la cinquantième heure on trouve environ 15 grammes, puis 10 grammes après 80 heures, 7 grammes après 100 heures et un chiffre voisin de 4 grammes pour des durées de jeûne variant entre 120 et 170 heures. Bien qu'il existe des différences individuelles marquées, on obtient une courbe assez régulière jusqu'à 120 heures, puis un palier.

Chez les animaux irradiés, la diminution des graisses au départ est très accusée ; elle suit à peu près la variation observée chez les témoins, mais elle s'atténue beaucoup plus tôt, et, à partir de 70 heures jusqu'à 150 heures, elle s'établit sur une zône en plateau avec une valeur voisine de 9 grammes. Il y a donc là une différence très nette avec les animaux témoins. Cette différence pourrait s'expliquer soit par l'inhibition d'un système d'utilisation lipidique, soit par l'accélération de la synthèse sous l'influence du rayonnement. Mais il y a de plus un autre élément qui apparaît très nettement et qui réside dans la très grande irrégularité du phénomène à l'échelle individuelle. La dispersion des résultats est beaucoup plus marquée que chez les témoins. Par contre, si on procède au dosage des acides gras à deux, trois et quatre doubles liaisons, on constate que leur présence ne semble pas affectée ni par le jeûne, ni par le rayonnement, au moins jusqu'à la soixante-dixième heure. La quantité totale d'acide arachidonique par animal reste constante jusqu'à la cent-trentième heure. Elle ne commence à diminuer qu'au moment où l'animal présente des troubles pré-mortem. Le tocophérol contenu dans le tissu adipeux ne présente guère de variations après administration de 1.000 r. Il ne semble également pas affecté par le jeûne.

(2) *Résorption intestinale.* On a cru pendant longtemps que la résorption des graisses par l'intestin était modifiée par l'irradiation. Martin et Rogers[35,36] avaient observé une diminution de résorption chez le chien après administration d'une dose faiblement erythémateuse. Dodds et Webster[37] avaient également décrit une altération de la résorption des graisses chez l'homme après irradiation.

Mead, Decker et Bennett[38] arrivent cependant à des conclusions tout à fait différentes. Ils ont administré, à des rats, de l'oléate de méthyle contenant 10 pour cent d'octadécadiénoate de méthyle dont les doubles liaisons, situées en 9 et 11, sont conjuguées et, de ce fait, facilement décelables par mesure du spectre d'absorption ultraviolet à 2.340 A. Ils ont tout d'abord procédé au dosage des graisses fécales de souris soumises à trois régimes différents et ayant reçu des doses de rayons X de 500 r. Ils n'ont relevé d'augmentation de l'excrétion fécale de graisse que chez les animaux soumis à un régime ne contenant pas de graisse. Les graisses fécales ne peuvent donc pas être d'origine alimentaire et doivent provenir d'une sécrétion intestinale, peut être de la desquamation de la muqueuse.

Ces auteurs ont également procédé au dosage des graisses dans les différents fragments du tube digestif après administration des lipides par une sonde stomacale. Ils n'ont pas constaté d'altération de la résorption des graisses au niveau de l'intestin, mais très souvent la résorption des lipides est retardée,

le bol alimentaire étant retenu beaucoup plus longtemps dans l'estomac par suite d'une augmentation du tonus du sphincter pylorique. Les auteurs ont également constaté une augmentation de la motilité de l'intestin grêle.

Coniglio *et al*[39] ont étudié l'excrétion fécale de graisses chez des rats après irradiation, mais en comparant les animaux irradiés à des témoins recevant la même quantité de nourriture que celle absorbée par les animaux irradiés. Dans ces conditions ces auteurs n'ont pas retrouvé de différence dans l'excrétion de graisse fécale entre les animaux témoins et les animaux irradiés et ceci pendant 28 jours pour des doses de rayons X de 500 et 650 r.

Bennett *et al*[40] ont examiné la résorption de la vitamine A alcool au niveau de l'intestin du rat. Ils ont trouvé cette absorption normale, sinon un peu accélérée, après administration de dose de rayons X de l'ordre de 625 r. Si l'irradiation ne modifie pas la résorption des lipides, il semble cependant que l'on puisse relever un certain nombre d'altérations biochimiques au niveau de l'intestin.

Coniglio et Hudson[41] ont étudié l'incorporation d'acétate marqué au C_{14} dans les lipides de la tunique intestinale. Ils ont administré 650 et 750 r en champ général à des rats et 22 heures plus tard ils ont injecté par voie intrapéritonéale de l'acétate marqué dans la fonction carboxyle. Les animaux ont été sacrifiés après 20, 40 et 60 minutes après l'injection. Les auteurs ont observé une diminution de l'incorporation de l'acétate marqué dans les lipides de l'intestin. Dans toute cette expérience les témoins ont reçu la même quantité de nourriture que celle absorbée par les animaux irradiés.

Hevesy et Dreyfus[42], avaient également relevé des modifications dans l'incorporation d'acétate marqué dans les lipides de l'intestin, mais sans attribuer à leurs résultats une valeur significative.

Conard[43] a étudié la choline-estérase de l'intestin de rats soumis à des doses de rayons X de 500 r. Il a constaté une diminution importante de l'activité enzymatique 15 à 20 heures après l'irradiation. Le phénomène est maximum le quatrième jour où la choline-estérase voit son activité diminuée de 60 pour cent. Elle retrouve des valeurs normales vers le vingtième jour. Il semble donc que la radiosensibilité bien connue de l'intestin s'accompagne de troubles affectant la motilité et de certains métabolismes biochimiques sans que cependant la résorption des lipides soit nettement affectée.

L'administration de doses élevées de rayons X à des animaux entraîne cependant une anorexie importante et Smith *et al*[44] se sont demandés si cette anorexie ne pouvait être responsable de la mort des animaux irradiés. Dans ce but les auteurs ont exposé à des doses léthales de rayons X des souris ordinaires et des souris rendues obèses, c'est-à-dire ayant des réserves lipidiques importantes. Ils ont trouvé la même courbe de variations de poids et la même courbe de mortalité après irradiation dans les deux lots d'animaux, la totalité des souris irradiées, obèses ou non, étant soumise au jeûne pendant toute la durée de l'expérience. Les auteurs en concluent que l'anorexie n'est pas responsable de la mort des animaux irradiés.

(3) Constituants lipidiques du sérum. L'administration de radiations ionisantes entraîne des modifications de toute une série de constituants lipidiques du sérum, modifications qui portent d'une part, sur certaines lipoprotéines du

sang, et d'autre part, sur le cholestérol, les vitamines liposolubles et l'activité de la lipase sanguine.

(*a*) *Modifications du taux des lipides et phospholipides totaux du sérum.* Steadman[45a] a étudié les modifications des lipides sanguins du lapin sous l'influence du rayonnement ; il a constaté une augmentation considérable des lipides totaux du sérum, une augmentation plus faible des phospholipides. Kohn *et al*[45b] ont observé également chez le rat une élévation du taux des lipides, élévation qui était grossièrement parallèle à la variation du rapport albumine-globuline. L'auteur n'a pas relevé de modifications du taux des phospholipides après irradiation. Buchanan *et al*[46] ont étudié les phospholipides plasmatiques de la rate femelle après irradiation par des rayons X pour des doses de l'ordre de 500 r. Les auteurs n'ont pas trouvé de modifications de la choline des phospholipides. Par contre 48 heures après une irradiation comprise entre 500 et 2.000 r. Buchanan note une élévation de la choline et du phosphore lipidique plasmatique, ce qui confirme les résultats antérieurs déjà observés par Entenman et Neve[47] qui avaient observé une augmentation du taux de phosphore lipidique pour les doses supérieures à 500 r.

(*b*) *Modifications des lipoprotéines sanguines.* Rosenthal[48] a constaté qu'après administration d'une dose léthale de rayons X à des lapins, il observait une opalescence du sérum, opalescence qui apparaît 24 heures après l'exposition aux radiations pour disparaître complètement après 72 heures. Cette opalescence présentait le caractère particulier d'être supprimée par agitation du sérum avec l'éther. Il s'agissait donc d'une substance liposoluble. L'auteur a également pu établir qu'il n'y avait pas de relation entre cette opalescence et la destruction, soit de globules blancs, soit de globules rouges, destruction consécutive à l'irradiation. La même année, Muntz *et al*[49] ont étudié par électrophorèse les modifications des différentes protéines sanguines après irradiation chez le chien. Trois jours avant la mort des animaux ils ont constaté une élévation des globulines α 3, mais cette élévation des globulines α 3 ne parait pas liée à une modification du taux des lipides, mais probablement à un état infectieux.

Gjessing et Chanutin[50] ont étudié les modifications du sérum sanguin des rats après irradiation totale en administrant des doses variant de 500 à 2.000 r. Ils ont procédé au fractionnement du plasma par fractionnement alcoolique et ils ont recueilli cinq fractions différentes. Chaque fraction a été étudiée par électrophorèse et les auteurs ont procédé au dosage des lipides contenus dans chacune des fractions. Ils ont observé des modifications importantes dans le taux des lipides de la fraction 4 ; après le deuxième, troisième, quatrième et cinquième jour, une augmentation qui, peu à peu, revient à la normale. Kohn[51] a examiné les modifications du rapport albumine/globuline du sérum de cobaye après administration des doses variant entre 200 et 600 r. Il a constaté une élévation très importante du rapport albumine/globuline pendant les quatre premiers jours consécutifs à l'administration du rayonnement lorsque la détermination des albumines et globulines est faite par précipitation saline ou par ultracentrifugation. Par contre, lorsque le taux de globulines et d'albumines est déterminé par électrophorèse, on ne trouve pas de modifications du rapport albumine/globuline. De même si le plasma est extrait à l'éther juste avant la détermination du taux de globulines et d'albumines, on retrouve un taux d'albumine/globuline

normal. L'adjonction *in vitro* à du plasma ou à du sérum normal de la substance extraits à l'éther élève également le rapport albumine/globuline du sérum de rat normal. Kohn a examiné aussi l'influence de l'hypophysectomie ou de l'adrénalectomie chez le rat, après irradiation. Il a retrouvé la même variation des rapports albumine/globuline chez les animaux hypophysectomisés et irradiés que chez les témoins irradiés seulement. Par contre, chez les animaux adrénalectomisés l'élévation du rapport albumine/globuline est plus forte que chez les témoins. L'auteur a également constaté que l'injection d'erythrocytes de mouton bloquait l'élévation du taux albumine/globuline, ce qui laisse supposer une intervention possible du système réticulo-endothélial.

Hewitt *et al*[52] ont repris l'étude des lipoprotéines du sérum de lapin après irradiation par ultracentrifugation. Ils ont constaté 12 heures après l'irradiation une augmentation brutale des lipoprotéines de faible densité ; augmentation qui était liée à l'apparition de l'opalescence du sérum. Ces auteurs ont constaté également que l'administration d'héparine fait disparaître très rapidement les lipoprotéines de faible densité, en les transformant en lipoprotéines de densité plus élevée. L'injection de bleu de toluidine, de sulfate de protamine ou de quinidine, produit des modifications des lipoprotéines identiques à celles de l'irradiation. En conséquence, les auteurs supposent que l'apparition de cette opalescence dans le sérum liée à un taux élevé de lipoprotéines de faible densité n'est pas due à une action directe sur les lipoprotéines mais probablement à un manque d'héparine consécutif à l'action du rayonnement.

(*c*) *Modifications du cholésterol.* Steadman[45a] avait constaté chez le lapin une élévation considérable du cholestérol lipidique après irradiation par les rayons X. Kohn *et al*[45b] a fait la même observation chez le rat et le cobaye et il a observé les mêmes variations du cholestérol plasmatique après irradiation d'animaux adrénalectomisés. Par contre, après hypophysectomie, l'élévation du taux de cholestérol n'est pas supprimée, mais légèrement modifiée. Cet auteur a constaté également que l'administration de dibénamine, de 2-dibenzyl-aminoéthanol, d'extrait lipidique corticosurrénal et de désoxycorticostérone, supprimait l'élévation du taux de cholestérol chez le rat après irradiation. Gjessing et Chanutin[50] après fractionnement alcoolique des protéines du plasma, avait constaté que l'élévation du taux de cholestérol se retrouvait surtout dans la fraction 4.

Roth *et al*[53] ont étudié la lipase sérique chez le rat après administration de 600 r. Ils ont observé une diminution nette de l'activité enzymatique du troisième au cinquième jour après l'irradiation suivie d'un retour à la normale le huitième jour. La même observation a été faite par del Buono[54] sur la tributyrinase du lapin.

(*4*) *Lipides hépatiques.* Le foie est généralement considéré comme un organe radio-résistant. Cependant toute une série de modifications biochimiques consécutives à l'irradiation indiquent des perturbations du métabolisme lipidique dont il est le siège. Forssberg et Hevesy[55] ont constaté une modification importante de la fixation de phosphate marqué dans le foie de jeunes souris à qui ils avaient administré 2.000 r, 15 minutes avant l'injection. Entenman et Weinman[56] ont étudié la fixation de ^{32}P dans les phospholipides du foie de rats irradiés avec des doses variant de 1.000 à 2.500 r

et ont également noté une modification importante de cette fixation après irradiation. Hevesy et Dreyfus[42] ont injecté de l'acétate, marqué au C_{14} dans le groupe carboxyle, à des souris auxquelles ils avaient administré une dose de 880 r. Ces auteurs ont constaté une modification importante de l'incorporation de l'acétate marqué aux lipides hépatiques sans accorder à leurs résultats une valeur statistiquement significative. Coniglio et Hudson[41] ont étudié le même problème en 1944 chez le rat. Dubois *et al*[57] ont constaté également chez le rat, après administration d'une dose de 800 r, une perturbation importante du métabolisme de l'acide citrique au niveau du foie. Enfin, un certain nombre d'auteurs ont étudié le comportement de la choline-oxydase après irradiation.

Tous ces faits semblent bien indiquer que l'irradiation entraîne une perturbation du métabolisme lipidique et il faut s'attendre à observer des modifications dans la constitution lipidique de cet organe. Cependant après administration de doses uniques de rayons X on ne relève généralement aucune stéatose hépatique ou tout au plus des signes de stéatose extrêmement discrets. Pohle et Bunting[58] ont administré à des souris, des doses de rayons X variant entre 600 et 2.500 r sans relever des signes histologiques des lésions hépatiques autre qu'un oedème cellulaire sporadique. Ariel[59] a repris l'étude des lésions hépatiques dues à l'administration des doses uniques, cette fois chez le lapin. Les rayons ont été administrés sur un champ hépatique et les doses ont été 300, 3.000, 30.000, 50.000 et 100.000 r, doses qui ont été administrées en une seule fois. L'auteur a noté des signes histologiques d'oedème, d'infiltration leucocytaire et de nécrose, lésions variables suivant les doses administrées, mais il n'a jamais relevé de stéatose.

Chevallier et Burg[11] ont administré à des rats des doses léthales uniques de 800 et de 3.000 r sans relever de lésions différentes de celles observées sur le lapin[59] ou sur la souris[58]. Entenman et Neve[47] ont administré également à des rats, en champ général, des doses de 2.500 r et ils n'ont pas relevé de modifications de la concentration en lipides totaux, phospholipides ou lipides non phospholipidiques.

Coniglio *et al*[39] ont administré des doses de 500 r à des rats et ils ont examiné le taux des différents lipides hépatiques 28 jours plus tard. Ils ont constaté une élévation nette du taux de triglycérides alors que le taux de phospholipides était normal. Pensant que cette élévation du taux de lipides hépatiques pouvait être due au jeûne consécutif à l'anorexie que présentent ces animaux, l'auteur a repris ses expériences en comparant le taux des lipides hépatiques des animaux irradiés à celui d'animaux témoins recevant la même quantité de nourriture que les animaux irradiés. Dans ces conditions l'auteur a constaté qu'il n'y avait pas de modifications des lipides hépatiques consécutives à l'irradiation, mais que les modifications entrevues étaient dues au jeûne.

Cependant Ord et Stocken[60], après administration de 1.000 r à des cobayes, ont relevé des signes discrets de stéatose hépatique. L'aspect morphologique des lésions hépatiques, après irradiation, est très différent lorsque la même quantité de rayons X est administrée en une série de petites doses quotidiennes au lieu d'être concentrée en une dose massive.

Ludin[61] a entrepris des irradiations systématiques localisées à la région hépatique chez le lapin et chez le cobaye. Il a relevé une stéatose hépatique

nette chez la plupart des animaux qui recevaient des doses quotidiennes faibles et qui, de ce fait, survécurent pendant longtemps, respectivement 21, 24 et 39 semaines. Doub *et al*[62] reprirent les expériences de Ludin[61], mais cette fois sur le chien. Dans ces conditions ils observaient, tout comme Ludin, une stéatose hépatique nette.

Chevallier *et al*[63] en administrant, en champ général, à des rats des doses quotidiennes de 50 r sous 200 kV, ont pu constater avec régularité

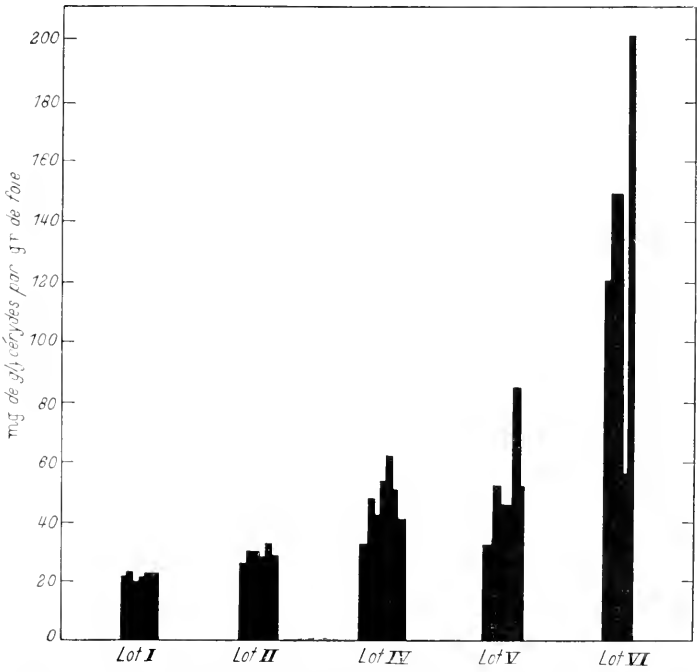


Figure 7. Taux de glycérides hépatiques en fonction de la dose quotidienne de rayons X administrée

I. Lot de 10 rats normaux provenant de l'élevage
II. Lot de 6 rats ayant reçu quotidiennement de l'auréomycine pendant une période allant de 80 à 90 jours, sans administration de rayons X
IV. Lot de 15 rats recevant de l'auréomycine et une dose quotidienne de 200 r
V. Lot de 9 rats recevant de l'auréomycine et une dose quotidienne de 100 r
VI. Lot de 21 rats recevant de l'auréomycine et une dose quotidienne de rayons de 50 r.

des stéatoses hépatiques extrêmement nettes, alors qu'avec des doses quotidiennes de 100 r l'accumulation de lipides dans le foie est moins nette et qu'elle devient insignifiante lorsque la dose quotidienne arrive à 500 r (*Figure 7*).

Un certain nombre de travaux ont été consacrés également à l'étude de la vitamine A hépatique après irradiation. Bennett *et al*[40] ont administré à des rates femelles des doses de 625 r en champ général et ils ont dosé la vitamine A hépatique pendant la semaine qui suivait l'irradiation. Les auteurs ont procédé de la façon suivante : ils ont administré une dose unique de vitamine A et ils ont tué les animaux soit 6 heures après l'administration de la vitamine, soit 10 heures après. Ils ont constaté une diminution très

importante du taux de vitamine A hépatique chez les animaux irradiés. Ils ont également observé que 6 heures après l'administration de la vitamine, on trouve dans la carcasse un taux de vitamine A beaucoup plus élevé que chez les témoins. Mais 10 heures après l'administration de vitamine, la carcasse montre un taux de vitamine qui est redevenu normal, comme si la vitamine A déposée dans la périphérie était rapidement métabolisée.

Coniglio[39] a dosé la vitamine A du foie de rat ayant reçu une dose de rayons X variant entre 500 et 650 r et il a comparé les résultats obtenus au taux de vitamine A de rats témoins ayant reçu la même quantité de nourriture que celle absorbée par les animaux irradiés. Dans ce cas, l'auteur n'a pas observé de différence entre les taux de vitamine A hépatique des animaux irradiés et des témoins.

Roth *et al*[53] ont recherché, l'activité de toute une série d'enzymes après irradiation. Entre autres, ils n'ont pas relevé de modification, ni de la choline-oxydase, ni de la lipase hépatique.

(5) *Lipides du cerveau.* Un certain nombre d'auteurs ont étudié également l'action du rayonnement X sur les lipides du cerveau. Warren *et al*[64] considèrent qu'à dose thérapeutique les rayons X ne produisent pas de modifications histologique, du moins chez l'adulte.

Florsheim *et al*[65] ont irradié à des doses de 500 et 800 r des souris adultes et n'ont pas trouvé de modification de la fixation de phosphate marqué au niveau du cerveau. Des doses de 19.000 r également n'ont pas révélé d'altération du métabolisme du phosphore. Hevesy et Dreyfus[42] ont constaté une augmentation statistiquement significative de l'incorporation d'acétate marqué dans les lipides du cerveau de souris ayant reçu une dose de 880 r. Schwartz *et al*[66] ont étudié par spectrographie infra-rouge les différentes fractions de cerveau de foetus de rat et de rats adultes. 24 heures après administration de 15.000 r en champ général, les rats adultes montrent un abaissement statistiquement significatif de la fraction lipidique ne présentant pas de fonction amide du cerveau moyen et du cerveau antérieur.

L'irradiation de femelles gestantes à doses de 150 r cause des modifications analogues dans différentes parties du cerveau des animaux examinés à l'état adulte. Par examen spectroscopique ces auteurs ont pu établir que ces modifications sont dues à une diminution des phospholipides et du cholestérol (Schwartz, communication personnelle).

Il faut enfin noter un travail effectué par Altman *et al*[67] sur la synthèse d'acides gras par la moelle osseuse à l'aide d'acétate marqué chez le lapin. Immédiatement après l'irradiation, ces auteurs constatent une élévation considérable de la synthèse, aussi bien des acides saturés, que désaturés. Par contre après 48 heures, si la synthèse des acides saturés est redevenue normale, la synthèse des acides désaturés est tombée de 18 pour cent en-dessous de la normale. 158 heures après, au contraire, la synthèse d'acides désaturés est de 283 pour cent supérieure à la normale, alors que la synthèse des acides saturés est tombée à 58 pour cent en-dessous.

REFERENCES

[1] Gray, L. H. *Brit. J. Radiol. N.S.* 1947, Suppl. 1, 7 ; 1953, **26** 609.
Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S. et Scott, O. C. *Brit. J. Radiol. N.S.* 1953, **26** 638.

[2] Cormack, D. V. et Johns, H. E. *Brit. J. Radiol. N.S.* 1952, **25** 369.
[3] Franck, J. et Sponer, H. Contribution à l'étude de la structure moléculaire, p. 169. Liège, 1948.
[4] Fano, V. *Symp. Radiobiol., Oberlin College*, (June) 1950, 13. New York, Wiley.
[5] Platzman, R. L. *Symp. Radiobiol., Oberlin College*, (June) 1950, 97. New York, Wiley.
[6] Livingstone, R. S. *Trans. 5th Conf. J. Macey Jr Fdn : Biol. Anti-oxidants*, p. 17. New York, 1950.
[7] Wallenstein, M., Wahrhaftig, A. L., Rosenstock, H. et Eyring, H. *Symp. Radiobiol., Oberlin College*, (June) 1950, 70. New York, Wiley.
[8] Sheppard, C. W. et Burton, V. C. *J. Amer. chem. Soc.* 1946. **68** 1636.
[9] Chalmers, T. A., Goodwin, T. W. et Morton, R. A. *Nature, Lond.* 1945, **53** 513.
[10] Hannan, R. S. et Boag, J. W. *Nature, Lond.* 1952, **169** 152.
[11] Chevallier, A. et Burg, C. *Ann. Nutr., Paris*, 1953, **7** C81.
[12] Hartmann, S. et Glavind, J. *Acta chem. scand.* 1949, **3** 954.
[13] Mead, J. F. *Science*, 1952, **115** 470.
[14] Bolland, J. L. et Ten Haven, P. *Trans. Faraday Soc.* 1947, **43** 201.
[15] Proctor, B. E. et Goldblith, S. A. *Nucleonics*, 1949, **5** 56.
Proctor, B. E. et Goldblith, S. A. *Advanc. Food Res.* 1951, **3** 119.
[16] Dale, W. M. *Brit. J. Radiol. N.S.* 1947, Suppl. 1, 46 ; 1951, **24** 433.
Dale, W. M. *Symp. Radiobiol., Oberlin College*, (June) 1950, 177. New York, Wiley.
[17] Weiss, J. *Nature, Lond.* 1944, **153** 748.
[18] Lea, D. E. *Brit. J. Radiol. N.S.* 1947, Suppl. 1, 59.
[19] Stein, G. *Disc. Faraday Soc.* 1952, **12** 227.
[20] Allen, A. O. *J. phys. Colloid Chem.* 1948, **52** 479.
[21] Bonet-Maury, P. *Brit. J. Radiol. N.S.* 1951, **24** 422.
[22] Loiseleur, J., Latarjet, R. et Caillot, T. *C.R. Acad. Sci., Paris*, 1941, **213** 370.
[23] Loiseleur, J. *C.R. Acad. Sci., Paris*, 1942, **214** 76.
[24] Polister, B. H. et Mead, J. F. *J. agric. food Chem.* 1954, **2** 199.
[25] Keller, H. et Weiss, J. *J. Chem. Soc.* **1950** 2709 ; **1951** 25, 1247.
[26] Chevallier, A. et Dubouloz, P. *Bull. Soc. Chim. biol., Paris*, 1936, **18** No. 4, 704.
[27] Weiss, J. *Ciba Colloq. Endocrin.* 1953, **7** 142.
[28] Hannan, R. S. et Shepherd, H. J. *Nature, Lond.* 1952, **170** 1021.
[29] Hannan, R. S. et Shepherd, H. J. *Brit. J. Radiol. N.S.* 1954, **27** 36.
[30] Kung, H., Gaden, E. L. et King, C. G. *J. agric. food Chem.* 1953, **1** 142.
[31] Dubouloz, P., Dumas, J. et Vigne, J. *C.R. Soc. biol., Paris*, 1950, **144** 1080.
[32] Bacq, Z., Burg, C., Chevallier, A. et Heusghem, C. *J. Physiol. Path. gén.* 1951, **43** 640.
[33] Horgan, V. J. et Philpot, J. St L. *Trans. Faraday Soc.* 1953, **49** Part 3.
[34] Horgan, V. J. et Philpot, J. St L. *Brit. J. Radiol. N.S.* 1954, **27** 63.
[35] Martin, C. L. et Rogers, F. T. *Amer. J. Roentgenol.* 1923, **10** 11.
[36] Martin, C. L. et Rogers, F. T. *Amer. J. Roentgenol.* 1924, **11** 280.
[37] Dodds, E. C. et Webster, J. H. *Lancet*, 1924, **i** 533.
[38] Mead, J. F., Decker, A. B. et Bennett, L. R. *J. Nutr.* 1951, **43** 485.
Cf. Mead, J. F., Bennett, L. R., Decker, A. B. et Schoenberg, M. D. *J. Nutr.* 1951, **43** 477.
[39] Coniglio, J. G., Darby, W. J., Wilkerson, M. C., Stewart, R., Stockell, A et Hudson, G. W. *Amer. J. Physiol.* 1953, **172** 86.
[40] Bennett, L. R., Bennett, V. C., Shaver, A. et Grachus, Th. *Proc. Soc. exp. Biol., N.Y.* 1950, **74** 439.
[41] Coniglio, J. G. et Hudson, G. W. *Fed. Proc.* 1944, **5** 13, 578.
[42] Hevesy, G. et Dreyfus, G. *Ark. Kemi*, 1952. **4** 337.
[43] Conard, R. A. *Radiology*, 1953, **61** No. 2, 312.
[44] Smith, W. W., Chapman, W. H. et Alderman, I. M. *Amer. J. Physiol.* 1952, **169** 511.

[45a] Steadman, L. T. *Quart. tech. Rep. Univ. Rochester*, 1949.
[45b] Kohn, H. I., Swingley, N., Robertson, W., Kirslis, M., Ledford, E., Click, G. et Lane, J. *Amer. J. Physiol.* 1951, **165** 27.
[46] Buchanan, D. J., Darby, W. J., Bridgforth, E. B., Hudson, G. W. et Efner, J. A. *Amer. J. Physiol.* 1953, **174** 336.
[47] Entenman, C. et Neve, R. *U.S.A.E.C. Rep. No. AD-307 B)*, 1951.
[48] Rosenthal, R. L. *Science*, 1949, **110** 44.
[49] Muntz, J. A., Barron, E. S. et Prosser, C. L. *Arch. Biochem. (Biophys.)* 1949, **23** 434.
[50] Gjessing, E. C. et Chanutin, A. *Arch. Biochem. (Biophys.)* 1950, **27** No. 1, 191 ; see also : *J. biol. Chem.* 1947, **169** 657.
[51] Kohn, H. I. *Amer. J. Physiol.* 1950, **162** 703.
[52] Hewitt, J. E., Hayes, T. L., Gofman, J. W., Jones, H. B. et Pierce, F. T. *Amer. J. Physiol.* 1953, **172** 579.
[53] Roth, J. S., Eichel, H. J., Wase, A., Alper, C. et Boyd, M. J. *Arch. Biochem. (Biophys.)* 1953, **44** 95.
[54] del Buono, G. *Boll. Soc. ital. Biol. sper.* 1951, **27** 290.
[55] Forssberg, A. et Hevesy, G. *Ark. Kemi*, 1952, **5** 93.
[56] Entenman, C. et Weinman, E. O. *Fed. Proc.* 1952, **11** 44.
[57] Dubois, K. P., Cochran, K. W. et Doull, J. *Proc. Soc. exp. Biol., N.Y.* 1951, **76** 422.
[58] Pohle, E. et Bunting, C. *Acta radiol., Stockh.* 1932, **13** 117.
[59] Ariel, I. M. *Radiology*, 1951, **57** 561.
[60] Ord, M. G. et Stocken, L. A. *Physiol. Rev.* 1953, **33** No. 3, 356.
[61] Ludin, M. *Strahlenther*, 1925, **19** 138.
[62] Doub, M., Bollinger, A., et Hartman, F. *Radiology*, 1927, **8** 142.
[63] Chevallier, A., Burg, C. et Spehler, H. *C.R. Soc. biol., Paris*, 1953, **147** 497.
[64] Warren, S., McMillan, J. C. et Dixon, F. J. *Radiology*, 1950, **55** 375, 557.
[65] Florsheim, W., Doernbach, C. et Morton, M. E. *Proc. Soc. exp. Biol., N.Y.* 1952, **81** 121.
[66] Schwartz, H. P., Riggs, H. E., Glick, C., McGrath, J., Cameron, W., Beyer, E., Bew, E. et Childs, R. *Proc. Soc. esp. Biol., N.Y.* 1952, **80** 467.
[67] Altman, K. I., Richmond, J. E. et Salomon, K. *Biochim. biophys. Acta*, 1951, **7** 460.

DISCUSSION

Z. M. Bacq : La teneur α-tocophérol des lipides naturel suffit-elle à assurer une protection maximale des lipides contre l'oxydation par les radiations ionisantes ?

Je pose cette question parce que plusieurs auteurs, dont Herve et nous-même, ont observé que l'injection d'α-tocophérol n'augmente pas la radiorésistance.

Peut-être serait-il préférable pour connaître le rôle de l'α-tocophérol, de voir si les animaux carencés en cette vitamine sont plus radiosensibles ?

H. A. Chevallier : Il est certain que l'on est frappé par le fait que les hydroperoxydes qui ont pû être mis en évidence après irradiation des lipides chez un animal vivant ne correspondent qu'à des quantités extrêmement petites, à la limite de la sensibilité des méthodes de mesure, malgré des doses importantes de rayons administrées à l'animal.

Il semble logique de penser que leur production est entravée par la présence de substances anti-oxygènes dans les milieux vivants, ces substances agissant là comme *in vitro* et la prolongation de l'irradiation n'aboutissant peut être qu'à la destruction des peroxydes eux-mêmes par le rayonnement.

Il serait sans doute intéressant d'examiner si certaines actions biologiques classiquement connues consécutives à l'action des radiations ionisantes sur le vivant apparaissent de la même manière chez des animaux soumis au régime producteur de la diathèse exsudative, celui de Dam, par exemple.

THE NATURE OF THE PEROXIDE-LIKE SUBSTANCES FORMED IN MICE BY X-RAYS

V. J. Horgan and J. St. L. Philpot

Medical Research Council Radiobiological Research Unit, Harwell

In our last publication[1] we gave evidence that a lethal dose of X-rays in a mouse caused an immediate increase in peroxide-like material (called 'peroxide' for short) with mean ionic yield about 80, suggesting that the well-known chain reaction of autoxidation could be initiated by X-rays *in vivo*. We noted, however, that the peroxide was not as reactive as expected in that the cuprous-catalysed reaction with aerobic leucoindophenol was far from complete in 10 minutes at room temperature. The necessity for air as well as peroxide in the reaction with leucoindophenol complicated the interpretation and we looked for an anaerobic reagent. Stannous chloride reacted anaerobically but required heating even with ordinary 'reactive' peroxides (hydroperoxides) of the expected sort, so was useless for distinguishing 'reactive' peroxides from the 'unreactive' ones apparently present in the mouse. Leuco-brilliant cresyl blue, catalysed by alkaline cuproiodide, was mentioned as a promising new reagent but was awaiting a proper test.

EXPERIMENTAL

Mice, irradiated or not with 1000r of 250kV X-rays, were killed and extracted with 100ml of peroxide-free *n*-butanol in a Waring blender or top-drive macerator, sometimes in an atmosphere of nitrogen. After filtering 2ml were diluted to 9ml and deoxygenated by distillation of the solvent[1] or better by 3 minutes' bubbling with nitrogen in a vessel that had been heated at 100° *in vacuo* to remove absorbed oxygen. The peroxide reagent was then added, consisting of 1ml of a 0·002M solution of buffered leuco-brilliant cresyl blue in *n*-butanol. The leuco-dye was made by running the dye through a column of copper or cadmium powder, with lithium iodide or other complexing agent in the solution to combine with the metal ions. With the copper column an amount of cuproiodide catalyst equivalent to the dye was automatically introduced in this way. After 10 minutes at room temperature or 100°C the reaction mixture was introduced anaerobically into the cell of a Hilger Uvispek Spectrophotometer and measured at 600mμ where the molar extinction coefficient of brilliant cresyl blue was found to be 18,800.

RESULTS AND DISCUSSION

The present communication is even more of an interim report than the last, and continual changes in technique make it even harder to quote satisfactory experiments, but the following generalizations will give some idea of the trend of the research.

Anaerobic alkaline leuco-brilliant cresyl blue reacts significantly more with extracts of irradiated than of unirradiated mice ; but with cuproiodide catalyst there is little evidence of unreactive peroxide since the reaction is almost complete in 10 minutes at room temperature.

Experiments with brilliant cresyl blue using less or no cuprous catalyst show the presence of unreactive peroxide which is increased by irradiation.

If the maceration of the mouse is performed in nitrogen rather than in air the ionic yield is somewhat less. This suggests that some of the increased peroxide is formed during the maceration if air is present, as a result of either destruction of antioxidant by X-rays or production of some very easily autoxidizable material.

One of our troubles with leuco-brilliant cresyl blue was that small quantities of peroxide turned it pink instead of blue. This was due to an impurity extractable with butyl acetate. Although the impurity is of unknown structure it has advantages over brilliant cresyl blue as a peroxide reagent, being more stable in alkaline solution and being apparently better at distinguishing unreactive from reactive peroxide.

We have searched for model substances with properties resembling 'unreactive peroxide', and the results are summarized in *Table I*, which shows the approximate percentage reaction in ten minutes at room temperature and 100° C. With peroxides of unknown concentration contained in autoxidized substances the percentage is arbitrarily based on the extent of reaction at 100° with aerobic leuco-2,6-dichlorophenol-indophenol by our modification of the Hartmann-Glavind method[1]. We thought that 'unreactive peroxides' might be merely aldehydes, but these showed no reactivity whatever under the conditions used. All the hydroperoxides tried and also benzoyl peroxide and hydrogen peroxide were too reactive, going to completion in 10 minutes at room temperature. Diethyl peroxide was about right. In our previous report we excluded this type of peroxide because it does not react with stannous chloride even at 100° C ; but since the mouse extract contains reactive as well as unreactive peroxide its reaction with stannous chloride may have been due solely to the former, and the latter can still be classed with diethyl peroxide.

Disubstituted peroxides like diethyl peroxide are not supposed to be formed when simple unsaturated fats like oleates or linoleates are autoxidized. Consistently with this *Table I* shows that the peroxide in autoxidized ethyl oleate is wholely of the reactive type. It is known that conjugated double bonds can give disubstituted peroxides by 1,4 addition, and consistently with this *Table I* shows some unreactive peroxide among the autoxidation products of isoprene and diacetyl. Neither of these substances has, however, been found in mammals and preliminary experiments with the polyconjugated natural substances carotene and vitamin A gave discouraging results. We have also found 'unreactive peroxide' in autoxidized morpholine and triethylamine (used as buffers in our peroxide reagents). The nature of these is not yet clear.

Very recently our attention has been directed to Squalene (dihydrohexaisoprene) by some work of Sobel and Marmorston[2], who have shown that it induces the autoxidation of carcinogens. Bolland and Hughes[3] have shown that squalene undergoes chain autoxidation whereby one

Table I.—Extent of reaction of various peroxides, etc. with cadmium reduced brilliant cresyl blue

Pure Substances	*Percentage Theoretical*	
	R.T.	100°
Butyric Aldehyde	0	0
Benzaldehyde	0	0
Ethyl Hydroperoxide	111	111
tert. Butyl Hydroperoxide	108	108
Tetralin Hydroperoxide	43	43
Cyclohexyl-1-Hydroxyl-1-Hydroperoxide	110	110
Benzoyl Peroxide	108	108
Hydrogen Peroxide	67	67
Diethyl Peroxide	0	54

Impure Substances (Autoxidized)	*Indophenol*		*Brilliant cresyl blue*	
	R.T.	100°	*R.T.*	100°
Ethyl Oleate	100	(100)*	95	95
Isoprene	25	(100)*	2·1	9·3
Diacetyl	25	(100)*	24	100

* Arbitrary standard.

hydroperoxide group and one cyclic peroxide group are introduced per molecule. This would be approximately consistent with our findings in mouse extract if the surprising assumption were made that practically the whole of the X-ray induced autoxidation in the mouse involves squalene or similar substances (1,5 dienes) rather than oleic or linoleic acids (monoenes or 1,4 dienes). Squalene has been known for some time to occur in sebum and to be mainly responsible for skin peroxides, and although only traces have been found inside the mammalian body Langdon and Bloch[4] have proved that it is an intermediate in the synthesis of cholesterol from acetyl groups through co-enzyme A, with the rapid turnover time of about 30 minutes. This suggests that radiation may act through chain autoxidation of squalene and its near neighbours in the metabolic pathway, leading perhaps to the formation of abnormal sterols or to blocking of the formation of normal sterols, or to interference with some function of squalene itself, *e.g.* in cell membranes, or to direct toxicity of squalene peroxide. In any case, if the primary hypothesis is true, it might be expected that temporary blocking of squalene synthesis *via* acetyl CoA might diminish radiosensitivity by temporarily diminishing the steady-state concentration of squalene and its neighbours. Bacq has pointed out that cysteamine, probably the best known antiradiation drug, is a component of co-enzyme A, and seemed at one time to imply that it might act by helping to restore damaged co-enzyme A. The new squalene hypothesis suggests that cysteamine and other —SH compounds and amines may act in the opposite direction, *e.g.* by preventing the oxidation of lipoic acid to the disulphide form needed for transferring acetyl groups to co-enzyme A (*cf.* Gunsalus[5]). A good

anti-radiation effect should be shown by pantothenic acid analogues such as pantoyltauryl-p-anisidide, which according to KLEIN and LIPMANN[6] suppresses steroid and fatty acid synthesis in liver slices by blocking acetylation.

SUMMARY

(*1*) Alkaline cuprous-catalysed leuco-brilliant cresyl blue is oxidized by *n*-butanol extracts of lethally X-irradiated mice significantly more than by control extracts, the mean ionic yield being about 80.

(*2*) There is some evidence that part of the oxidizing material is less reactive than the α unsaturated hydroperoxides resulting from the autoxidation of unsaturated lipides.

(*3*) Similar behaviour is shown by autoxidized isoprene.

(*4*) It is suggested that the precursor of the oxidizing material in mouse extracts may be squalene and its metabolic neighbours.

REFERENCES

[1] HORGAN, V. J. and PHILPOT, J. ST. L. *Brit. J. Radiol.* 1954, **27** 63.
[2] SOBEL, H. and MARMORSTON, J. *Nature, Lond.* 1954, **174** 553.
[3] BOLLAND, J. L. and HUGHES, H. *J. Chem. Soc.* 1949, 492.
[4] LANGDON, R. G. and BLOCH, K. *J. Biol. Chem.* 1953, **200** 129.
[5] GUNSALUS, I. C. *Symposium on Mechanisms of Enzyme Action*, p. 545. Baltimore, Johns Hopkins Press, 1953.
[6] KLEIN, H. P. and LIPMANN, F. *J. Biol. Chem.* 1953, **203** 101.

HYDROGEN PEROXIDE PRODUCTION UNDER VARYING CONDITIONS OF IRRADIATION

M. Ebert

Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London

Aerated water is decomposed by ionizing radiation to molecular oxygen and molecular hydrogen, and hydrogen peroxide is formed. These reactions are of general interest in radiation biology for two reasons : (*a*) because they provide information on radical reactions and interactions in aqueous systems, and (*b*) because hydrogen peroxide is an important substance biologically.

Aqueous chemical systems exposed to radiation are known to be affected by dissolved oxygen and other gases and often show a marked pH dependence[1]. In radiation biology the effect of oxygen has been widely demonstrated[2, 3], but little is known about the effect of pH. The influence of these two variables on the formation and decomposition of hydrogen peroxide will be discussed in this paper.

It has been shown[4] that the concentration y of hydrogen peroxide formed in aerated water by a radiation dose d can be described by the function

$$y = a(1 - e^{-bd})$$

where a and b are constants (*see Figure 2*) which are characteristic for one set of experimental conditions, including radiation quality, dose rate, oxygen concentration and pH. This function reaches an equilibrium value for high doses, at which the hydrogen peroxide concentration is not altered by a further increase in dose. At these dose levels the forward and back reactions proceed at the same rate, *i.e.* formation and break-down of hydrogen peroxide balance each other.

Effect of dissolved gases

The initial yields of hydrogen peroxide in water quoted in the literature are very conflicting, the disagreement being much larger than can be ascribed to errors in dosimetry, or analytical procedures[5]. Some of the difficulty may be due to the fact that molecular hydrogen and oxygen are formed in water in yields comparable to those of hydrogen peroxide, and both these gases react with the primary free radicals. In some experimental conditions the gases formed cannot escape from the bulk of the solution.

The importance of this point is illustrated by an experiment in which varying amounts of hydrogen peroxide were added to neutral aerated water, which was then irradiated with 1·2 MeV electrons at high dose rates. The total doses delivered were approximately ten times higher than those needed to produce the equilibrium concentration of hydrogen peroxide. As shown

in *Figure 1*, the final yield for a given dose was proportional to the amount of hydrogen peroxide added before irradiation. The slope of the curve depended both on the dose rate and on the depth of the solution, being steeper for higher dose rates and deeper solutions. In the experiment illustrated, the irradiation vessels were Petri dishes 35 mm in diameter, and the solution was only 3·5 mm deep, conditions which would at first sight appear to favour the ready diffusion of gases. These results suggested that oxygen was formed by radiolysis of the hydrogen peroxide initially present, and, at the high dose rates used, could not escape, and therefore supersaturated the solution. To avoid this complication further experiments have been carried out under conditions in which the concentration of dissolved gases was controlled.

The base of the irradiation vessel was a sintered glass filter through which a gas stream could be forced. This, breaking up into small bubbles, ensured rapid equilibration of the solution with the desired gas, and the removal of gases formed during the irradiation. Gas mixtures were prepared by passing the individual gases through 'Rotameter' flowmeters, and

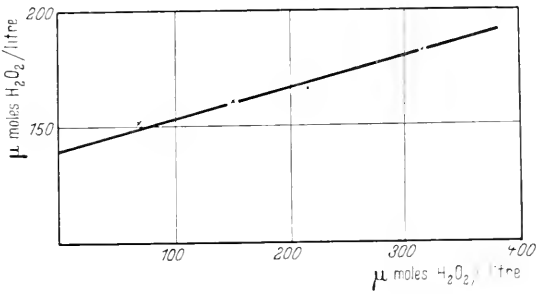


Figure 1. The amount of H_2O_2 *formed by* 7×10^6 rad *as a function of the initial concentration of* H_2O_2

the concentrations of the dissolved gases were assumed to be proportional to the partial pressures in the gas phase.

The above experiment was repeated with this technique. It was found that the hydrogen peroxide equilibrium concentration no longer depended on the initial concentration. It is clear that the bubbling technique removed the gaseous products of hydrogen peroxide decomposition.

Oxygen and nitrogen—The influence of oxygen on the formation of hydrogen peroxide was studied by using mixtures of oxygen and nitrogen. Each curve in *Figure 2* shows the yield of hydrogen peroxide against dose for one oxygen–nitrogen mixture. No hydrogen peroxide was detected with pure nitrogen. Within experimental error the initial rates of formation of hydrogen peroxide were independent of the oxygen concentration, whereas the equilibrium values were directly proportional to the oxygen concentration.

This result confirmed the interpretation of the experiment in which dilute hydrogen peroxide solutions were irradiated at high dose rates without bubbling. In these conditions, the oxygen formed by the radiolysis of hydrogen peroxide determined the equilibrium values, which were therefore proportional to the initial hydrogen peroxide concentrations.

The formation of hydrogen peroxide is usually ascribed to two distinct mechanisms. If two excited water molecules are close enough together, they combine directly to form hydrogen peroxide *via* reactions of the type A, a

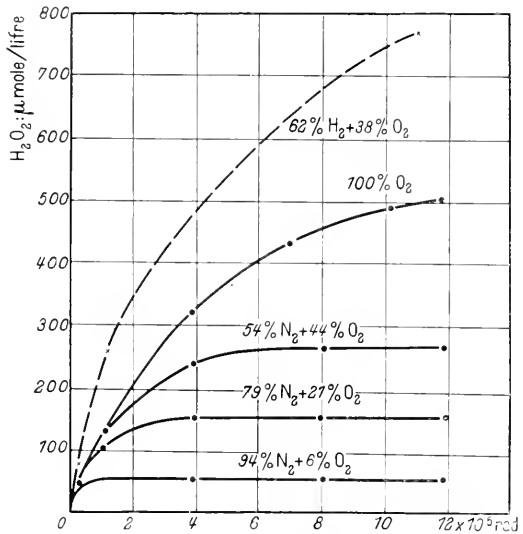


Figure 2. Hydrogen peroxide formed in water, various gas treatments

process which appears to be independent of chemical conditions. The second mechanism B leads to radical formation. Oxygen is then needed for the formation of hydrogen peroxide.

$$A\ 2H_2O \longrightarrow H_2O_2 + H_2$$
$$B\ H_2O \longrightarrow H + OH$$

$$H + O_2 \rightarrow HO_2 \quad . \ . \ . \ . \ (1)$$
$$2HO_2 \rightarrow H_2O_2 + O_2 \quad . \ . \ . \ . \ (2)$$
$$H + HO_2 \rightarrow H_2O_2 \quad . \ . \ . \ (3)$$

Reactions leading to the break-down of hydrogen peroxide involve all the radicals produced by irradiation in water :

$$H_2O_2 + OH \rightarrow H_2O + HO_2 \quad . \ . \ . \ . \ (4)$$
$$HO_2 \rightleftarrows H^+ + O_2^- \text{ or } HO_2 + OH^- \rightarrow H_2O + O_2^- \quad . \ . \ . \ . \ (5)$$
$$H_2O_2 + O_2^- \rightarrow OH + OH^- + O_2 \quad . \ . \ . \ . \ (6)$$
$$H_2O_2 + H \rightarrow H_2O + OH \quad . \ . \ . \ . \ (7)$$

Some of the experimental evidence suggests that reaction (6) is more likely to occur than a direct interaction between hydrogen peroxide and the radical HO_2[4, 7-9].

The simplified scheme set out implies that hydrogen peroxide is formed by radical interactions only in the presence of oxygen. Any hydrogen peroxide formed by direct interaction of excited water molecules would, in the absence of oxygen, be decomposed *via* (4) and (7). With oxygen present reaction (1) will occur giving rise to hydrogen peroxide formation *via* (2) and (3), and the rate of reaction (7), decomposition of hydrogen peroxide, will become slower as the hydrogen radicals are removed by reactions (1) to (3).

Hydrogen and oxygen—To study the effect of hydrogen on the formation of hydrogen peroxide[10], hydrogen alone and mixtures of hydrogen and nitrogen, and of hydrogen and oxygen were bubbled through neutral water. No

hydrogen peroxide was detected with hydrogen alone or with mixtures of hydrogen and nitrogen, but with mixtures of hydrogen and oxygen, hydrogen peroxide yields were higher than those obtained with oxygen alone. *Figure 3* summarizes these results. The three curves were obtained with $2{\cdot}9 \times 10^4$rad, $10{\cdot}5 \times 10^4$rad and 115×10^4rad of 1·2 MeV electrons.

Comparison of the curves shows that the lower the dose, the smaller the range of oxygen tension which influences the yield obtained. Thus at $2{\cdot}9 \times 10^4$rad, the hydrogen peroxide yield no longer increased, *i.e.* the yield became constant with oxygen concentration in the oxygen–nitrogen mixtures above 12 per cent oxygen. With the highest dose, on the other hand, the yield of hydrogen peroxide increased with oxygen tension up to 100 per cent oxygen in the mixture. The same tendency is seen in the curves for hydrogen–oxygen mixtures. At the lowest dose, hydrogen peroxide

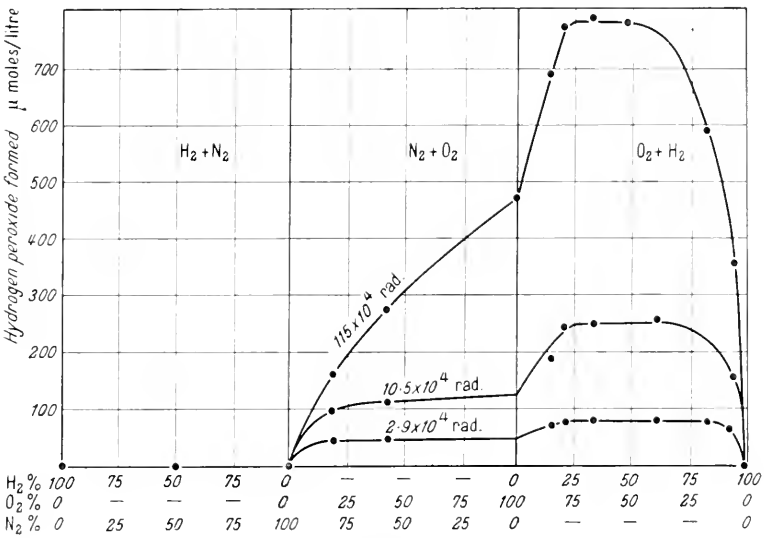


Figure 3. Effect of gas treatment on hydrogen peroxide yield

yields were constant over a range of 10 per cent to 90 per cent hydrogen or oxygen respectively. It seems justifiable to extrapolate these results to still lower doses, at which the dependence of the yield of hydrogen peroxide on the amount of oxygen present (or hydrogen and oxygen in the case of hydrogen–oxygen mixtures) would be critical over a very small range indeed.

The hydrogen peroxide yields with hydrogen–oxygen mixtures may be accounted for by an increase in the concentration of hydrogen radicals arising from reaction (8)[11].

$$H_2 + OH \rightarrow H_2O + H \qquad \ldots\ (8)$$

If hydrogen is present, but no oxygen, the break-down of hydrogen peroxide *via* reaction (7) predominates, and the probability of reaction (4) will be reduced. If oxygen is present as well as hydrogen, it will remove hydrogen radicals by reactions (1) and (3), and the reaction rates of break-down processes, (4) and (7), will be accordingly decreased. Accordingly, not only the

initial rate of formation, but also the equilibrium values are higher if hydrogen and oxygen are used than with oxygen alone.

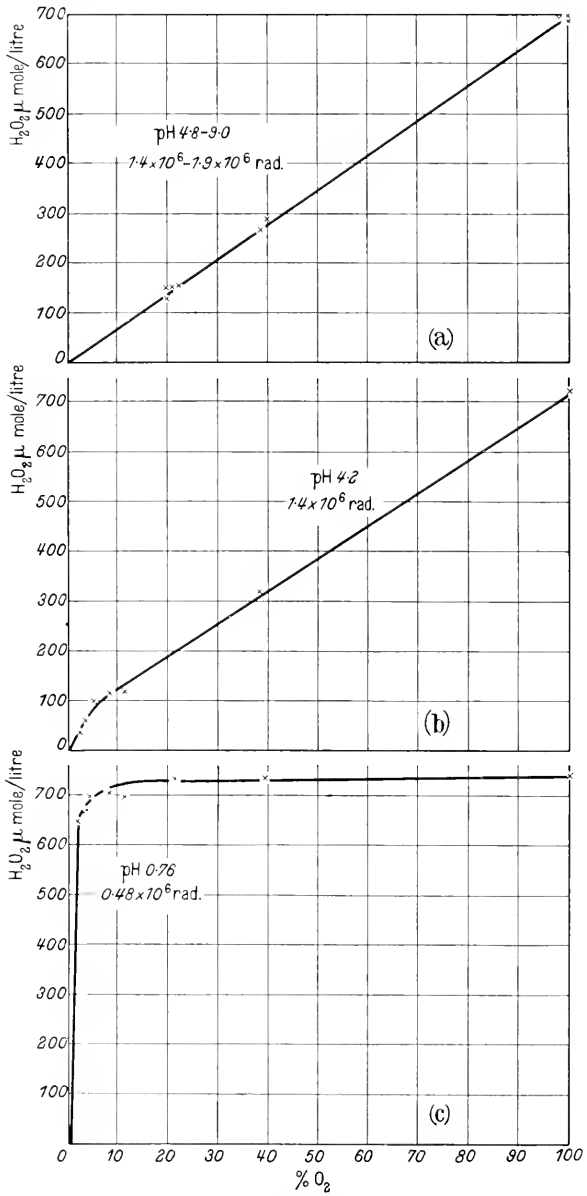


Figure 4. H_2O_2 *yield for various* N_2-O_2 *mixtures at different* pH

Effect of pH *and dissolved oxygen*—The effect of pH on the formation and decomposition of hydrogen peroxide was studied under various oxygen tensions. The results of the experiments are summarized in *Figure 4*[12].

The interpretation of these curves is aided by reference to *Figure 3*. It can be seen that at doses which are very far below those needed for equilibrium values it is only at low oxygen tensions that the yield depends on oxygen tension. The curves of *Figure 4* represent three stages with respect to equilibrium values of hydrogen peroxide. Curve 1, in the pH range 4·8–9·0, shows that the dose was high enough so that the hydrogen peroxide yield was at its equilibrium value, and therefore proportional to oxygen concentration. At pH 4·2 (curve 2) the hydrogen peroxide yield was not at equilibrium, but not far below. Curve 3, at pH 0·76, was of the form which indicates a yield very far below equilibrium value. The rate of formation of hydrogen peroxide at pH 0·76 was independent of the dissolved oxygen for all oxygen–nitrogen mixtures containing more than 3 per cent oxygen.

As the equilibrium values are attained only with very high doses at low pH, the back reaction was studied by the decomposition of hydrogen peroxide.

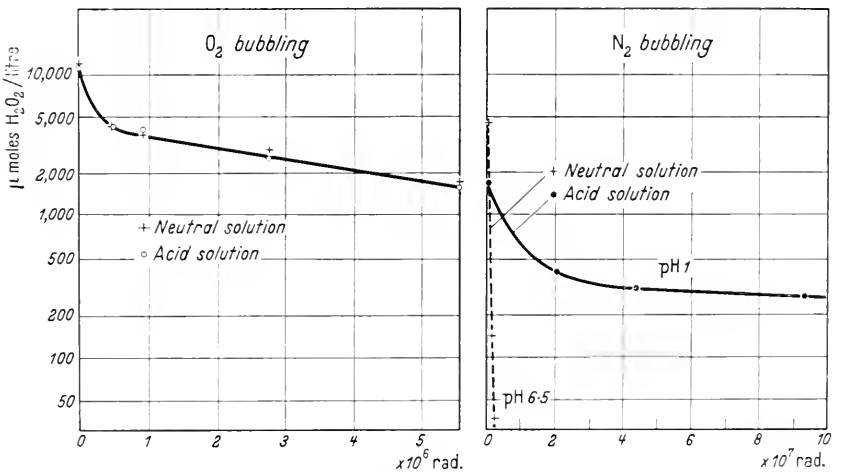


Figure 5. H_2O_2 *decomposition in neutral and acid solutions*

Figure 5 shows that the rate of decomposition of hydrogen peroxide was independent of pH if oxygen was bubbled. When nitrogen alone was used, the decomposition of 0·01 M hydrogen peroxide was carried to completion with a dose of 4×10^6 rad at pH 6, whereas at pH 2 the decomposition was much slower and appeared to approach equilibrium. In the reaction scheme suggested the break-down of hydrogen peroxide into molecular oxygen goes *via* the reaction

$$H_2O_2 + O_2^- \rightarrow OH + OH^- + O_2 \qquad \ldots\ (6)$$

This follows the only reaction which is pH dependent

$$HO_2 \rightleftarrows H^+ + O_2^- \text{ or } HO_2 + OH^- \rightarrow H_2O + O_2^- \qquad \ldots\ (5)$$

so that at low pH the rate of reaction (6) will be much reduced and molecular oxygen will therefore be lost very slowly.

Even in acid solutions decomposition of hydrogen peroxide takes place *via* reactions (4) and (7), but the forward reactions (2) and (3) are still able

to occur, thus avoiding the major loss of bound oxygen *via* reaction (6). The loss of oxygen from the solution should be of no significance when oxygen is in excess, so that hydrogen peroxide decomposition is pH independent with oxygen bubbling.

Discussion

The function

$$y = a(1 - e^{-bd})$$

fits the experimental data very closely, and seems to govern the production of hydrogen peroxide for doses used in these experiments. It was shown that the initial yield was independent of oxygen tension. The yield can be derived by differentiating y with respect to d, and for small doses it is

$$\frac{\partial y}{\partial d} = ab = c_1$$

It has been found that for large doses the hydrogen peroxide concentration was proportional to the oxygen tension f. Under these conditions the exponential term of y approaches 0,

$$y = a = c_2 f$$

$$\text{Thus } y = c_2 f\left(1 - e^{-\frac{c_1 d}{c_2 f}}\right)$$

$$\text{and } \frac{\partial y}{\partial d} = c_1 e^{-\frac{c_1 d}{c_2 f}}$$

The equilibrium value of hydrogen peroxide concentration y will be achieved if $\frac{\partial y}{\partial y} \to 0$. This condition is fulfilled if $\frac{c_1 d}{c_2 f}$, *i.e.* $\frac{d}{f}$, is very large. The hydrogen peroxide concentration rises to within 10 per cent of the equilibrium value when

$$\frac{d}{f} = \frac{c_2}{2{\cdot}3c_1}$$

It follows that if the dose is low, a small change in oxygen tension leads to a large change in yield at very low but not at high oxygen tensions. It is possible to calculate for different doses d the oxygen tensions f at which the hydrogen peroxide formation becomes independent of oxygen tension. In *Table I* are listed these doses and the corresponding oxygen tensions.

Table I. Calculated oxygen tensions for given doses, at which the yields of hydrogen peroxide have reached 90 per cent of the equilibrium values

d dose (r)	*f, oxygen tension as per cent of* 1 atm. *of oxygen*
10	9×10^{-3}
100	9×10^{-2}
1,000	9×10^{-1}
10,000	9

The values in *Table I* reflect the relative probabilities of reactions (1) and (7) for the stated conditions. In living tissue, however, the principal

competition for H-radicals is between oxygen molecules and organic molecules. It can be expected therefore that the values in *Table I* will be applicable to biological systems only qualitatively. In fact, most of the observed oxygen effects in radiation biology show increasing damage with increasing oxygen tensions as long as the oxygen tension is low. For high oxygen tensions an increase does not result in an increase in damage.

SUMMARY

(*1*) At low doses and moderate oxygen tensions the yield of hydrogen peroxide is directly proportional to the dose.

(*2*) The yield of hydrogen peroxide changes with changing oxygen tension. At high doses, which give equilibrium values, the yield is directly proportional to oxygen tension. At low doses, on the other hand, a small change in oxygen tension leads to a large change in yield, if the oxygen tension is low, but to a much smaller change if the oxygen tension is moderate or high. This effect is enhanced at low pH.

(*3*) At small doses the admixture of small amounts of hydrogen to oxygen, or *vice versa*, increases the change in yield of hydrogen peroxide considerably. The addition of large quantities of hydrogen does not change the yield at low doses.

The doses used in radiation chemical experiments are usually high from the radiobiological point of view, and some reluctance may be felt in applying the data to biological systems. It is undecided whether hydrogen peroxide molecules are formed in tissue *via* the radical mechanisms discussed, with doses used in radiobiology, and whether hydrogen peroxide can play a significant part in causing radiation lesions. However, radical reactions are bound to occur in irradiated tissue, and the investigations reported here serve to show how much some radical reactions are influenced by variation in both oxygen tension and hydrogen ion concentration, particularly with small doses and low oxygen tensions.

ACKNOWLEDGEMENTS

I am greatly indebted to Miss T. Alper and Mr. P. Howard-Flanders for helpful discussions during the progress of the work.

REFERENCES

[1] Dewhurst, H. A., Samuel, A. H. and Magee, J. L. *Rad. Res.* 1954, **1** 62.
[2] Gray, L. H. *Brit. J. Radiol.* 1953, **26** 608.
[3] Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S. and Scott, O. C. A. *Brit. J. Radiol.* 1953, **26** 638.
[4] Ebert, M. and Boag, J. W. *Disc. Faraday Soc.* 1952, **12** 189.
[5] Allen, A. O. *Rad. Res.* 1954, **1** 85.
[6] Allen, A. O. *Disc. Faraday Soc.* 1952, **12** 79.
[7] Barb, W. G., Baxendale, J. H., George, P. and Hargrave. *Trans. Faraday Soc.* 1951, **47** 462.
[8] Bray, J. *J. Amer. Chem. Soc.* 1938, **62** 3357.
[9] Weiss, J. *Trans. Faraday Soc.* 1953, **31** 668.
[10] Ebert, M. and Alper, T. *Nature, Lond.* 1954, **173** 987.
[11] Hochanadel, C. J. *J. phys. Chem.* 1952, **56** 587.
[12] Alper, T. and Ebert, M. *Science*, 1954, **120** 608.

DISCUSSION

M. Magat : Il me parait hasardeux, dans l'état actuel de nos connaissances, d'essayer d'attribuer la perturbation, par des molécules organiques, du schéma cinétique de Ebert, à la réaction de ces molécules avec un type de radicaux donné. En effet, les différents radicaux sont assez peu sélectifs et en général il faut tenir compte de la compétition des molécules organiques pour tous les radicaux présents, H, OH, HO_2, dont le dernier est probablement le moins réactif. Le schéma cinétique devient difficile à écrire et encore plus difficile à résoudre. Il va dépendre de la molécule organique en question, de son pouvoir de réagir préférentiellement avec l'un ou l'autre des radicaux et de la nature de cette réaction. En absence de données expérimentales, c'est une discussion théorique assez serrée qu'il faut faire dans chaque cas. Il est évident que dans une cellule, où un grand nombre de composés chimiques sont présents, le nombre des réactions initiales possibles à priori devient très élevé et les réactions ultérieures deviennent imprévisibles. Plusieurs années de travail s'écoulèront avant que l'action des rayonnements sur l'eau pure soit connue en détail et que nous puissions dire avec certitude ce qui arrive dans la cellule vivante.

BACTERIOPHAGE INACTIVATION UNDER VARYING CONDITIONS OF IRRADIATION

TIKVAH ALPER

Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London

ALTHOUGH in recent years bacteriophage has been shown to have some degree of organization[1, 2], it is probably still the simplest living, or self-reproducing, entity with which we can experiment. In the free state, *i.e.* when not in contact with bacteria, phage does not metabolize, so far as is known. A stock may retain all its properties for months, or even years. As soon as they are placed in contact with sensitive bacteria, however, active phage particles attach themselves, penetrate and reproduce. Inactivation of the phage may be due to an interference with any one of these steps. It is well known, for example, that after exposure to ultraviolet radiation, phage particles are inactivated because they do not reproduce, although their ability to adsorb to, and penetrate, bacteria may be unimpaired. On the other hand, phage may be inactive through a failure to adsorb, as is the case with the 'tryptophane-deficient' mutant of phage T4, which will not adsorb to its normal host, coli B, unless tryptophane is present[3]. In this paper, no attempt will be made to discuss which steps in the reproductive cycle are affected by the various inactivating agents to be described. The 'inactivation' of a phage particle will be defined as a failure to form a plaque on a confluent growth of host cells (*Figure 1*) (page 40).

Ionizing radiations may inactivate phage through a variety of mechanisms. LEA and SALAMAN[4] exposed the small dysentery phage S13 in the dry state to ionizing radiations of different ion densities, and concluded from their data that a single ionization taking place within a particle could inactivate it. This is usually referred to as the direct effect of radiation, and ultraviolet inactivation appears to be of the same type. When the phage is suspended in an aqueous medium, it may be affected also by the decomposition products of water. This is usually called the indirect effect of radiation. If the suspension is very dilute, the probability of inactivation by direct effect may be so small, compared with that of indirect effect, that it may be neglected. This paper will deal only with such suspensions, and direct effect will therefore not be considered.

It is possible to show that indirect effect itself may proceed through one of three mechanisms :

(*1*) If hydrogen peroxide is formed by radiation, it has an inactivating effect on the phage.

(*2*) The phage may be changed by free radicals, so that although it is still able to form plaques if allowed to adsorb to bacteria soon after irradiation, it is rendered hypersensitive to certain inactivating

agents (hydrogen peroxide and ascorbic acid). I have called this 'part-inactivation' of the phage.

(3) The phage may be rendered inactive immediately by free radicals.

To some extent it has been possible to study these mechanisms separately. The effect of irradiated suspending medium, *i.e.* of radiation-produced H_2O_2, was studied by introducing non-irradiated phage into irradiated buffer solution. Part-inactivation was studied by comparing the susceptibility of irradiated and non-irradiated phage to radiation-formed hydrogen peroxide, commercial hydrogen peroxide, or ascorbic acid. The *immediate* effects of radical action could to some extent be separated from the other two mechanisms by irradiating at a high dose rate, so that radiation was

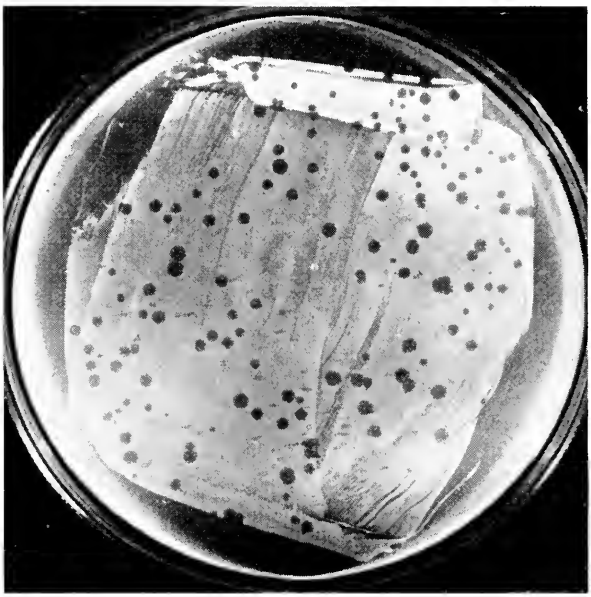


Figure 1

completed within a time which was short, compared with that required for the action of formed hydrogen peroxide. It was necessary, in these experiments, to sample as quickly as possible after the end of each period of irradiation. The immediate effects of free radicals on phage were studied in this way, in varying conditions of gas treatment and of hydrogen ion concentration.

The techniques followed in the phage experiments were those used by Dr. EBERT[5] in examining formation of hydrogen peroxide. Information was almost always derived by plotting survival curves, which involved taking several samples of the phage during the course of each irradiation. Such curves can of course be regarded as reciprocal yield–dose curves, since survivors are counted instead of inactivated particles. It was reported previously[6, 7, 8] that the immediate indirect effects of radiation on phage appear to be reductions. This was fully confirmed by irradiating phage in

the presence of various gases or gas mixtures. As shown by *Figure 2*, the survival curves were steepest with hydrogen bubbling, and flattest with hydrogen and oxygen mixtures. In the particular experiment illustrated, the inactivation doses (37 per cent doses) were 900, 1,800, 3,600 and 11,600 rads for H_2, N_2, O_2 and a $H_2 + O_2$ mixture respectively. The relative yields (based on the curve in nitrogen) were respectively 2, 1, 0·50, and 0·16. As has been shown[9], these results conform with the idea that phage particles are reduced by H radicals. When the only dissolved gas present is nitrogen, the free radicals formed are

$$H_2O \rightarrow H + OH \qquad . \; . \; . \; . \; (1)$$

when hydrogen is present, we have

$$H_2 + OH \rightarrow H + H_2O \qquad . \; . \; . \; . \; (2)$$

so that the probability of a phage inactivation by means of a hydrogen radical, with hydrogen present, is twice that with nitrogen present.

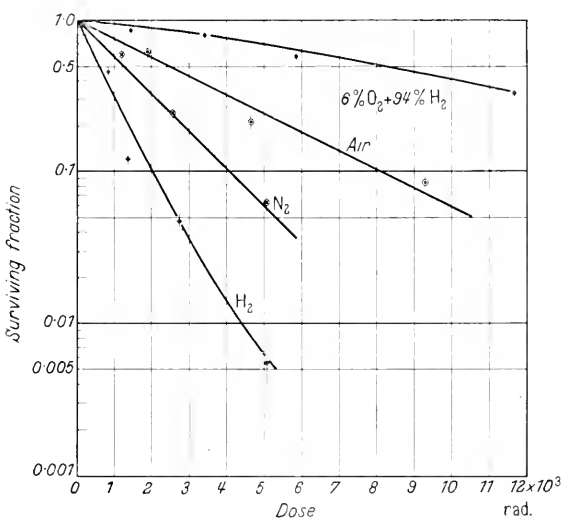


Figure 2. Survival curves of S13 under various gas treatments

It is usually agreed that, when oxygen is present in the suspension, the reaction

$$H + O_2 \rightarrow HO_2 \qquad . \; . \; . \; . \; (3)$$

occurs. This would explain why the presence of oxygen *protects* the phage against inactivation, at high dose rates. Nevertheless, inactivation does take place, and this may be due to reduction by the radical ion O_2^-, arising from the dissociation

$$HO_2 \rightleftarrows H^+ + O_2^- \qquad . \; . \; . \; . \; (4)$$

This possibility was supported by the results of irradiating in hydrogen and oxygen mixtures, in which both HO_2 and H radicals are removed by the reaction

$$HO_2 + H \rightarrow H_2O_2 \qquad . \; . \; . \; . \; (5)$$

which prevents the *immediate* reaction of reducing radicals with the phage. In fact the act of formation of H_2O_2 can be regarded as a protective mechanism against immediate action on the phage, a fact which is emphasized by comparing the effects of gas treatment on H_2O_2 formation and on phage inactivation (*Figure 3*). To bring out the complementary nature of the mechanisms of phage inactivation and H_2O_2 formation, inactivation doses (*i.e.* reciprocals of yields) have been plotted. The general form of the curve applying to phage follows most closely that for H_2O_2 production at the

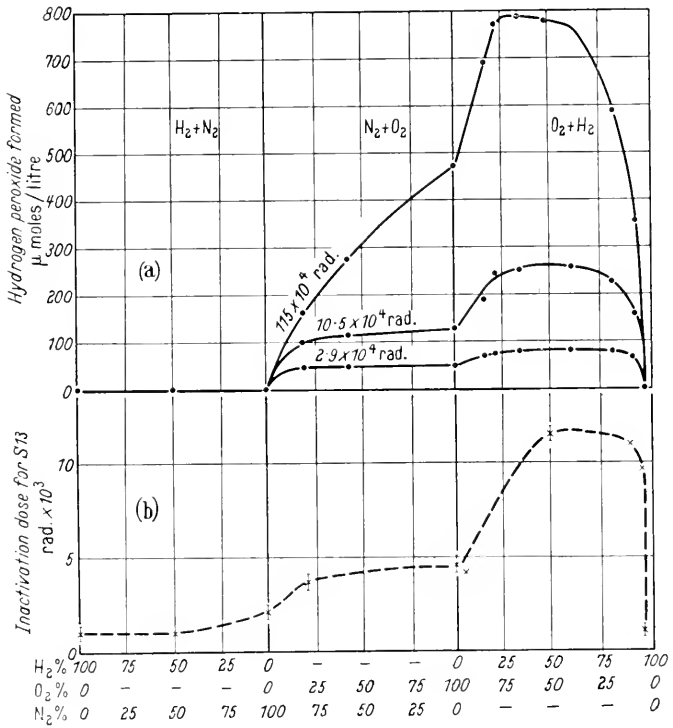


Figure 3. Effect of gas treatment on :—
(a) *Hydrogen peroxide yield*
(b) *Inactivation dose for bacteriophage S13*

lowest dose level used by Dr. EBERT[9], which were considerably higher than the phage inactivation doses. It can be seen that the full protective effect of oxygen–hydrogen mixtures was obtained with small amounts of oxygen.

Reaction 4, *viz.*

$$HO_2 \rightleftarrows H^+ + O_2^-$$

is clearly dependent on hydrogen ion concentration, so that oxidation yields should be greater at higher hydrogen ion concentrations, and reduction yields should be smaller. pH dependence is well known in various radiation chemical systems, including formation and decomposition of H_2O_2[5]. It was found that when phage was exposed to the indirect action of

radiation in suspensions of pH about 9, 7 and 5, the inactivation yield was greatest in the most alkaline suspension, and least in the most acid. Since reaction (4) occurs only in oxygenated suspensions, it is in these that pH dependence should occur, if reaction with the O_2^- radical ion is responsible for inactivation in the presence of oxygen. The pairs of curves presented in *Figure 4* show that there was more marked pH dependence under oxygenation than with hydrogen bubbling. An explanation must, however, be sought for the fact that some pH dependence was found with no oxygen present, and it seems that this may lie in the reaction

$$H + H^+ \rightleftarrows H_2^+, \qquad \ldots \quad (6)$$

which was originally postulated by Weiss[10] to account for pH dependence of the ferrous–ferric system in deaerated solutions. This pH dependent

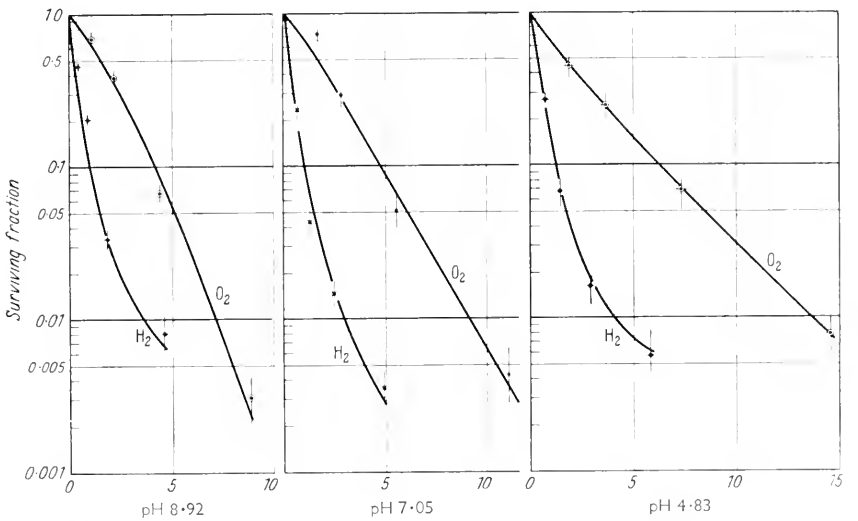


Figure 4. Effect of pH *on survival curves of S13 (Abscissa: minutes' irradiation at 1800 rad/min)*

reaction would act as a scavenger mechanism for H radicals. It appears that H_2^+ is fairly stable in aqueous solution[11]. It may be eliminated by reaction with a reducing substance, in a reaction of the type

$$R^- + H_2^+ \rightarrow R + H_2 \uparrow,$$

or possibly by reactions which lead ultimately to water and gas molecules.

From the experiments which have been described, it can be seen that bacteriophage in dilute suspensions can be used as an indicator for radiation chemical reactions, and, since phage is inactivated by reduction, it is useful as a complementary system to those in which oxidations are studied, which at present are in the majority. It is worth remembering, however, that phage is a self-reproducing entity, and if the effectiveness of radiation is so strongly influenced by factors such as gas treatment and hydrogen ion concentration, it seems reasonable to suppose that these factors operate also in the living cell.

It will probably have been noticed that there is an apparent paradox in the relationship between bacteriophage and hydrogen peroxide : I have described it as an inactivating agent for phage, and yet have shown that its formation acts as a protective mechanism. The paradox is resolved if the time factor is taken into account ; the formation of hydrogen peroxide protects the phage only when radiation is delivered within a fairly short time, and the phage sampled immediately afterwards. At low dose rates the hydrogen peroxide can attack the phage during irradiation, the more so since part-inactivated phage particles are counted as survivors in short-term experiments, but may, with longer time lapses, be inactivated by the peroxide. There is evidence that phage is attacked by hydrogen peroxide only when it decomposes, and then only when it decomposes *via* reducing radicals. In fact it seems justifiable to postulate that any radicals which are removed at the time of radiation by H_2O_2 formation may act as inactivating agents when the H_2O_2 decomposes. The H_2O_2 therefore acts as a bank for radicals, in any experiment in which dose rates are low, or which is of such a nature that a time lapse occurs between the end of irradiation and the measurement. One can take this argument a step further. In biological experiments in which the end product of radical action is not defined in chemical terms, it is often assumed that enhancement of radiation effects implies an oxidative mechanism. But the presence of oxygen allows of the formation of hydrogen peroxide, and therefore, in effect, of the storage of free radicals which would otherwise be lost by recombination into water or into H_2 and O_2 molecules. The H_2O_2 may decompose, either under the action of the radiation, or after irradiation ceases, and so give the radicals a further probability of reacting with sensitive systems. If the nature of a radiation experiment is such that dose rates are low, or long term effects are involved, it seems possible that an important role of oxygen may be to make radicals available for later reaction, rather than exclusively to enhance oxidative reactions.

SUMMARY

From high dose rate irradiations of bacteriophage under various gas treatments, and at various hydrogen ion concentrations, it has been concluded that phage is inactivated by H radicals, in deoxygenated suspensions, and by O_2^- radical ions, in oxygenated suspensions. In the former, protection may arise in acid suspensions from the reaction $H + H^+ \rightleftarrows H_2^+$. The formation of hydrogen peroxide acts as a protective mechanism for the phage, when irradiations are completed in a short time, but the H_2O_2 may later inactivate the phage by decomposing into the radicals which entered into its formation. It is suggested that the presence of oxygen may enhance radiation effects not exclusively through enhancement of oxidative reactions, but also because the oxygen acts as a means for 'storing' radicals which may later become available for reaction with sensitive systems.

REFERENCES

[1] Anderson, T. F., Rappaport, C. and Muscatine, N. A. 'Le Bactériophage,' *Ann. Inst. Pasteur*, 1953, **84** No. 1, 5.

[2] Hershey, A. D. and Chase, M. *J. Gen. Physiol.* 1952, **36** 39.

[3] Garen, A. and Puck, T. *J. exp. Med.* 1951, **94** 177.

[4] Lea, D. E. and Salaman, M. H. *Proc. roy. Soc.* 1946, **133B** 434.
[5] Ebert, M., 1954. This Conference.
[6] Alper, T. 'Radiation Chemistry,' *General Discussions Faraday Soc.* 1952, No. 12 234
[7] Alper, T. *Abstracts, VI. International Microbiology Congress, Rome. Riassunti delle Communicazione*, 1953, **2** No. 528, 181.
[8] Alper, T. *Brit. J. Radiol.* 1954, **27** 50.
[9] Ebert, M. and Alper, T. *Nature, Lond.* 1954, **173** 987.
[10] Weiss, J. *Nature, Lond.* 1950, **165** 728.
[11] Coulson, C. A. *Proc. roy. Soc.* 1952, **211A** 396.

DISCUSSION

L. K. Mee : Alper has mentioned the work of Anderson and McDonald on the continued inactivation of pepsin and trypsin after irradiation. They had shown that this 'after effect' is dependent on temperature.

We have noticed a similar effect on the normally thermostable enzyme ribonuclease. When irradiated enzyme solutions are kept at 0° C very little further inactivation occurs, but at 25° C and higher temperatures the enzyme continues to lose activity. The continued inactivation appears to be faster at higher temperatures. No inactivation of the enzyme has been detected when irradiated solution is added to the unirradiated enzyme.

This slow reaction appears to depend on a change in the protein during irradiation which does not affect its enzymic activity immediately but makes it unstable in some way.

AFTER-EFFECTS OF IRRADIATION OF DNA

J. A. V. Butler and (Miss) P. Simson

Chester Beatty Research Institute, Royal Cancer Hospital, London

'After-effects' of irradiation are not unusual. The irradiation initiates a sequence of effects which continues for some time. This is particularly the case with living organisms, which are dynamic systems, in which interference with one part may produce a long sequence of changes, ending in death. After-effects can be observed in some cases in purely chemical systems such as DNA. Their interest is mainly in the opportunity they give of determining the mechanism of the processes involved.

We should like to add some new observations to those previously given on the subject. The slow decrease of the viscosity of DNA which occurs after irradiation in the presence of oxygen has been ascribed[1,2] to (1) the formation of substances of a peroxidic nature which are formed by processes such as

$$RH + OH \rightarrow R^{\bullet} + H_2O\ ;\ R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}\ ;\ RO_2^{\bullet} + H \rightarrow RO_2H.$$

It would follow that such peroxides would undergo a slow decomposition. (2) the formation of labile phosphate owing to the oxidation of the C4 atom of the sugar, giving a keto-sugar from which the phosphate is capable of slow hydrolysis[3].

We have made some further experiments in order to define the circumstances under which the latter process occurs. Weiss and Scholes have stated that although only small amounts of phosphate are liberated in the action of X-rays, considerably greater amounts are formed by hydrolysis with acids after such treatment.

We have used as a hydrolysing agent 0·5 N, H_2SO_4 at 70°. This produces practically no phosphate in 1 hour with unirradiated DNA, but in longer times the amount of phosphate liberated increases rapidly (*Figure 1*). Now acid treatment of this nature is known to liberate purines from the DNA and the initial lag period may be ascribed to the time taken for an appreciable amount of depurination to occur since the intact nucleotide chain is apparently not susceptible to hydrolysis until the purines have been removed. Another factor is the fact, already discussed[4], that two adjacent phosphate-sugar links have to be broken before inorganic phosphate is liberated. That is, the breakage of one phosphate-sugar link, although it breaks the nucleotide chain, can only result in the formation of a terminal phosphate group. The free phosphate produced by hydrolysis of X-ray treated DNA might then be the result of the acid hydrolysis of the terminal phosphate groups. It is found (*Figure 1*) that the effect of irradiation with X-rays is to shorten the initial lag period. Comparatively large doses are required to produce a marked effect. It is difficult to give an unambiguous explanation of this, as it might be due to (*a*) an increase in the susceptibility of the nucleotide chain to hydrolysis owing to loss of purines ; (*b*) the partial

oxidation of the sugar moiety, resulting in a greater ease of hydrolysis of the phosphate, or (*c*) the hydrolysis of terminal phosphate which has been produced by the primary action of the radiation.

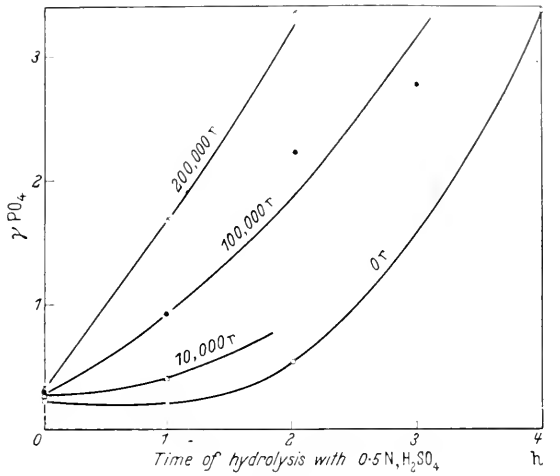


Figure 1

However, it is not certain that the viscosity after-effect can be ascribed to the same process as gives rise to hydrolysable phosphate, as very little change in the amount of free phosphate occurs on standing in *neutral* solutions, even after massive doses of X-rays, although the acid treatment can liberate considerable amounts, as shown in *Table I.*

Table I

	PO_4 (γ per ml.)	*Same after* 1 hr *hydrolysis with* H_2SO_4
DNA initial	0·28	0·28
DNA after irradiation with 200,000 r	0·35	1·68
Same after 16 hr *standing*	0·39	1·80

A marked change of viscosity may, of course, be brought about by the breakage of comparatively few bonds.

In order to exclude the possible hydrolysis of terminal phosphate, experiments were made with the ribonucleotides (not *deoxy*), adenosine-2′ (or 3′)-phosphate and adenosine-5′-phosphate. The phosphate group of the former compound is easily hydrolysed by sulphuric acid (70°) but that of the 5′-phosphate is barely affected in 1 hour. Treatment of the latter compound with X-rays, or with hydroxyl radicals photochemically generated by ultraviolet light on H_2O_2, causes a marked increase in the amount of phosphate hydrolysed by sulphuric acid.

It thus appears that the effect of X-rays is to increase the hydrolysability of phosphate which is initially terminal. The actual increase of hydrolysable

Table II.—Effect of X-rays on adenosine-5′-phosphate

	PO_4 (γ per ml.)	*Same after* 1 hr *hydrolysis with* H_2SO_4
Adenosine-5′-phosphate (initial)	0·26	0·34
Same after 10,000 r	0·32	0·48
Same + 16 hr *standing*	0·34	0·48
Same after 100,000 r *in* N_2	1·30	2·04
Same + 16 hr *standing*	—	1·92

phosphate is however comparatively small and of the same order as the amount of phosphate liberated during the irradiation. Similar results have been obtained after treating adenosine-5′-phosphate with photochemically generated hydroxyl radicals (*Table III*).

Table III.—Effect of photochemically produced OH *radicals on adenosine-5′-phosphate*

	PO_4 (γ per ml.)	*Same after* 1 hr *hydrolysis with* H_2SO_4
Adenosine-5′-phosphate	0·26	0·34
After 1 hr *ultraviolet treatment with* H_2O_2	3·3	6·8
Ditto after 16 hr *standing*	4·4	6·5

It can be concluded that treatment with X-rays or OH radicals does produce some phosphate which is more easily hydrolysed by acids, and that this labilization is due either to the removal of the purine or to a partial oxidation of the sugar. In neutral solution the rate of the hydrolysis is rather small.

We are indebted to Mr. E. W. Johns for assistance in some of the analyses, and to Dr. M. Ebert for the irradiations with large doses of X-rays.

This investigation has been supported by grants to the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

REFERENCES

[1] Butler, J. A. V. and Conway, B. E. *J. Chem. Soc.* 1950, 3418.
[2] Conway, B. E. *Brit. J. Radiol.* 1954, **27** 42 ; *Nature, Lond.* 1954, **173** 579.
[3] Scholes, G. and Weiss, J. *Nature, Lond.* 1953, **171** 920, 1153.
[4] Butler, J. A. V. and Conway, B. E. *Proc. Roy. Soc.* 1953, **141** 562.

PHYSICO-CHEMICAL METHODS OF PROTECTION AGAINST IONIZING RADIATIONS

PETER ALEXANDER and ARTHUR CHARLESBY

Chester Beatty Research Institute, Royal Cancer Hospital, London, and Atomic Energy Research Establishment, Harwell, respectively

THE primary step when a biological system is exposed to ionizing radiations is the utilization of the absorbed energy in a chemical reaction. Since the energy required to produce a biological lesion is often very small and, moreover, since it is initially deposited at random throughout the irradiated material, it would seem to be necessary that the biologically important reaction is with a macromolecule because the proportion of biologically active substances of low molecular weight (*e.g.* ATP or glutathione) which are changed by irradiation with a few hundred roentgens is negligibly small. We assume, therefore, that the observed lesion is the result of the chemical change of some vital macromolecules present in very limited numbers (*e.g.* the DNA of the chromosome threads).

Since in most biological systems radiations having a high specific ionization are more effective for the same amount of energy deposited than hard X-rays or gamma rays, it seems possible that some morphological structure must be damaged by changing a number of macromolecules close together. Whatever the primary chemical events are, these cannot in general (*i.e.* excepting perhaps chromosome breakage) be detected in mammals because the doses necessary to produce a biological lesion, of the order of 1,000r or less, cannot bring about a measurable chemical change *in vivo*.

In principle there are three general methods of protecting against the initial chemical changes and these may be called physico-chemical, as opposed to physiological protection which interferes with the development of the primary chemical changes into biological end-effects.

(*a*) A substance can be added which influences the conversion of the energy taken up in such a way that less chemical change occurs in the 'vital' macromolecules.

(*b*) Repair by an added substance of the damage produced in the macromolecule immediately after the primary reaction and before any irreversible change has occurred. In many cases this is not possible since the first reaction is irreversible, *e.g.* when pure stearic acid is irradiated with alpha rays[1] the following chemical change occurs which cannot be 'repaired'

$$CH_3(CH_2)_{15}CH_2COOH \rightarrow CO_2 + CH_3.(CH_2)_{15}CH_3.$$

In other cases the primary action may be the loss of, for example, a hydrogen atom, to give a free radical and this can be repaired. Since successive chemical reactions of a molecule changed in this way by ionizing radiations will occur extremely rapidly, the protective substance bringing about the repair must be present before the irradiation.

(*c*) Shielding of a vulnerable group in a molecule (*e.g.* the prosthetic group of an enzyme) with another substance which can be removed after irradiation. The protection of an enzyme by its substrate[2] is believed to function by this principle which will not be considered further here.

The present paper reports an investigation on the changes produced in a number of synthetic macromolecules under a variety of conditions when protection by different mechanisms was encountered. We hope to be able to establish what type of compounds are most effective in providing protection by the different mechanisms. It may then become possible to deduce by analogy the mechanism of protection in various biological systems by comparing the protective action of a number of substances *in vivo* with their activity in the synthetic systems.

COMPETITION FOR FREE RADICALS

When the action of the ionizing radiations is indirect (*i.e.* the energy from the radiations absorbed in the solvent produces highly reactive entities which react with the solute) a protecting substance can protect the solute by competitively removing the active entities. DALE[3] was the first to find that added substances (notably thiourea) protected enzymes, by a competition mechanism, when these were irradiated in dilute aqueous solution. DAINTON[4] established experimentally, following the original suggestion by RISSE[5] in 1929, that the free radicals $OH^{\bullet}$ and possibly $H^{\bullet}$ are formed on irradiating pure water. In the presence of dissolved oxygen the radicals formed are $OH^{\bullet}$ and $HO_2^{\bullet}$. In DALE's experiments the enzymes were probably inactivated by OH radicals[6] and the activity of the protective agents in these systems is therefore determined by their reaction with OH radicals.

The degradation of polymethacrylic acid in dilute aqueous solution by X-rays is due to HO_2 radicals[7] and the protective action of more than 100 compounds in this system has been studied[8]. These compounds protect by competing for HO_2 radicals and the protective action is therefore a measure of reactivity with HO_2 radicals. In another system, the polymerization of methacrylic acid, we have studied protection by competition for OH radicals[9]. The order of effectiveness of a series of compounds is not the same in the two systems. The activity of substances in protecting mice against the lethal effects of X-rays follows closely the HO_2 series and not the OH series[8, 9]. We deduce that competition for HO_2 radicals plays an important part in the protection of mice.

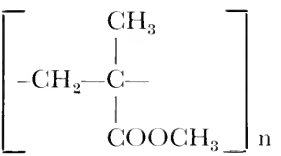
PROTECTION AND DIRECT ACTION

At first sight it would appear that where the action of the ionizing radiations is direct (*i.e.* the energy is absorbed by the actual material undergoing change) no protection is possible. According to this view, which is widely held, once a macromolecule has absorbed sufficient energy to undergo a chemical change, the ensuing reaction is inevitable and cannot be prevented. Nevertheless an experiment carried out fifteen years ago by SVEDBERG and BROHULT[10] pointed to the possibility that direct action was more complex. These workers found that a very specific dissociation of the giant protein molecule haemocyanin into two equal parts could be induced by irradiation

with α-particles, and that the passage of one α-particle anywhere through the molecule was sufficient to produce this change. This indicated that energy absorbed in one part of the molecule could be transferred to those bonds responsible for holding the two halves together. If energy transfer of this kind can occur, then protection against direct action is theoretically possible. The term 'energy transfer' does not imply any particular mechanism but is used as a general description of processes we do not at present understand. Thus before it was established that indirect action in aqueous solution was brought about by the intervention of free radicals the process could have been referred to as energy transfer from the solvent to the solute. Recently MILTON BURTON and his colleagues[11] have found evidence for energy transport from one molecule to another when a mixture of two liquids such as benzene and cyclohexane is irradiated. In this study, transfer of charge by collision between an ionized and un-ionized molecule plays an important part. How far such a process can occur in our solid systems cannot at the moment be assessed.

Protection of Polymers.—We decided to test the possibility of protecting against the direct action of ionizing radiations by admixing different chemicals with synthetic polymers and irradiating these as solids. Some polymers are crosslinked while others are degraded[12] when irradiated with gamma rays *

Polymethylmethacrylate,



is degraded on irradiation and the number of breaks produced is directly proportional to the radiation dose. For every 61 eV of energy from gamma rays which is absorbed by the polymer one main-chain bond is broken. This does not mean that all the 61 eV is used to break the bond, and other chemical reactions, notably side-chain breakdown, also occur.† Films of this polymer were prepared which contained a small quantity of a low molecular weight additive and the energy required to produce one main chain bond in the polymer was again measured. Some substances exerted a truly remarkable protective action and the presence of 10 per cent of

* This difference in behaviour has been explained[13, 14] as follows : Degradation can only occur when a chemical rearrangement of the main chain is possible to give two non-interacting products. Direct rupture of a bond into two radicals will not produce a permanent break since the two parts, being in close proximity (cage effect), will immediately recombine. When such a rearrangement of the main chain cannot occur the predominant chemical reaction will be confined to the side chains with the result that active centres capable of forming inter-chain bonds (crosslinking) will be produced. Crosslinking will not occur exclusively and some rupture of main chains is usually also found : this is to be expected on our hypothesis since a proportion of main-chain radicals are bound to escape from the cage and result in breaks. Escape from the cage is more likely to occur with radiations of high specific ionization and these should be less effective for producing crosslinked polymers.

† Much of the energy does not bring about any chemical change but is wasted in heating up the polymer by a minute amount.

dimeta-tolyl-thiourea, aniline or allylthiourea raised the energy which had to be taken up by the polymer per main-chain break to 227, 152 or 143 eV respectively. Other substances such as long-chain paraffins and ethyl urea do not protect.

We can see two possible interpretations : (1) the energy absorbed by the polymer is not immediately utilized to bring about a chemical change and in the interval the energy is transferred (see footnote on page 51) from the polymer to the additive. (2) The protection is brought about by repair of the polymer by the added substance which increases, for example by crosslinking, the molecular weight and thereby hides some of the main-chain breaks. We believe that the energy transfer mechanism occurs for the following reasons. (*a*) A detailed quantitative study by Toms (unpublished) relating the protection to the concentration of the protector and to the radiation dose indicates energy transfer. (*b*) The list of substances which protect is so diverse that it is highly improbable that they share a chemical property such as ability to produce crosslinks. (*c*) Preliminary experiments indicate that the same substances which protect polymethyl methacrylate against degradation protect other polymers against crosslinking.

The decisive test is to see if the energy required to modify chemically a molecule of the additive is less when incorporated in a polymer than when irradiated by itself. This experiment is difficult to carry out since even when extremely large doses of radiation are used, only a very small fraction of the low molecular weight additive is changed. Preliminary experiments showed that a considerably greater *proportion* of aniline was changed when this was irradiated in a film with polymethylmethacrylate than by itself.

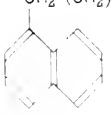
Instead of treating this effect as one of protection the polymer can be considered to enhance the decomposition of the additive by handing on to it some of the energy absorbed (*i.e.* the polymer then fulfils the same function as the solvent in the case of indirect action). In this way the energy which was initially absorbed uniformly in the system* concentrate at certain points.

Energy transfer within molecules.—Two series of experiments indicate that energy transfer can occur over considerable distances within a macromolecule. The energy required to form a crosslink by irradiation with gamma rays in a straight chain hydrocarbon was independent of its molecular weight[15]. We examined the energy to form a crosslink in a hydrocarbon to which an aromatic group (naphthyl) was attached to see if energy transfer to this group occurred (*i.e.* if some of the energy absorbed in the hydrocarbon chain was transferred to the aromatic group). As can be seen from *Table I* the energy required to produce a crosslink is greater in the substituted dodecane derivatives and the protection is most marked when the naphthyl group is in the centre of the chain. We believe that a reasonable interpretation is that energy originally absorbed by the hydrocarbon chain is transferred to the aromatic group and that the transfer is not efficient over a distance of more than a few carbon–carbon bonds. To eliminate the possibility that the decrease in crosslinking found was due to steric interference by the naphthyl group, a hydrocarbon was examined which was substituted

* The absorption coefficients of different organic materials to hard X- and gamma rays and to particulate radiations other than neutrons do not vary by more than a few per cent.

with an equally bulky group, cyclo-decalyl, but one which we knew from experiments with polymethylmethacrylate to be a very feeble energy transfer protector. *Table I* shows that the energy required to crosslink cyclo-decalyl-dodecane is only slightly greater than that of the pure hydrocarbon and we conclude that steric factors played at most a minor part in the protection of the substituted dodecanes.

Table I.—Influence of an aromatic group substituted in different positions along the chain of the straight-chain hydrocarbon dodecane on the energy from ionizing radiations which has to be absorbed to produce one crosslink[16].

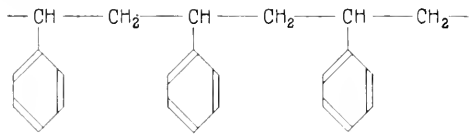
Substance		Energy per crosslink (eV)*
$CH_3 \cdot (CH_2)_{10} \cdot CH_3$	dodecane	20
$CH_3 \cdot (CH_2)_{10} \cdot CH_3$ 	naphthyl-1-dodecane	32
$CH_3 \cdot (CH_2)_2 \cdot CH \cdot (CH_2)_7 \cdot CH_3$ 	naphthyl-4-dodecane	46
$CH_3 \cdot (CH_2)_4 \cdot CH \cdot (CH_2)_5 \cdot CH_3$ 	naphthyl-6-dodecane	49
$CH_3 \cdot (CH_2)_4 \cdot CH \cdot (CH_2)_5 \cdot CH_3$ 	cyclo-decalyl-6-dodecane	27

* The absolute values are open to some uncertainty but the relative values are reliable.

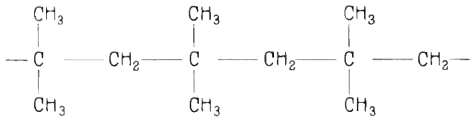
Similar results have been obtained when co-polymers of isobutylene and styrene *(see Figure 1)* were irradiated. Polyisobutylene is degraded[17] and behaves very similarly to polymethylmethacrylate for which detailed results have been published[13]. Polystyrene, on the other hand, is extremely radiation resistant but will crosslink, though the energy required is approximately one hundred times that for the straight-chain hydrocarbons†. The behaviour of the co-polymers is complicated and changes as the radiation dose is increased[19]. At first they degrade in a regular manner *i.e.* the

† This is a case of internal protection by the phenyl side chains to which energy absorbed by the rest of the molecule is transferred. The benzene nucleus is known to be very resistant to radiation[11] and can, because of its aromatic structure, dissipate a great deal of energy without chemical change.

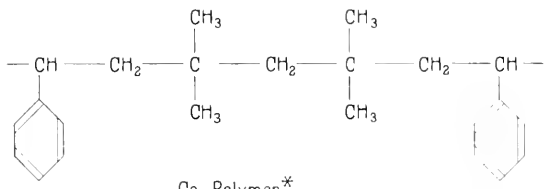
number of main-chain bonds broken is proportional to the radiation dose). Then fairly suddenly the average molecular weight of the material (from viscosity measurements) is no longer changed by irradiation and the main-chain breakdown in the polyisobutylene units is counterbalanced by the



Polystyrene



Polyisobutylene



Co-Polymer*

* The components are assumed to be distributed at random in the proportion in which they are present.

Figure 1.—Composition of Polymers

crosslinking of the polystyrene units. At still higher doses crosslinking predominates and part of the material is converted to an insoluble gel. The energy necessary to break a main-chain band in the polyisobutylene part of the molecule can be obtained from experiments with relatively low doses and from *Table II* it can be seen that the polystyrene exerts a very definite

Table II.—Degradation of co-polymers by ionizing radiations

Polymer	*Energy deposited in the polymer per one main-chain break*
Polyisobutylene	17 eV
Co-polymer of 20 per cent styrene + 80 per cent isobutylene	32 eV
,, ,,50 ,, ,, + 50 ,, ,,	55 eV
,, ,,80 ,, ,, + 20 ,, ,,	~ 100 eV
Polystyrene	~ 2,000 eV †

† Polystyrene is not degraded but becomes crosslinked and the energy quoted is that required to produce one crosslink.

protective effect. By making reasonable assumptions concerning the distribution of the isobutylene and styrene units in the macromolecule we

computed[19] from the irradiation data that one styrene unit provides complete protection to two isobutylene units either side. This is, of course, an oversimplification since the probability of protection will decrease gradually with the distance from the phenyl residue. However, this calculation gives a value for the distance over which energy transfer can occur which is very similar to that obtained from the substitution of naphthyl groups in different positions along the dodecyl chain.

REPAIR OF DAMAGED MOLECULES

As already mentioned on page 49 radiations can produce chemical reactions which involve the complete disruption of the molecule and are clearly not reversible. In many cases, however, the first chemical change is the loss of a hydrogen atom from an organic molecule either by direct action or as the result of abstraction by a free radical (*e.g.* $>CH_2 + OH^{\bullet} \rightarrow >CH^{\bullet} + H_2O$). This type of damage can be restored by what may be called a transfer reaction and this was found to occur when aqueous solutions of polyvinyl alcohol were irradiated [20]. In the absence of oxygen the molecule does not degrade but crosslinks to give a stiff gel. Some added substances, notably —SH compounds, protect by repairing the activated molecules before these can interact to give a polymer network.

The general reaction may be illustrated as follows:

$$\underset{\text{part of polymer molecule}}{-CH_2-} \xrightarrow[\text{hydrogen}]{\text{loss of}} \underset{\text{polymer radicals which combine with one another to form a gel}}{-CH^{\bullet}-}$$

The protective agent (PH) transfers a hydrogen atom to reconstitute the polymer before the polymer radical has had time to undergo further reactions such as crosslinking.

$$PH + -CH^{\bullet}- \rightarrow \underset{\text{reconstituted polymer molecule}}{-CH_2-} + \underset{\text{radical from the protective agent which is of low chemical reactivity.}}{P^{\bullet}}$$

In the presence of oxygen the polymer radical will be converted to an unstable peroxy radical

$$-CH^{\bullet}- + O_2 \rightarrow -CHOO^{\bullet}-$$

which will undergo further changes and may in some cases decompose in such a way as to bring about main-chain breakdown. This degradation can in principle be prevented by the protector as follows :

$$-CHOO^{\bullet}- + PH \rightarrow -CHOOH- + P^{\bullet}$$

In this way the polymer is changed but decomposition is prevented by the formation of a stable compound.

We have no evidence that hydrogen transfer plays a part in biological protection. PRÉVOT-BERNAS[21] observed that cysteine and cysteamine act as chain terminators in polymerization processes by hydrogen transfer and postulated that the protective action of these substances in mice was due to their ability to act as transfer agents and not as competitors for free radicals. This hypothesis can be rejected for the following reasons. (*a*) Many protective agents which are active *in vivo* with mice and *in vitro* with polymethacrylic acid do not act as transfer agents in polymerization reactions under conditions where the —SH compounds do.[9] (*b*) Amines are as active as protectors in the cationic as in the un-ionized form whereas they can only transfer a hydrogen atom when un-ionized[8]. Crosslinking of polyvinyl alcohol is prevented by un-ionized methylamine but not by ionized methylamine. This further supports the view that in this system protection occurs by transfer. (*c*) All —SH compounds act as transfer agents while only very few are capable of protecting mice[8].

PROTECTION AND TARGET THEORY

In the earlier formulation of the target theory (see for example LEA[26]) a chemical change was postulated to occur whenever an ionization had occurred ; the energy for this event plus its associated excitation was assumed to be the same as that required to ionize an atom in air (*i.e.* about 32 eV). Current radiochemical research has shown that this second postulate need not apply and that when pure materials are irradiated a chemical change often occurs only when very much more than 32 eV of energy has been absorbed. We have recently shown (*see* page 54) that for some reaction such as the breaking of main-chain bonds in polyisobutylene considerably less than 32 eV may be sufficient to produce a discrete chemical change. Thus, if radiation of low specific ionization were used to determine a molecular weight from the apparent 'target size' in, for example, polystyrene (using crosslinking as a criterion), a very low value would be obtained, while with polyisobutylene the molecular weight derived would be too high.

These experiments may make it necessary to re-evaluate the data obtained for the sizes of the sensitive volumes of viruses and enzymes from 'target area' calculations, since the chemical change may not be confined entirely to the track of the ionizing particle. In particular the occurrence of energy transfer throws doubt on the validity of the calculation for the number of sensitive sites in an irradiated organism from the variation in apparent 'target size' (or relative biological effectiveness, RBE) with the specific ionization of the radiation used. This problem has recently been dealt with in great detail by ZIRKLE and TOBIAS [22] who interpret the RBE of different radiations in terms of the diffusion of free radicals. Transfer of energy initially deposited at certain points may provide an alternative explanation for the observed facts.

THE MECHANISM OF 'DIRECT-ACTION'

Largely as the result of Dale's work direct and indirect action have been clearly differentiated. The least ambiguous test for distinguishing between the two processes is to examine how the percentage inactivation, of for

example an enzyme, varies with concentration in solution (*i.e.* if the inactivation is inversely proportional to concentration, the action is indirect). This test cannot, however, be readily applied at the cellular level since it is usually not possible to vary at will the concentration of constituents within the cells.

Based on the concepts that direct action is not influenced by the condition of the environment of the material, and that once sufficient energy has been absorbed (or a given number of ionizations have occurred) the subsequent damage to a molecule is unalterable, three other tests are used to distinguish between the two processes. The action of radiations of low specific ionization (*e.g.* hard X-rays or gamma rays*) is said to be indirect (*a*) if the presence

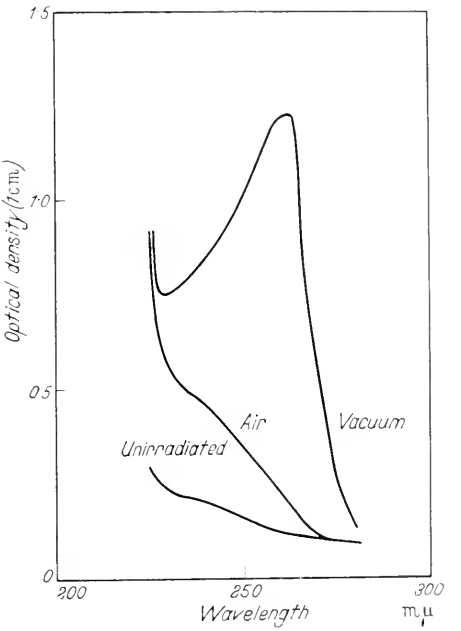


Figure 2.—The ultraviolet absorption of polyisobutene showing the effect of radiation in the presence and absence of oxygen (dose approx. $1{\cdot}5 \times 10^8$ r) (0·13 per cent *solution in n-hexane)*

of oxygen in the external medium enhances the biological effect ; (*b*) if the action of the radiation is decreased on freezing all the water in the system thereby making it impossible for free radicals to diffuse ; (*c*) if the presence of other substances can reduce (*i.e.* protect) the irradiated system.

Since protection[14] and an effect of oxygen[23] is almost invariably found when living systems are irradiated with X-rays or gamma rays it has been concluded that indirect action plays an important part in bringing about the observed effects.

Our work on the irradiation of dry polymers where the action must be direct shows that the last test is not decisive for indirect action, since

* The oxygen effect and protection cannot be used to determine whether the action of densely ionizing radiations (*e.g.* neutrons or alpha rays) is direct or indirect, since they produce radicals in water at very high local concentration and their reactivity cannot therefore be influenced by the presence of oxygen or protective agents in the water.

protection is now known to be possible against direct action. In addition we have observed that the absorption spectrum (*see Figure 2*) of polyisobutylene is modified much more extensively after irradiation under nitrogen than under air[17]. This is a clear example of an oxygen effect when the action is direct since exposure of the polymer to oxygen after irradiation does not modify the absorption spectrum. We can conclude that irradiation produces different chemical products in the presence and absence of oxygen even where the process is ' direct '.

The degradation of both polyisobutylene[17] and of polymethylmethacrylate[13] depends on temperature. The energy required to break a main-chain bond in polyisobutylene is nearly doubled on decreasing the temperature from 70° C to —60° C. A possible interpretation is that transfer of energy into the required bond is more efficient at higher than at lower temperature (*cf.* fluorescence). A similar observation has been made with biological materials. Dry enzymes[24] and dry bacteriophage[25] were reported to be inactivated more readily (*i.e.* their target size appears to be larger) at higher temperature. This has been interpreted by POLLARD[24] in terms of an inherent change in sensitivity of the enzyme with temperature and by BACHOFER *et al*[25] as due to the diffusion of an active intermediary within the dry phage. Since a temperature dependence has now been found with a completely synthetic material where neither of these explanations can apply, it may be necessary to assume an energy transfer process. Although these experiments in no way disprove that the action of ionizing radiations on living systems is indirect they show that an important contribution by direct action cannot be excluded. Since in the cell and particularly in the cell nucleus proteins and nucleic acids are present in relatively high concentrations, it has always been difficult to understand why the action of ionizing radiation should be predominantly indirect.

SUMMARY

The possible ways in which the primary chemical effects of ionizing radiations can be reduced are reviewed.

Examples are given of protection against indirect action by competition for free radicals and of repair of a damaged macromolecule by transfer agents present during the irradiation. Irradiation of solid polymers has shown that protection is possible against direct action and an undefined energy transfer process is suggested as the mechanism. Such an effect will require modification of the 'target theory' in its more general form.

In addition to protection by added chemicals, direct action can also be influenced by the presence of oxygen and by changes in temperature. This shows that 'direct' action need not be an unalterable event and that it may play a more important part in the irradiation of living systems than is generally assumed.

This work has been supported by grants to the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

REFERENCES

[1] Breger, I. A. *J. phys. Chem.* 1948, **52** 551.
[2] Doherty, G. D. *Fed. Proc.* 1952, **11** (part 1) 35.
[3] Dale, W. M. *Biochem. J.* 1942, **36** 80 ; *J. cell. comp. Physiol.* 1952, **39** Supplement 1, 39.
[4] Dainton, F. S. *J. phys. Chem.* 1948, **52** 490.
[5] Risse, O. *Strahlentherapie*, 1929, **34** 578.
[6] Holmes, B. and Dainton, F. S. *Nature, Lond.* 1950, **165** 266.
[7] Alexander, P. and Fox, M. *Nature, Lond.* 1952, **169** 572 ; *Trans. Faraday Soc.* 1954, **50** 605.
[8] Alexander, P. *Brit. J. Radiol.* 1953, **26** 413.
Alexander, P., Bacq, Z. M., Cousens, S. F., Fox, M., Herve, A. and Lazer, J. *Radiation Res.*, in press.
[9] Alexander, P. and Fox, M. *J. chim. phys.* 1953, **50** 415.
[10] Svedberg, T. and Brohult, S. *Nature, Lond.* 1939, **143** 938.
Brohult, S. *Nova Acta Regiae Societatis Scientarium Upsaliensis*, 1940, **12** No. 4.
[11] Manion, J. P. and Burton, M. *J. phys. Chem.* 1952, **56** 560.
Burton, M. and Patrick, W. N. *J. phys. Chem.* 1954, **58** 421.
Patrick, W. N. and Burton, M. *J. phys. Chem.* 1954, **58** 424.
[12] Charlesby, A. *Nature, Lond.* 1953, **171** 167.
[13] Alexander, P., Charlesby, A. and Ross, M. *Proc. Roy. Soc.* A. 1954, **223** 392.
[14] Bacq, Z. M. and Alexander, P. *Fundamentals of Radiobiology*. London, Butterworth, 1955.
[15] Charlesby, A. *Proc. Roy. Soc.* A. 1954, **222** 60.
[16] Alexander, P. and Charlesby, A. *Nature, Lond.* 1954, **173** 578.
[17] Alexander, P., Black, R. M. and Charlesby, A. (to be published).
[18] Charlesby, A. *J. poly. Sci.* 1953, **11** 513.
[19] Alexander, P. and Charlesby, A. (to be published).
[20] *British Empire Cancer Camp. Report*, 1954, **31** 13.
[21] Prèvot-Bernas, A. *J. chim. Phys.* 1953, **50** 445.
[22] Zirkle, E. R. and Tobias, A. C. *Arch. Biochem. Biophys.* 1953, **47** 282.
[23] Gray, L. H. *Radiation Res.* 1954, **1** 189.
[24] Pollard, E. C. *Advanc. biol. med. Phys.* 1954, **3** 153.
[25] Bachofer, C. S., Ehret, C. F., Mayer, S. and Powers, E. L. *Proc. Nat. Acad. Sci.* 1953, **39** 744.
[26] Lea, D. E. *Actions of Radiations on Living Cells*. London, Cambridge Univ. Press, 1946.

DISCUSSION

M. Magat : Je voudrais faire deux remarques. D'abord au sujet de l'utilisation de la méthode viscosimétrique et la perturbation éventuelle de la viscosité par les ions. La variation du pH influence, d'aprés tout ce que l'on sait, uniquement la viscosité des solutions polyélectrolytes, dont DNA est un exemple. Or, à l'exception de l'acide polyméthacrylique, aucun des polymères utilisés par Alexander n'est un polyélectrolyte. Sa méthode expérimentale est donc parfaitement justifiée.

Ensuite, je voudrais préciser quelque peu le mécanisme de protection contre l'effet direct. Deux cas sont à distinguer—celui où la molécule contient elle-même un noyau phénylique et celui où les molécules aromatiques servent de solvant. Dans le premier cas, l'excitation électronique intervenant dans n'importe quel point de la molécule, se propage à l'intérieur de la molécule sous forme de ce que j'appelerai un exciton, et tombe comme dans un piège, dans le noyau phénylique. Au lieu de provoquer une rupture de liaison, l'énergie sert à exciter un électron et se trouve éliminée probablement par émission d'un photon. Alexander a montré, et je

crois que c'est un résultat très remarquable, que la propagation de l'exciton est limitée dans l'espace à quelques 6 liaisons C—C. Remarqons que cet effet protecteur du noyau, phénylique a été pour la premiére fois signálé par Burton, qui l'a appelé ' effet éponge '.

Dans le deuxième cas, le mécanisme initial n'est pas bien compris. On a observé effectivement, aussi bien au laboratoire de Burton que dans le mien, qu'en présence de solvant aromatique (ou même simplement insaturé) une partie plus importante de l'énergie dissipée par le rayonnement est utilisée par le composé aromatique qu'il nesserait justifié, vue sa concentration. Les composés aromatiques ont un très petit rendement en radicaux libres, une grande partie de l'énergie étant réémise sous forme de luminescence (c'est le principe des compteurs à scintillation). Ageno à Rome, a pu montrer que les photons émis par une molécule sont absorbés par des molécules voisines, réémis par elles, et ainsi de suite jusqu'au moment où ils sont réémis vers l'extérieur le point d'émission pouvant être distant de quelques millimètres du point d'excitation.

TWO CLASSES OF PROTECTIVE AGENTS IN THE OXIDATIVE DEGRADATION BY GAMMA RAYS OF POLYSTYRENE IN CHLOROFORM

M. Fox

Laboratoire de Chimie Physique de la Faculte des Sciences de Paris*

A NUMBER of compounds have been examined as possible protective agents against degradation of polystyrene dissolved in chloroform in the presence of air by gamma rays from a ^{60}Co source at an intensity of 23 r/min. The degradation was measured by the change in viscosity of the solution. *Table I* shows the results obtained for compounds which have been found effective in a similar investigation using an aqueous polymer system.[1] Degradation is reduced by amines, hydroxy compounds and mercaptans (*1*), and by compounds having the structure $-S-C\begin{matrix}=N-\\ \diagdown\end{matrix}$ or $-S=C\begin{matrix}\diagup N<\\ \diagdown\end{matrix}$ as in thiourea, mercaptobenzothiazole and dithiocarbamate derivatives (*2*); aromatic compounds are generally more effective than the aliphatic homologues. In the case of protective agents which are chelating agents, such as 8-hydroxyquinoline and dithiocarbamate, chelation does not suppress the protective action although the complex is less active.

The viscosity of the polystyrene solution continues to drop for a considerable time after irradiation has ceased (*see Table II*). The effect of Co^{2+} ions is consistent with the view that the degradation is due to decomposition of a polymer peroxide resulting from electron transfer[2]:

$$ROOH + Co^{2+} = RO^{\bullet} + OH^{-} + Co^{3+}$$
$$\downarrow$$
decomposes.

The post effect is not found when the irradiation is carried out in the presence of protective agents of the thiourea type but occurs in other cases as shown in *Table I*. Moreover the former type of compound when added after irradiation reduces the post-effect, other protective agents act in the opposite sense, accelerating the degradation as shown in *Table I* (cf. the action of phenols on hydroperoxides[3]). It seems therefore that the two classes of protective agents react in a different manner. The kinetics of the reaction of β-naphthol as a function of the degradation have been investigated in some detail[4]. The results show that the degradation which is initially inhibited resumes its normal course, at a dose which is proportional to the concentration of β-naphthol. Assuming reaction of β-naphthol with primary solvent radicals it is found that the protective agent reacts with only a small

* British Council and Medical Research Council Exchange Fellow; Present address: Chester Beatty Research Institute, Royal Cancer Hospital, London, S.W. 3.

Table I

Compound	*Protective agent added before irradiation*				*Protective agents added after irradiation*				
	Immediate Degradation		*Degradation* after standing for* 16 hr *at* 40° C		*Degradation after* 16 hr *at* 40° C *in air*†		*Degradation after* 16 hr *at* 40° C *in vacuo*		
	$1 \cdot 10^{-3}$ M	$5{\cdot}0 \times 10^{-3}$ M	$1 \cdot 10^{-3}$ M	$5{\cdot}0 \times 10^{-3}$ M	$2{\cdot}5 \times 10^{-3}$ M	$5{\cdot}0 \times 10^{-3}$ M	1×10^{-3} M	$2{\cdot}5 \cdot 10^{-3}$ M	$5{\cdot}0 \cdot 10^{-3}$ M
Allyl Alcohol	94	68	—	54	—	—	—	—	
β-Naphthol	0	0	182	79	136	—	—	151	144
8-Hydroxyquinoline	43	0	—	—	—	—	—	—	
8-Hydroxyquinoline Copper Complex	—	30	—	—	—		—	—	
α-Phenylethylamine	96	28	153	107	—	—	—	—	
Aniline	44	0	77	64		159	—	—	
Octyl Mercaptan	61	20	—	132		189	—	—	124
β-Mercapto Ethylamine	89	12	25	0	—	170	—	—	—
m-Tolyl Thiourea	58	0	0	0	—		—	—	49·5
Diethyl Ammonium Diethyl Dithiocarbamate	65	0	0	0	43·5	22·5	84	23	5·0
Dithiocarbamate Copper Complex	—	26	—	—	—	—	—	—	—
2-Mercaptobenzothiazole	50	0	0	0	—	59	—	—	62·5
Tetramethyl Thiuram Disulphide	29	9	0	0	—	—	—	—	70

* Degradation is expressed as the proportion in percent of the drop in viscosity of a 2 per cent polymer solution irradiated with 35,000 r in the presence and absence of protective agent.
† Oxygen has a slight inhibiting effect on the post-effect degradation, which in the presence of air is *ca.* 85 per cent that *in vacuo*, for the control solution.

fraction of the total number of radicals produced. This eliminates in this case the possibility of protection by reaction with primary radicals. Reaction with peroxidized polymer is assumed from the following observations :

(*a*) Completely protected polymer shows a typical peroxide post effect (*see Table I*). (*b*) Such apparently non-degraded polymer is no longer completely insoluble in polar solvents such as alcohol which completely precipitate untreated polymer. (*c*) A solution of polymer was irradiated by gamma

Table II. Various methods for producing the post-effect

*Solution**	*Treatment during post-effect*	*Drop in viscosity (per cent) during post-effect*
Non-irradiated	16 hr at 40° in air	0
Irradiated	16 hr at 40° in air	23·5
Non-irradiated + irradiated $CHCl_3$	16 hr at 40° in air	0
Non-irradiated	16 hr at 25° + 10^{-3}M Co^{2+} in 90 per cent chloroform, 10 per cent acetic acid	0
Irradiated	16 hr at 25° in 90 per cent chloroform, 10 per cent acetic acid	7·5
Irradiated	16 hr at 25° + 10^{-3}M Co^{2+} in 90 per cent chloroform, 10 per cent acetic acid	15
Non-irradiated	30,000 r in vacuum	0
Irradiated	30,000 r in vacuum	28

* Solution is 2 per cent polymer and irradiation refers to a dose of 35,000 r.

rays in the presence of *p*-bromophenol, dialysed and the polymer obtained after solvent evaporation was irradiated in the pile. Radioactive ^{82}Br was detected of an activity 16 times that of a control sample subjected to similar treatment except for the gamma irradiation. This reaction probably occurs at the same stage as that postulated by Bolland[5] for the reaction of phenols with auto-oxidation chains.

The action of the second type of protective agents is less clear. It may be remarked that the action of the two types of protective agents is very similar to their antioxidant action in the aging of rubber. The peroxides formed in the presence of amines are thermally unstable ; however, Type 2 protective agents can react with the peroxides formed on the rubber to render them 'harmless'[6].

REFERENCES

[1] Alexander, P. and Fox, M. *J. Chim. Phys.* 1953, **50** 415.
[2] Bawn, C. E. H. and Williamson, J. B. *Trans. Faraday Soc.* 1951, **47** 721, 735.
[3] Bolland, J. L. and Ten Have, P. *Trans. Faraday Soc.* 1947, **43** 201
[4] Chapiro, A. Durup, J., Fox, M. and Magat, M. *International Symposium on Macromolecuar Chemistry*, Milan. Sept. 1954.
[5] Bolland, J. L. and Ten Have, P. *Faraday Soc. Discussion*, 1947, No. 2,252.
[6] Le Bras, J. *C.R. Acad. Sci.* 1943, **217** 297.
Le Bras, J. and Le Foll, J. *C.R. Acad. Sci.* 1950, **231** 145.
Le Foll, J. *Rev. Gen. Caoutchouc.* 1952, **29** 114.

ACTION PRESERVATRICE DE DERIVES PHENOLIQUES VIS A VIS DE L'IRRADIATION LETALE DE LA SOURIS*

A. Lacassagne, J.-F. Duplan et N. P. Buu-Hoï

Institut du Radium, Paris

On sait que parmi les méthodes utilisées pour diminuer chez les animaux la mortalité consécutive à une irradiation totale, certaines consistent à provoquer une anoxie anoxique ou cytotoxique, d'autres à introduire dans l'organisme des composés chimiques qui détournent des récepteurs cellulaires les peroxydes radioformés. Dans cet ordre d'idée, nous avons montré[1] que le pyrogallol n'est pas doué d'activité radiopréservatrice, mais que certains de ses dérivés cétoniques, injectés par voie intrapéritonéale avant une irradiation totale à une dose 100 pour cent létale, étaient susceptibles de réduire la mortalité des souris. Ce pouvoir protecteur appartient toujours à des acylpyrogallols dont la chaîne latérale possède au moins six carbones. Dans le but de préciser les configurations chimiques actives et d'étendre à d'autres phénols les résultats que nous avons obtenus avec le pyrogallol, nous avons préparé trois types de composés : (i) des 4-acyls dérivés du pyrogallol, du pyrocatéchol et du résorcinol ; (ii) des 1-acyls dérivés du 2-naphtol et des 2-acyls dérivés du 1-naphtol ; (iii) des 3-R-2-dihydroxyphényl-indole et des 3-R-2-trihydroxyphényl-indole, le radical R étant aromatique ou aliphatique (*Figure 1*).

Toutes les irradiations sont pratiquées sur des souris de la lignée pure XVII, âgées de 60 à 80 jours et pesant 20 à 22 g. Ces animaux, irradiés par groupe de six, reçoivent en une seule fois la dose de 700 r (180 kV, 10 mA, 0,3 Cu, 45 cm, 65 r/m) qui est, pour cette lignée, 100 pour cent létale en 12 jours. Une demi-heure à une heure avant l'irradiation, on injecte dans le péritoine de ces animaux 0,5 ml d'huile d'olive neutre dans laquelle on a fait dissoudre le produit à étudier ; seuls le pyrogallol, le résorcinol et le pyrocatéchol ont été dissous dans du sérum physiologique. Les résultats que nous avons obtenus sont résumés dans le *Tableau I* (page 66).

L'activité des dérivés du pyrogallol se trouve confirmée mais, par contre, celle des cétones dérivées des diphénols reste douteuse et demande confirmation. Aussi bien le pyrogallol que le résorcinol et le pyrocatéchol sont inactifs sous leur forme simple. Il faut remarquer que, dans ce groupe, le pyrogallol et le pyrocatéchol sont des poisons mitotiques[2] et, qu'au moins en ce qui concerne le premier, l'activité radiopréservatrice n'apparaît que pour une dose supérieure à la dose minimale antimitotique. Il est permis de penser que les dérivés cétoniques que nous avons utilisés sont, à dose

* Travail du Laboratoire Pasteur à l'Institut du Radium, réalisé avec une subvention du Commissariat à l'Energie Atomique.

équivalente, moins toxiques pour les mitoses que les corps simples correspondants.

Les cétones dérivées du naphtol montrent une activité indubitable en ce qui concerne le 1-béhénoyl-2-naphtol et plus douteuse quand il s'agit de dérivés du 1-naphtol, ce qui peut s'expliquer par la toxicité plus forte de ce dernier corps.

Les différents dérivés des 2-di et 2-trihydroxyphényl-indole sont tous inactifs, ce qui semble indiquer que la chaîne aliphatique saturée doit être

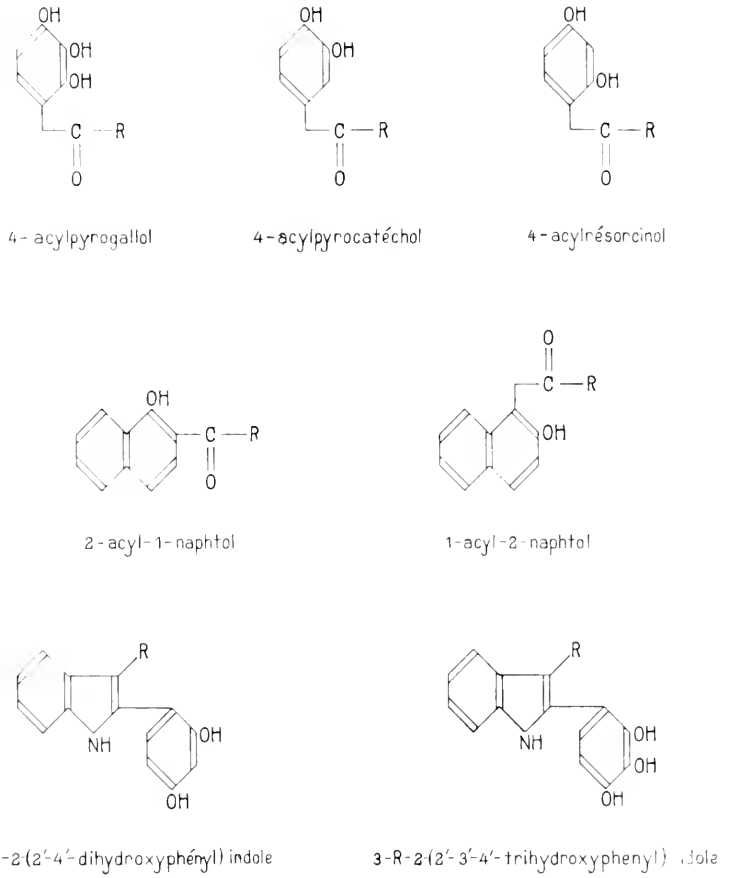


Figure 1

fixée directement sur le cycle phénolique. Il n'est, par contre, pas possible d'affirmer que la fixation de cette chaîne par l'intermédiaire d'une fonction cétone soit nécessaire.

Dans le groupe des phénols, à partir de six carbones, la longueur de la chaîne latérale ne semble pas influencer le pouvoir protecteur et ne paraît pas non plus modifier considérablement la durée de la protection mesurée par le temps qui s'écoule entre l'injection du composé et le moment de l'irradiation. Les résultats des expériences que nous venons de rapporter

et de notre travail antérieur[1] montrent que, pour des chaînes de 8 à 22 carbones, l'activité préservatrice est maximale 45 minutes environ après l'injection. Il est possible, par contre, que les naphtols soient doués d'activité même quand ils sont liés à des chaînes latérales plus courtes.

On a souvent pu démontrer qu'il existait un parallèlisme indiscutable entre les radioprotecteurs actifs *in vitro* et *in vivo*, tel est le cas de la cystéine [3, 4], de la thioürée[5, 6], des dithiocarbamates[7, 8] et d'un grand nombre

Tableau I

Substances	*Doses* mg/kg	*Temps entre injection et irradiation* min	*Taux de survie au 30è j.*	*Date des décès*
Témoins	—	—	0/18	7è au 12è j
Huile d'olive	—	20	0/12	6è – 10è j
Laurate de Na	300	35	0/6	8è – 12è j
Pyrogallol	165	10	0/6	5è – 10è j
4-Béhénoylpyrogallol	750	45	4/10	11è – 21è j
$R=C_{21}H_{43}$	750	150	0/6	9è – 18è j
	1.000	60	0/6	7è – 12è j
Résorcinol	120	10	0/6	6è – 9è j
4-Palmitoylrésorcinol	400	30	0/6	7è – 12è j
$R=C_{15}H_{31}$				
Pyrocatéchol	160	10	0/6	6è – 10è j
4-Bélénoylpyrocatéchol	600	45	1/10	8è – 16è j
$R=C_{21}H_{43}$	750	30	0/12	6è – 13è j
1-Béhénoyl-2-naphtol	250	35	6/10	10è – 20è j
$R=C_{21}H_{43}$				
2-Acétyl-1-naphtol	60	35	1/12	10è – 15è j
$R=C_2H_5$				
2-Palmitoyl-1-naphtol	160	30	0/6	7è – 12è j
$R=C_{15}H_{31}$				
2-Stéaroyl-1-naphtol	200	30	1/6	8è – 15è j
$R=C_{17}H_{35}$				
3-Docosyl-2(2'-4'-dihydroxyphényl)indole	600	30	0/6	7è – 12è j
$R=C_{20}H_{41}$				
3-Phényl-2(2'-3'-4'-trihydroxyphényl)indole	250	30	0/6	7è – 12è j
$R=C_6H_5$	400	30	0/6	6è – 10è j
3-n-*Tétradécyl-2(2'-3'-4'-trihydroxyphényl)-indole*	500	30	0/6	7è – 11è j
$R=n\text{-}C_{14}H_{29}$				
3-n-*Docosyl-2-(2'-3'-4'-trihydroxyphényl)-indole*	750	30	0/6	6è – 12è j
$R=n\text{-}C_{20}H_{41}$				

d'acides aminés[3, 6, 9]. On savait aussi que les phénols étaient des accepteurs d'oxygène radioformé [3, 10] et des protecteurs vis à vis de la dégradation des polymères[7], mais ils se montraient totalement inactifs quand ils étaient injectés à un animal avant irradiation. Les résultats que nous venons de rapporter montrent que, pour jouer dans la radioprotection biologique un rôle non négligeable, les phénols doivent être introduits sous une forme moins toxique et mieux adaptée aux fonctions organiques.

REFERENCES

[1] Lacassagne, A., Duplan, J.-F. et Buu-Hoï, N. P. *C.R. Acad. Sci. Paris*, 1954, **238** 1279.
[2] Parmentier, R. *C.R. Soc. Biol. Paris*, 1949, **143** 585.
[3] Loiseleur, J. *Bull. Soc. Chim. biol. Paris*, 1942, **24** 172.
[4] Patt, H. M., Tyree, E. B., Straube, R. L. et Smith, D. E. *Science*, 1949, **110** 213.
[5] Mole, R. H. *J. Chim. phys.* 1951, **48** 258.
[6] Alexander, P. et Fox, M. *J. Chim. phys.* 1953, **50** 415.
[7] Fox, M. *C.R. Acad. Sci. Paris*, 1954, **237** 1682.
[8] Bacq, Z. M., Herve, A. et Fischer, P. *Bull. Acad. roy. Méd. Belg.* 1953, **18** 226.
[9] Bacq, Z. M. et Herve, A. *J. suisse Med.* 1952, **82** 160.
[10] Loiseleur, J. *Bull. Soc. Chim. Biol. Paris*, 1943, **25** 22.

DISCUSSION

C. Burg : Après combien de temps l'huile d'olive injectée dans le péritoine est-elle résorbée ?

J. F. Duplan : Quelques jours.

C. Burg : On peut se demander quel est le sort, dans ce cas, des substances injectées dans le péritoine, lorsqu'elles sont en solution dans l'huile d'olive ?

J. F. Duplan : La lenteur de résorption du solvant ne permet pas de préjuger de la vitesse de diffusion, dans l'organisme, des corps qu'il sert à dissoudre. Il est probable que les composés phénoliques que nous avons étudiés ne diffusent qu'après s'être détachés de tout ou partie de leur chaîne grasse.

INFLUENCE OF OXYGEN ON DAMAGE TO MICRO-ORGANISMS BY IONIZING IRRADIATION

H. Laser

Molteno Institute, University of Cambridge

A number of bacteria representing strict and facultative aerobes and anaerobes, as well as some yeasts, were exposed to X-rays (190kvp, no added filtration, dose rate 6500r/min.) at doses from $6 \cdot 5$ to 65×10^3r. Known cell concentrations of washed cells were irradiated in phosphate buffer + glucose in absence of any added growth-promoting substance. During irradiation the liquid phase was either free from dissolved O_2 (the gas space containing N_2 or H_2) or it was in equilibrium with O_2 of decreasing percentage (100 per cent O_2 ; 20 per cent or 5 per cent O_2/N_2). In some experiments with 20 per cent and 5 per cent O_2 the nitrogen was replaced by carbon monoxide.

Immediately after irradiation a known number of cells were transferred either into fresh buffer + glucose or into a nutrient medium in manometer flasks and the rate of some metabolic processes measured for up to 10 hours. These included : O_2 uptake, CO_2 production, aerobic and anaerobic fermentation or acid production and utilization of H_2 (*Vibrio desulphuricans*). Under these conditions the increase, for example, in O_2 uptake or anaerobic fermentation with time is proportional to the increase of dry weight of bacteria, *i.e.* it is a true reflection of growth.

The main results are as follows :

(*1*) O_2 uptake, CO_2 production and anaerobic fermentation or acid production were not significantly affected, when measured in washed non-growing cells. Aerobic fermentation of baker's yeast was slightly inhibited.

(*2*) Growth inhibition became apparent only after a lag period which is the shorter, the higher the X-ray dose.

(*3*) All growing cells showed an ' O_2-effect ' on irradiation. The relative sensitivity for O_2-treated as compared to N_2-treated cells was approximately three-fold for some cells (*Staphylococcus albus*, *Lactobacillus Delbrückii*). On the other hand a strictly anaerobic organism (*Vibrio desulphuricans*) was unaffected by $6 \cdot 5 \times 10^{-3}$r in N_2 but almost completely inhibited by the same dose in air ; and the growth of vegetative forms from spores of *B. subtilis* was only inhibited after irradiation ($2 \cdot 6 \times 10^4$r) in presence of O_2 but not in N_2.

(*4*) In order to study whether the ' O_2-effect ' is dependent on a particular metabolic state of the cell or a particular equilibrium of an enzymic system involved in respiration, cells were irradiated in presence of O_2 while their respiration was inhibited by respiratory poisons. Thus, in the case of *Sarcina lutea*, it has been possible with an X-ray dose of 26,000 r to suppress almost completely the O_2-effect, *i.e.* the cells, after removal of the poison, behaved as if they had been irradiated in N_2. The effective inhibitors were

CO, KCN, hydroxylamine and azide. Urethane did not abolish or diminish the ' O_2-effect '.

The mode of the effective poisons in their rôle as respiratory inhibitors is known. They all block hydrogen transfer through the respiratory enzymic system by combining with oxidized cytochrome a_3 and stabilizing the remaining respiratory enzymic chain in the reduced form. Taking this mode of action as a guide in advancing a possible explanation for the ' O_2-effect ' in irradiation, it is suggested, at least for this bacterium, that the enhancement of irradiation damage (1) involves the enzymic respiratory mechanism, (2) requires at least part of the enzymic respiratory chain to be in the oxidized form during irradiation. This makes it possible, that the impedance has been caused by a reducing agent.

DISCUSSION

F. H. Sobels : I was interested to hear about Laser's observation that potassium cyanide exerts a protective effect against X-radiation in bacteria in view of the opposite results recently obtained by myself in *Drosophila*. Here injection of potassium cyanide prior to X-radiation significantly increases the mutation rate as compared to that induced by the same dose of X-rays alone. Similarly King, Schneiderman and Sax showed that carbon monoxide pretreatment increases the frequency of X-ray induced chromosome aberrations in *Tradescantia*. It seems as if the exactly opposite results of cyanide on radio-sensitivity spring from another metabolic situation in the different experimental materials used.

THE ACTION OF IONIZING RADIATION ON ENZYMES AND VIRUSES

ERNEST POLLARD

Biophysics Division, Sloane Physics Laboratory, Yale University

THE purpose of the studies which have been conducted for the past five years in the Biophysics Division at Yale University have been to develop radiation methods as a tool for the study of fundamental cellular processes. Three reviews of this work have appeared[1, 2, 3]. The work is, therefore, not so much directed at the understanding of radiobiological action on large organisms, as it is at developing a means of study.

The following features about radiation can be exploited for the study of cells. The high energy release produced by radiation, the fact that this is localized and that the localization can be varied by choosing the conditions of irradiation, and the fact that penetration into the cell without damage of the cell wall can be achieved by ionizing radiation.

The work to be described follows logically from the early work of LEA, SMITH, HOLMES and MARKHAM[4]. These workers studied the effect of high doses of X-rays on two dry enzymes and concluded from the nature of the inactivation that a moderately reliable estimate of the molecular size of the two enzymes ribonuclease and myosin could be obtained. The principle of this work is to assume that ionizing radiation produces randomly distributed high energy releases which produce the great majority of their effect inside any one molecular unit. The statistics of inactivation permit the derivation of a parameter which can be called the *inactivation volume* in the case of irradiation where the energy release is distributed randomly in volume and a *cross section* in the case where energy release is confined to dense swaths of ionization. Both of these methods of irradiation were clearly understood by Lea.

The techniques of modern nuclear physics permit irradiation in a very much more precise and flexible way than was available prior to the second world war. In particular the use of cyclotron-produced particles for irradiation is very attractive. A group at Yale has exploited this by modifying a relatively small cyclotron, with an external beam, which passes through a defocusing system on to a shutter in front of a series of samples. The shutter is operated when each sample is put in place and exposures of known beam for known times are given and in this way inactivation of quite small and relatively insensitive molecules can be obtained.

The energy release by fast charged particles is well known from a combination of theory and experiment. These have been described in the review articles already quoted. The important feature is that the spacing between energy releases can be changed by varying the speed of the bombarding particle and that these energy releases do not spread very far from the track of the particle itself. Therefore some idea of the localization of the

energy can be obtained in terms of the energy of the particle and the number of particles per cm^2 incident on the enzyme or the virus.

If an enzyme, such as trypsin, is subjected to bombardment by deuterons then it is inactivated semilogarithmically according to the relation

$$\ln n/n_o = -SD$$

In this formula n and n_o are the activities of the enzyme after and before irradiation respectively, D is the number of deuterons per cm^2 incident on the trypsin, and S is the *cross section*, which measures the statistical probability of inactivation of a trypsin molecule. It is found that the quantity S depends on the density of ionization of a bombarding particle. This fits quite well with the idea that the slower the particle the more dense the ionization and the closer the quantity S approaches to the expected physical cross section of the molecule of trypsin.

To illustrate the kinds of studies that have been carried out the inactivation of invertase may be considered. If this is subjected to bombardment by deuterons at varying energies and also by high speed electrons, which produce ionization effects which are substantially random in volume, then the loss of ability to digest sucrose is lost according to a semilogarithmic manner just as described for trypsin. So is the loss of ability to combine with specific antibody to invertase. The two do not, however, follow the same line. The serological activity is lost less rapidly, with more bombardment, than the enzymatic activity.

If the temperature at which the invertase is irradiated is raised then the sensitivity of the invertase begins to increase at a temperature just below that at which enzymatic activity would be lost due to temperature alone. If the temperature is reduced to liquid air temperatures the sensitivity of the enzyme diminishes. There is a range of 50°, or so, over which the sensitivity does not vary very much, and at which the observed cross section corresponds rather closely to that expected for the physical cross section of the molecule. If in place of commercial preparations, the enzymatic activity in yeast cells is studied, it is found that the same inactivation is observed. In other words the sensitivity of invertase in intact yeast cells is the same as that in commercial preparations.

It is perhaps easiest to see the nature of these effects of ionizing radiation on enzyme molecules in terms of a proposed explanation. According to this explanation we consider the enzyme to be made up of protein chains, crosslinked in some way, and that a part of this whole protein molecule is essential for the enzymatic activity, and also for other specific biological activities. The same two parts need not be involved in different functions. The arrival of ionizing radiation causes the ejection of an electron from any one of the atoms in the whole molecule without there being any special tendency to select one class of molecule. This results in the ejection of an electron and leaves behind an atom which is positively charged due to the loss of an electron. The fact that the electron is missing from an atom means that it will tend to restore its lack of balance. This it may do by transfer of an electron from a neighbouring atom and such transfer may be extremely rapid. In view of this it is probable that the localization of the ionized atom changes throughout the molecule as electrons are interchanged

between one atom and another. It is felt that this process of very rapid exchange can only take place within the molecular structure where the atoms are bonded by electron exchange mechanisms. The transfer of energy therefore takes place rapidly along the chains and continually occurs until such time as it is either lost by some method of de-excitation such as the return of an electron to an atom to restore the original condition. Until that time, however, there is a rapidly moving region of excitation, which also corresponds to a region of weak bonding since the electron exchange necessary for bonding is not present. If a molecule has an alternative configuration which can result from such a weakness then this may take place. This may correspond to the formation of an unwanted crosslinking in which case the molecule is permanently distorted and will no longer function in its biological way. Or it may be that due to thermal agitation certain weak links are present in the molecule at various places, and that the presence of an additional weak link induced by radiation near these regions may cause again the formation of an unwanted and semipermanent structure. In this way radiation induces the permanent distortion of the molecule into another form and so causes its inactivation.

It is clear that such a process, while largely confined to the molecule itself, may well have energy requirements imposed on it for effective action. Thus it may not be sufficient for one bond breakage to occur : it may be necessary that three or four be produced inside the molecule. This has been found to be the case in the studies made at Yale, particularly for ionizing action on the solubility of the molecule. For this to be lost it is generally found that three or four ionizations must occur inside the molecular structure.

In view of the effects which are covered by the explanation given above, which may still not be the true explanation but which gives a means of compact description of what is likely to be occurring, it is possible by radiation action involving heavy particles of different velocities, and also fast electrons, to determine the following three things regarding a molecule. (*1*) Its maximum cross section, (*2*) its sensitivity in terms of a number of ion pairs needed for inactivation, (*3*) its approximate thickness. These three quantities can only be determined if irradiations under many different conditions are used. These can be illustrated by considering the irradiation of *B. subtilis* cells by deuterons and electrons and subsequent assay of these cells for the two enzymes cytochromoxidase, and succinic-dehydrogenase.

Studies by Powell and the author[5] have shown that these enzymatic properties are lost under deuteron and electron bombardment and that the loss varies with ion density of the bombarding radiation in a manner which permits the deduction of molecular sizes and thicknesses for these two enzymes as follows.

Enzyme	*Molecular Weight*	*Length*	*Thickness*
Cytochrome oxidase	160,000	200 Å	36 Å
Succinic dehydrogenase	310,000	390 Å	36 Å

Irradiation of these *B. subtilis* cells, and of lysates of the cells, showed no difference between them and there is, therefore, for these enzymes, no property of ionizing radiation which has been shown to be dependent on the organization within the cell.

One very interesting application of ionizing radiation is the study of the structure of viruses. This has been employed by many workers notably LEA and SALAMAN, and BONET-MAURY and LATARJET[6]. The techniques are essentially those which have just been described and have been employed, for example, by Bonet-Maury and Latarjet to deduce a structure for the internal part of vaccinia virus. The type of study which is possible is the following. A well characterized virus usually has between three and six well-known properties. These are, for example, ability to infect the host cell, the ability to attach to the host cell, the ability to interfere with other virus infections, the ability to combine with specific antiserum, the ability to kill the host cell without necessarily multiplying in it, and the ability to agglutinate red cells in the case of a certain large class of animal viruses.

All of these properties can be subject to examination after irradiation by deuterons, alpha-particles and electrons. The result of doing this shows that the loss of properties is widely different. Thus the loss of ability to combine serologicaly is highly insensitive. If the method of analysis described above is applied to results on this serological affinity for the case of T-1 bacteriophage, the molecular size deduced[7] corresponds to a molecule of molecular weight 22,000. If the ability of Newcastle disease virus to agglutinate red blood cells is studied[8], the molecular size corresponds to a pair of units of molecular weight 220,000. If the infectivity is studied very much larger sizes are obtained and the manner in which this infectivity cross section varies with the ion density of the bombarding particle indicates that, in general, there must be some kind of internal structure, for most of the viruses that have been studied. This internal structure must be a relatively small part of the virus in the case of T-1 bacteriophage and a relatively large part of the virus in the case of southern bean mosaic virus. A detailed study of these properties leads to inferred structures for the inside of viruses which represent one way of describing the probable nature and morphology of viruses. It must be stressed that such pictures are a continuing matter, that as more radiation data appears the picture may change, and that in any event the inferred internal structure of viruses must be made to agree with other methods—physical, biochemical, and genetic. Nevertheless it would appear as though virus structure might well be elucidated, at least in part by this type of radiation study.

ACKNOWLEDGEMENT

The author wishes to acknowledge the great part played by his associates in the Biophysics Division at Yale University in conducting the researches on which this article is based.

REFERENCES

[1] POLLARD, E. *Advanc. biol. med. Phys.* 1953, **3** 153.

[2] POLLARD, E., GUILD, W. R., HUTCHINSON, F. and SETLOW, R. B. *Prog. Biophys.* 1955, **5** 72.

[3] POLLARD, E. *Advanc. Virus Res.* 1954, **2** 109.
[4] LEA, D. E., SMITH, K. M., HOLMES, B. and MARKHAM, R. *Parasitology*, 1944, **36** 110.
[5] POWELL, W. F. and POLLARD, E. *Radiation Res.*, in press.
[6] BONET-MAURY, P. and LATARJET, R. This symposium.
[7] POLLARD, E. and SETLOW, J. *Arch. Bioch. Biophys.* 1954, **50** 376.
[8] WOESE, C. and POLLARD, E. *Arch. Bioch. Biophys.* 1954, **50** 354.

DISCUSSION

M. ERRERA : Je voudrais signaler que JEENER *et al** a montré l'existence, dans des feuilles de tabac injectées de virus de la mosaïque, de deux antigènes de constantes de sédimentation et de vitesses d'électrophèse différentes, qui ont des propriétés immunologiques proches de celles du virus et qui en font probablement partie. Il pourrait bien s'agir de protéines identiques à celles dont on peut supprimer les propriétés immunologiques par irradiation des virus.

L. EHRENBERG : Pollard determined by irradiation the same molecular weight of saccharase when it was isolated and when it was situated in the intact yeast cells. It should be noted that there are indications that the saccharase activity is located on the surface of the yeast cell.† It would be important to know whether the yeast cells were viable.

* JEENER, R., LEMOINE, P., LAVAND'HOMME, C. *Bioch. Bioph. Acta*, 1954, **14** 321.

† THORSELL, W. and MYRBÄCK, K. *Arkiv Kemi*, 1951, **3** 323 ; *cf.* WILKES, B. G. and PALMER, E. T. *J. Gen. Physiol.* 1932, **16** 233.

L'IRRADIATION DES VIRUS

P. Bonet-Maury

Laboratoire Curie, Institut du Radium, Paris

Si longue est la liste des organismes vivants soumis, depuis la découverte des rayons X et de la radioactivité, à l'action des radiations ionisantes, qu'il serait surprenant de ne pas y voir figurer, au moins quelques virus.

Les premières expériences, réalisées en général, avec des techniques primitives et seulement avec l'idée de 'voir ce qui se passe' ont cependant montré que l'irradiation pouvait, comme d'autres agents physiques, détruire certaines propriétés des virus, comme le pouvoir de multiplication ou les effets pathogènes pour l'homme et les animaux.

Les travaux de Dessauer, Timoffeef-Ressosvky, Zimmer, Rajewsky en Allemagne, Crowther, Gray et surtout D. H. Lea en Grande-Bretagne, Lacassagne, Holweck, Mme Curie en France, Gowen, Wyckoff, Zirckle aux Etats-Unis, ont donné naissance à la radiobiologie quantique et la théorie de l'impact—treffer ou target theorie—a réussi à interprèter raisonnablement l'irradiation par les rayons X des organismes unicellulaires : bactéries, levures, protozoaires. Mais il faut attendre 1940, à la veille de la guerre, pour voir appliquer ces idées nouvelles aux virus et publier des travaux quantitatifs, comportant une courbe dose/effet ainsi que le calcul du volume sensible du virus, par Wollman, Holweck (Ultra-microscopie statistique), Lacassagne, Luria en France[1, 2, 3], Lea en Grande-Bretagne[4] et Gowen et Lucas aux Etats-Unis[5].

Durant la guerre, Lea poursuit des recherches remarquables sur la radiobiologie quantique des virus, tandis qu'en France, dans l'ignorance complète, pendant cinq ans, des travaux britanniques, mon laboratoire entreprend une ètude systématique de l'irradiation des phages et des virus pathogènes[6]—par les rayons α et X—pour mesurer leurs dimensions, étudier leur structure et la préparation possible de radiovaccines[7].

Nous avons utilisé généralement des virus pathogènes non purifiés, en suspension dans un milieu aqueux, renfermant des concentrations suffisantes de substances organiques protectrices, pour éliminer l'effet chimique indirect. Les virus, non purifiés, sont également protégés, contre les effets chimiques de surface par la gaine de tissus qui les enveloppe ; d'autres conditions contribuaient encore à cette protection : le titre aussi élevé que possible du virus, la détermination soignée de l'origine de la courbe dose/effet, pour les doses faibles et dans certains cas, l'irradiation dans l'air liquide (*Figure 1*).

Les rayons alphas du radon ont été choisis pour des raisons de commodité techniques et surtout parce que le calcul de la cible est plus simple que pour les rayons X. Pour que la rencontre d'un virus par une particule ionisante puisse être considérée comme le 'choc actif' de la théorie de l'impact, il faut deux conditions :

(*1*) La trajectoire doit passer par le virus (probabilité d'atteinte = 1).

(2) L'énergie cédée au virus durant la traversée doit être suffisante pour produire l'effet biologique cherché (probabilité d'action = 1).

La densité ionique élevée le long d'une trajectoire de particule alpha, permet de considérer la deuxième condition comme toujours remplie, même pour les petits virus. On peut alors remplacer la notion de volume sensible par celle de *section efficace* pour un bombardement alpha, dont le calcul ne comporte aucune hypothèse sur l'énergie exacte correspondant au choc actif.

La technique d'irradiation pour les rayons alphas est simple et avec une source de 30 millicuries de radium, nous avons inactivé les virus les plus résistants ; les rayons X étaient produit par un tube démontable Holweck, avec une longueur d'onde moyenne de 0·9A.

La partie la plus difficile de ces expériences est le titrage biologique du virus qui, facile pour les phages, nécessite pour les virus pathogènes un grand nombre d'animaux et des méthodes statistiques convenables.

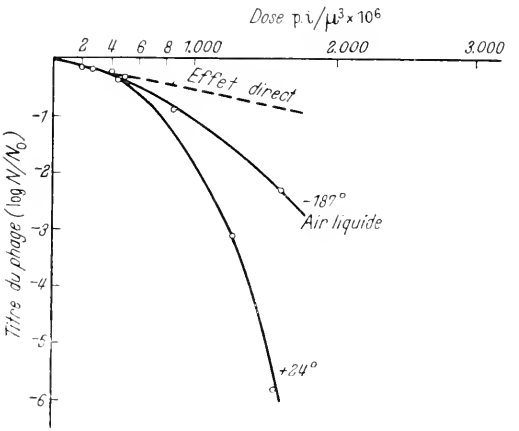


Figure 1. Irradiation avec les rayons α du radon du phage φ-X-174. Action de la température sur l'effet indirect

Toutes nos courbes d'inactivation, comme celles de Lea, et des précedénts auteurs, se sont montrées linéaires en coordonnées semi-logarithmiques, c'est-à-dire des exponentielles ou courbes de Poisson à un seul choc, d'après la théorie de l'impact.

Chaque corpuscule recevant en moyenne un choc actif, pour la dose *D37*, réduisant de 37 pour cent le titre du virus, on calcule le diamètre de la section efficace pour les rayons alphas, par la formule

$$d_\alpha = 6{,}45 \times 10^6 / V\, D37$$

où d_α = diamètre exprimée en mμ

D37 = dose exprimée en ionisations (33 eV) par μ3, pour permettre la comparaison des rayonnements très divers.

Le calcul du volume sensible, pour les rayons X, nécessite une hypothèse sur l'énergie du choc actif. Nous avons choisi, comme Lea, le groupe de 3 ionisations, correspondant sensiblement à 100 eV, utilisé maintenant en chimie des radiations pour calculer le rendement *G*. Le diamètre du volume sensible se calcule par la formule

$d_x = 1{,}76/V\, D37$ avec les mêmes unités.

Lea a donne un procédé général de calcul (méthode du volume associé) qui utilise la densité ionique linéaire (overlapping factor F) et permet la détermination du volume sensible pour tous les types de rayonnements, y compris les rayons alphas.

J'ai également utilisé, pour les rayons alphas, le graphique liant la section efficace aux dimensions moyennes du virus ; déterminées par les autres méthodes physiques (*Figure 2*).

Tous nos résultats, avec ceux d'autres auteurs, ont été groupés dans un tableau, comportant également les dimensions des virus obtenues par d'autres méthodes physiques : microscope électronique, centrifugation, filtration.

On peut dire, d'une façon générale, que les sections efficaces alphas sont en accord raisonnable avec les dimensions du corpuscule, mesurées par les diverses méthodes mécaniques. Le fait que tout le virus soit radiosensible parait surprenant pour les gros virus, auxquels on attribue généralement une

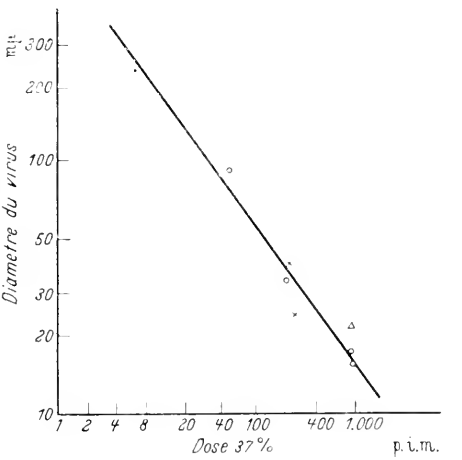


Figure 2. Relation entre la dose d'inactivation pour les rayons α et le diamètre du virus

Virus { o = *Bactériophage* ; × = *Des animaux* ; △ = *Des plantes*

structure ; on peut l'expliquer cependant par une migration, physique ou chimique, de l'énergie produite en excès, lors de la traversée alpha.

La comparaison des sections efficaces des volumes sensibles pour les rayons X, conduit à des constatations différentes. Si les diamètres X et alphas sont du même ordre pour les petits virus comme les phages S13 et φ-X-174 ou la fièvre aphteuse dermotrope, il y a une différence de plus en plus significative quand la taille du virus augmente et, pour la vaccine, le diamètre X n'est plus que le dixième du diamètre alpha, c'est-à-dire un rapport 1.000 pour les volumes.

Cet écart peut naturellement s'expliquer par un mauvais choix des hypothèses de calcul. Il est évident que la forme sphérique, utilisée en première approximation, ne convient pas pour les virus filiformes, comme la mosaïque du tabac et le choix du choc actif n'est pas à l'abri de la critique. Si on admet cependant que, pour les rayons X employés, le volume sensible correspond à une réalité biologique, toute différence significative entre les cibles X et alpha indiquera une structure hétérogène différenciée, c'est-à-dire un certain degré d'organisation biologique.

On sera conduit à attribuer alors aux petits phages comme le S13 ou le φ-X-174 une structure homogène alors que les gros, comme le C16 montrent déjà une organisation en territoires différenciés.

Dimensions des cinq phages par différentes méthodes :

Phages	Irradiation		Micr. électron. (Section efficace)	Centrifug.	Filtration fac. corr. 0.83
	X	α			
S 13	16	16	< 20	16–20	18
φ-X-174	17	18	< 20	16	18
C 36	22	35	35	23	33
St K	40	50	65	60	83
C 16	36	90	90	70–85	83

Il en est de même pour les virus des animaux, l'accord de la section efficace et du volume sensible pour la fièvre aphteuse dermotrope conduit à une structure homogène, tandis que nos expériences sur la vaccine, comme celles de LEA et SALAMAN[8] s'interprètent par une structure tout à fait hétérogène, que nous concevons d'ailleurs de manière un peu différente de Lea. Celuici considère un volume sensible fractionné en 110 corpuscules de 6 mμ, repartis dans une partie inerte, tandis qu'avec Frilley et Laterjet nous admettons[9] une construction en micro-briques de 22 mμ, entièrement radiosensible pour les alphas.

L'analyse de la structure des virus par l'irradiation met donc en évidence le passage progressif du type macromolécule homogène, attribué aux plus petits virus, au type hétérogène, annonçant la structure cellulaire des gros virus, différenciés en territoires inégalement sensibles au rayonnement.

Pour étendre ces conclusions, une plus large expérimentation avec d'autres rayonnements est nécessaire. A ce point de vue, la contribution de POLLARD[10] est d'un grand intérêt car, avec les deuterons accélérés artificiellement, il a pu faire varier la densité d'ionisation et étudier systématiquement l'influence sur la section efficace de la perte linéaire d'énergie, c'est-à-dire de l'espacement des ionisations, pour en déduire la forme et les dimensions du volume radiosensible, par une méthode différente de calcul.

Si l'application aux virus des principes de la radiobiologie quantique présente un grand intérêt pour les virologistes, il est au moins égal pour les radiobiologistes. L'irradiation des virus, organismes vivants les plus simples, constitue, en effet, le premier chapitre de la radiobiologie et un champ d'étude particulièrement favorable pour approfondir le mécanisme élémentaire de l'action biologique des radiations.

REFERENCES

[1] HOLWECK, LURIA et WOLLMAN. *C.R. Acad. Sci. Paris*, 1940, **210** 639.

[2] LACASSAGNE, A. et WOLLMAN. *Ann. Inst. Pasteur*, 1940, **64** 5.

[3] WOLLMAN et LACASSAGNE, A. *Ann. Inst. Pasteur*, 1940, **64** 5.

[4] LEA, D. E. *Action of Radiations on Living Cells*. Cambridge University Press, 1946.

[5] GOWEN et LUCAS. *Science*, 1939, **90** 621.

[6] BONET-MAURY, P. L'irradiation des Virus dans *Les Ultras-virus des Maladies Animales*. Maloine, 1943 ; et *Les Ultras-virus des Maladies Humaines*, Maloine, 1948.

[7] BONET-MAURY, P. et OLIVIER. *C.R. Acad. Sci. Paris*, 1939, **209** 459.

[8] LEA, D. E. et SALAMAN, M. H. *Brit. J. exp. Path.* 1942, **23** 27.

[9] BONET-MAURY, P. *Ann. Inst. Pasteur*, 1945, **71** 46.

[10] POLLARD, E. This symposium.

INACTIVATION, PAR LES RAYONS X, D'UN AGENT TRANSFORMANT DU PNEUMOCOQUE

H. Ephrussi-Taylor et R. Latarjet

Institut de Génétique et Institut du Radium, Paris respectivement

Le TP Sr, qui confère au pneumocoque la résistance à une concentration de 2×10^{-3} pour cent de streptomycine, a été inactivé en solution aqueuse par des rayons X de 0,7–0,9 Å donnés avec une intensité de 1 kr par seconde. Les faits suivants ont été mis en évidence.

(*a*) La courbe de survie est exponentielle, mais présente une cassure pour une survie d'autant plus basse que la solution est moins concentrée (*Figures 1 et 2*). Cette cassure dénote une hétérogénéité. Celli-ci ne provient pas de la présence de particules d'ADN différant héréditairement des autres

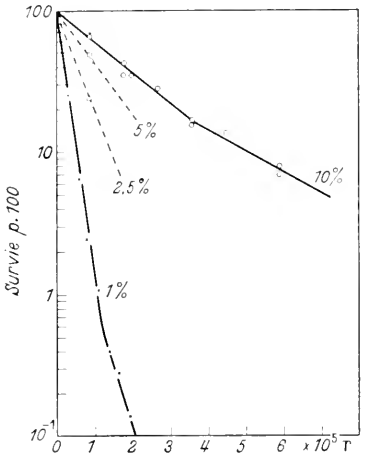


Figure 1. Courbes d'inactivation du TP en solution à 10^{-3} pour cent dans des extraits de levure à 1 pour cent, 2,5 pour cent, 5 pour cent et 10 per cent

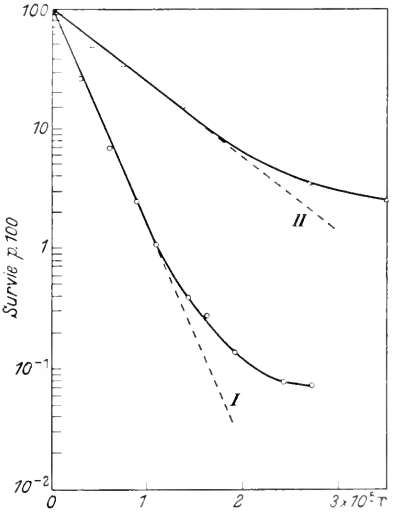


Figure 2. Courbes d'inactivation du TP en extrait de levure à 1 pour cent. I TP 10^{-3} pour cent. II TP 10^{-1} pour cent. Auto-protection manifeste (en pointiller, le prolongement de la partie rectiligne de la courbe met la cassure en évidence)

par une radiorésistance plus élevée : les molécules qui survivent à une très forte dose de rayons X n'induisent pas chez le pneumocoque la formation de TP résistant. Il s'agit d'une hétérogénéité dans la structure de la solution, actuellement à l'étude.

(*b*) Le TP est extrêmement sensible aux actions indirectes du rayonnement. Tandis qu'à la concentration de 1 pour cent l'extrait de levure

protège complètement les enzymes et les petits bactériophages contre l'effet indirect, à la concentration de 10 pour cent cet extrait n'assure pas encore une protection complète du TP (*Figure 1*). L'autoprotection du TP est également très important (*Figure 2*). Ce fait est sans doute en relation avec l'absence de membrane protéique autour de l'ADN, et aussi avec la forme filamenteuse très dissymétrique, qui, pour un volume donné d'ADN, offre aux radicaux libres du milieu ambiant une très grande surface acceptrice.

(*c*) On atteint une protection à peu près complète en opérant sur des solutions en extrait de levure à 10 pour cent congelées à $-70°$ C. Dans ces conditions d'effet direct à peu près pur, la courbe d'inactivation (*Figure 3*)

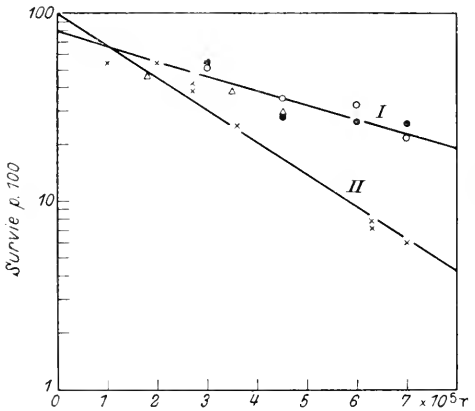


Figure 3. Courbes d'inactivation par effet direct. I *du TP en extrait de levure à 10 pour cent congelé.* II *du bacteriophage S-13*

fournit une dose $D_{0,37}=530$ krad, qui correspond, pour le poids moléculaire du TP, à la valeur

$$P \leqslant 7{,}3\times10^5$$

Dans le calcul de ce poids, les incertitudes liées au groupement des ionisations ont été très diminuées en irradiant conjointement le petit bactériophage S13, dont le volume est approximativement connu (diamètre moyen 15,4 mμ) —*Figure 3*.

(*d*) La présence d'oxygène est sans influence notable sur l'inactivation du TP. Si cette inactivation est, par sa nature, voisine de ce qu'on appelle une radiomutation génique, on peut concevoir que cette dernière, résultant d'une action primaire sur l'acide nucléique, serait également indifférente à la présence d'oxygène.

Comme les ruptures de chromosomes lui sont en revanche très sensibles, on peut penser qu'elles résultent de l'atteinte primaire de substances différentes de l'ADN.

L'étude de l'influence de l'oxygène permettrait ainsi de dire si une altération génétique consécutive à l'irradiation résulte, ou non, de l'atteinte primaire de l'acide nucléique.

RADIATION DEATH IN MAMMALS

B. Rajewsky

Max Planck – Institut für Biophysik, Frankfurt/Main

DOSE AND SURVIVAL TIME

The complete dose-mortality curves obtained on irradiating with X-rays mice, rats, and guinea-pigs show several distinct ranges of total dose over which the biological mechanism of bringing about death is different. This fact is especially true for whole body irradiation. *Figure 1*, for example, represents the complete survival curve of white mice after a whole body irradiation by roentgen rays. The final response to irradiation is generally the premature death of the animal. A special case arises when medium doses are applied over a long period of time (for about one year or more), and if a very small irradiation intensity is used. That is the field of carcinogenesis. Here cancer is the cause of the death of animals, but in this case their mean survival time has, in general, not been reduced considerably. If one looks at the entire survival curve as it is reproduced in *Figure 1*, one notices first that the curve covers the dose range from some 100r to some 100,000r. When small doses (under 100r) are applied, changes take place in the irradiated body, the meaning of which as regards injury of the organism is still not known. They can be recognized mainly by changes in the blood picture. In addition there are some further observations which, however, did not lead to a well defined conception so far as the mode of action of these radiation doses is concerned. The very large doses of the order of magnitude of 200,000r result in the practically instantaneous death of the animals. Between these extremes there are dose ranges which differ from one another in a distinctive way. These ranges are approximately as follows : *(1)* 100r to 1,200r, *(2)* 1,200r to 15,000r, *(3)* 15,000r to about 30,000r, *(4)* 30,000r to about 100,000r, *(5)* exceeding 100,000r. From the results available it can be concluded that each of these dose ranges is connected with different mechanisms of injury, which finally cause the death of the animals.

In the first dose range numerous changes occur in different hormonal and other systems of the body. A large part of the observed symptoms is due in this case to irritation of the haematopoietic system and its connections with other functional centres of the organism. In this range an essential part is played by the time intensity factor of the radiation effects. Here the direct injury of cells is also of great importance. Especially regarding these direct cellular effects and the effects of local irradiation we generally consider this region as being the field of radiation therapy. This field is being studied in greatest detail at the present time. The effects of doses under 100r, mentioned above, can probably also be classed in this range.

In the second dose range final reaction to irradiation is independent of the magnitude of the applied dose. The results of the investigations we carried out show that the damage to *certain organs* and their functions predominate if compared with direct cell-damage and other injuries of the

tissue of the body. The third dose range is characterized by a rapid reduction of survival time of the irradiated animals. The mechanism producing the final effects in this dose range is apparently completely different from the mechanism prevailing in the second dose range.

In the fourth dose range animals die with symptoms that seem to point to a considerable participation of the central nervous system in the mechanism of injury.

The fifth dose range is marked by a process of damage that takes place rapidly (instantaneous death). The mechanism of these effects proceeds

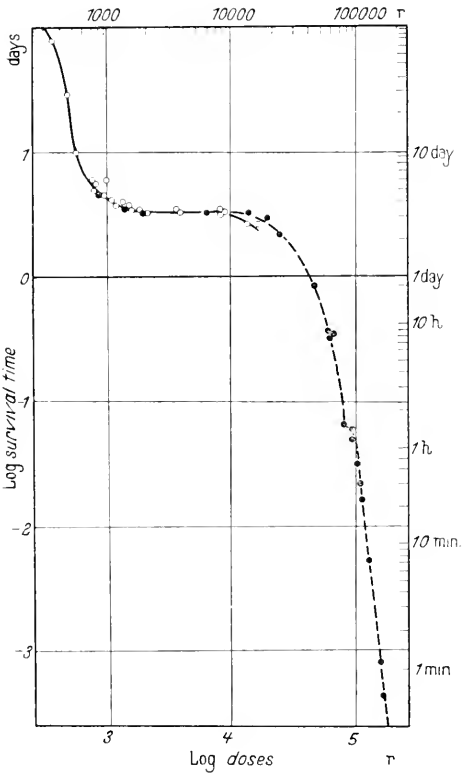


Figure 1. Dose mortality curve of white mice after whole body roentgen-irradiation. o———o *Curve obtained in 1943.* ●– – –● *Curve obtained in 1953.*

probably from the destruction of a relatively large quantity of practically all basic substances of the organism (molecular death). We shall see subsequently that probably each of these ranges really includes several mechanisms of injury. This can be said definitely of the first and second dose ranges. It was possible to demonstrate that the second dose range includes at least three mechanisms which are probably connected with each other.

Single doses, as small as 100r to 200r, already lead to a premature death of the animals. Especially remarkable is the occurrence of a dose range, varying slightly with the species and resistance of the animals, at least from 1,200r to 15,000r. The mean survival time for white mice amounts to 3·5 days and is independent of the applied dose. This horizontal part of the

curve is followed with increasing doses by a steep fall in the survival time till instantaneous death of the animals occurs (few seconds). A new symptom of injury appears when doses of approximately 30,000r to 100,000r are used. Then the irradiated animals suffer from tetanic convulsions. The lower curve of *Figure 3B* representing the dependence of the appearance of convulsions on the dose is similar to the general curve of injury.

The injury in the 'dose-independent' range is to a great extent independent of the intensity of radiation. The range studied was for single doses lasting from 2·5 seconds to 10 hours (*i.e.* radiation intensities varied by a factor of 14,400).

Similar effects also appear in the cases of other animals. The dose range for constant survival time differs for different species both in their extent

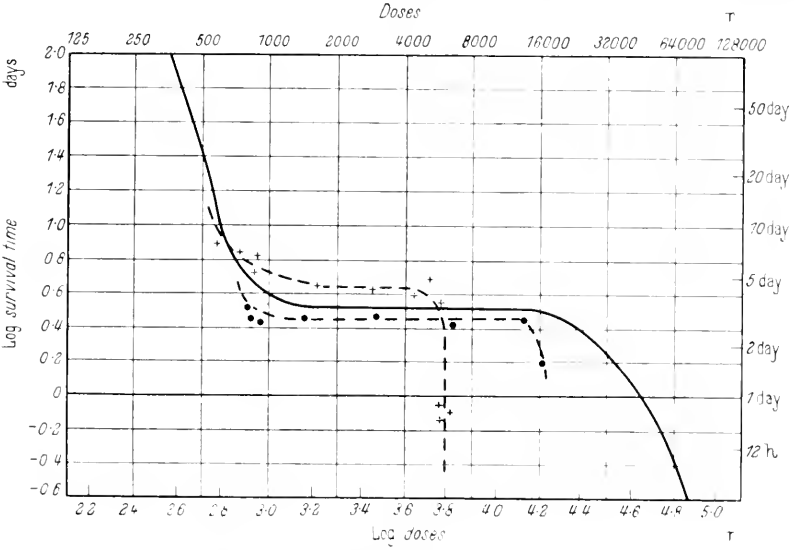


Figure 2. Dose mortality curves of white mice (———), *guinea pigs* (+ — — +) *and rats* (• — — •) *after whole body irradiation*

and in the length of the mean survival time. The differences, however, are not big. *Figure 2* represents the results of corresponding irradiations of white mice, guinea-pigs, and rats. In the following we will refer for the sake of brevity to a 3·5 day-effect also with rats.

PHYSIOLOGICAL PROCESSES

While instantaneous death (*i.e.* within a few seconds) after application of large doses may probably be regarded as 'molecular' death caused by destruction of the organism's basic substances, the 3·5-day death is undoubtedly connected with more or less complicated physiological processes in the irradiated body. Therefore this effect (first described by the author in 1943) was of special interest. Further experiments in this direction were interrupted unfortunately by the war. The studies were resumed in 1948 with my co-workers[1] (Heuse, Aurand, Wilhelm, Pauly, Gerber, Parchwitz and Winkler) using an X-ray set (Heuse and Rajewski[2])

of very high power, the construction of which had already been begun in 1940. The plant operates at a maximum of 50 kV, 2 A tube current (permanently) and supplies up to 1,000,000 r/min. Irradiation of animals is carried out in rotating plastic containers so as to obtain a homogeneous irradiation.

The results of these experiments[4] led to the recognition of a second effect (*i.e.* a 7-day effect) which is observed when certain parts of the body are shielded. In the first series of experiments either the head was covered with lead and the trunk alone was irradiated or the trunk was covered with lead and the head alone was exposed. Irradiation of the trunk again gave the 3·5-day effect. With larger doses, however, the survival time decreased more slowly than with whole body irradiation. When only the head is

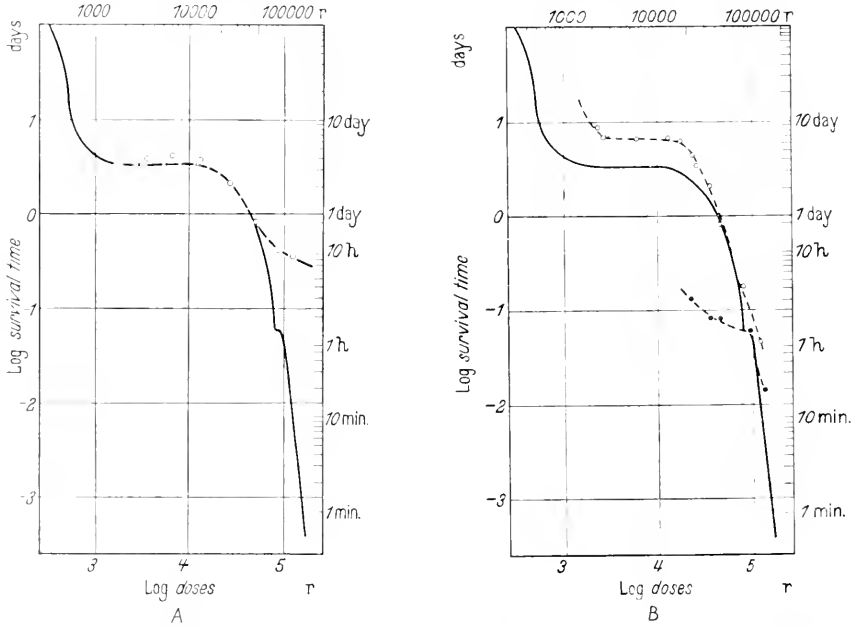


Figure 3. Survival time of white mice after irradiating the whole body (———), *and the head only* (o——o) *and beginning of convulsions after head irradiation* (•——•)

irradiated the entire course of the survival curve was essentially the same as for whole body irradiation, except that the survival time for the dose-independent range was *seven days* and the range is shortened (approximately from 2,500 r to 12,000 r). We were led to conclude that two distinct mechanisms were operating in whole body irradiation in the dose-independent range of the survival curve. With whole body irradiation a 3·5-day effect, showing itself more rapidly, naturally hides the 7-day effect, which can only appear if the mechanism leading to the 3·5-day effect does not act. If large doses are applied the injuries connected with the 7-day effect probably come to the fore with a shorter latent period. Furthermore, it would appear that the 3·5-day effect is essentially connected with irradiation of the trunk. *Figure 3* represents the results of the tests with white mice described above.

Three interrelations for the 3·5-day effect seem possible :

(*1*) An important organ of the body is critically injured after application of a certain dose. The injury requires a latent period of 3·5 days until death takes place. Further injuries, which appear when the dose is increased, take a longer time to be lethal and are therefore without any significance as regards the 3·5 day effect.

(*2*) After reaching the minimum dose in the independent range a process is started in the body of the irradiated animal by various irradiation injuries, which finally causes the death of the animal within 3·5 days.

(*3*) The changes produced in the body in the dose-independent range are of such kind that continued injuries are compensated by the function of one or several organs. This system of compensation breaks down if a much too large dose is applied.

Based on the studies of Miller, Hammond, Tompkins and Shorter[3], injury of the intestine, leading to an increased permeability to bacteria and consequently to bacteraemia, appears as a possible mechanism, and Quastler (verbal communication), after confirming the 3·5-day effect,

Table I.—Effect of antibiotics on survival time

Species	*No. of animals*	*Dose* r	*Antibiotic used*	*Mean survival time*	σ
Mice	62	1,500	penicillin and streptomycin	3·5d+3h	±13·9h
Mice	25	1,500	untreated	3·5d−8h	±10·8h
Rats	15	1,500	penicillin and streptomycin	3·5d+2h	±7·5h
Rats	15	1,500	untreated	3·5d−7h	±7·2h

believed that it is mainly due to damage of the intestinal epithelia. It was necessary, therefore, to examine this question. For this purpose white rats and white mice were treated with penicillin and streptomycin before irradiation and then were irradiated with different doses. The 3·5-day effect was maintained and the treated animals showed no significant difference from the untreated animals (*see Table I*). Blood cultures from the control and antibiotic-treated animals were all negative.

Consequently bacteraemia cannot be the cause of the 3·5-day effect.

The two other possibilities mentioned above were therefore examined using a different technique of lead shielding. The experiments were carried out in such a way that in one series the amount of shielding of the animal's body was increased progressively from the cranial end. In the other series shielding was started at the caudal end portion and increased towards the head.

As can be seen from *Figure 4A* the two injury curves overlap at a point which corresponds to a life span of 3·5 days ; (*I*) shows both the 3·5-day and the 7-day effect, while from the other curve (*II*) only the 3·5-day effect can be observed, which hides the 7-day effect in whole body irradiation.

The anatomical location of the cross over 'point' for the 3·5-day effect can be seen from the radiograph of the mouse used as abscissa in *Figure 4A* and *B*.

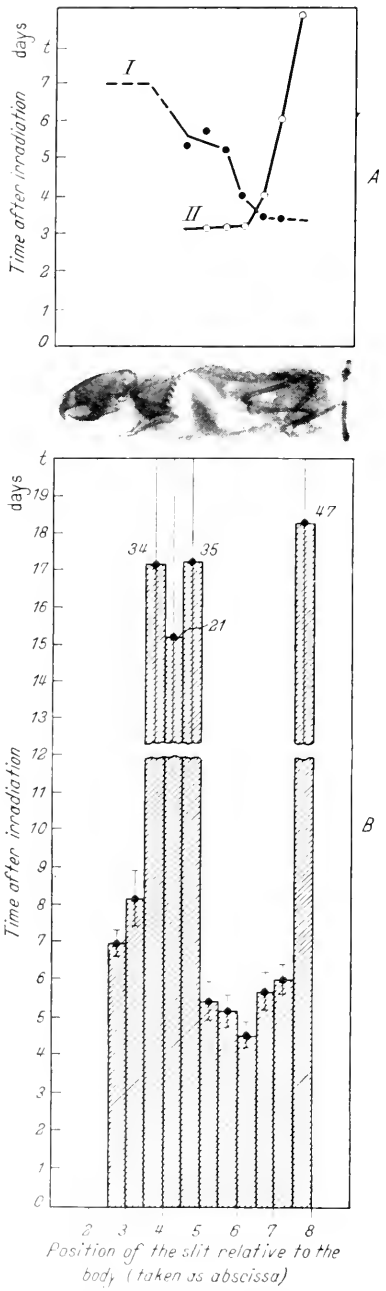


Figure 4. Survival time of white mice after partial body irradiation with 8,000 r. *Diagram A shows the survival time as a function of the amount of the body shielded with lead. I Caudal region of the body shielded up to point* •. *II Cranial region of the body shielded up to point* o. *Diagram B shows the survival time after slit irradiation in relation to the position of the* 5 mm *slit in regard to the body*

It corresponds to the region where important organs of the body (liver, spleen, kidney, and a part of the intestine) are located.

Curve (*I*) in *Figure 4* also presents a further dose-independent range of about 5-day mean survival time between the two constancy ranges of the 3·5-day and 7-day effects. The meaning and more precise localization of this new effect has not yet been clearly recognized. Anatomically considered, however, the effect is not connected with irradiation of the adrenal, or the pituitary gland region. Further investigations on this point are in progress.

'Slit irradiation' was attempted to locate more exactly the critical organs. With these irradiations the whole animal's body is shielded by lead with the exception of a 5-mm wide slit. In this way only the part of the body actually placed under the slit was irradiated. The slit was brought above different regions of the animal's body in a series of irradiations. The results of these experiments are summarized in the diagram *B* in *Figure 4*. It can be seen that the irradiation of only a narrow strip of the animal's body is able to produce both the 3·5-day and the 7-day effects. The 3·5-day effect can be obtained by irradiating only the region of the abdominal organs mentioned above, especially by irradiating a zone containing the adrenal and the kidney, while the 7-day effect can be obtained by irradiating the region of the pituitary gland. Slit irradiation of other parts of the body decreased the mean survival time, although to a very much smaller degree than irradiation of the critical regions just mentioned. These results indicate that the two dose independent survival times were essentially due to local injuries and the next step was to determine the relative importance of the different organs located in the sensitive regions.

The following experiments were carried out with this end in view :

(*1*) Animals were irradiated, which were splenectomized, adrenalectomized or hypophysectomized before irradiation.

(*2*) Individual organs of the animals were irradiated *in situ* : adrenal, kidney, liver, spleen, intestine, and pituitary gland.

(*3*) Different combinations of these organs were irradiated *in situ*.

(*4*) An attempt was made to influence the reactions by the administration of certain substances.

Details of these experiments will be given elsewhere[5] and the most important results only will be summarized.

Adrenal—White rats were exposed to radiation over the whole dose-independent range from 800 to 15,000 r, but no constant survival time was observed in adrenalectomized animals, and a significant dependency of survival time on the applied dose (*i.e.* a rapid fall of the medium survival time with increasing doses ; curve + ——— + in *Figure 5*) was found. Irradiation of the adrenal *in situ*, while all other parts of the animal's body were shielded with lead, did not produce a direct effect ; during the time of observation which lasted for 6 weeks, the animals stayed alive. In another series of irradiations the adrenalectomized animals were treated with 5 mg of cortisone twice daily after irradiation. These cortisone-treated animals showed again the full dose-independent range of the 3·5-day effect. It is, however, remarkable that the dose range over which the 3·5-day effect occurs was not extended by treatment with cortisone—after further increase of the dose up to approximately 15,000 r a rapid fall of the animals' mean survival time occurred according to the general injury curve.

Pituitary gland—Animals that had been hypophysectomized and irradiated with different doses showed a reaction similar to that of the adrenalectomized animals. Here also the 3·5-day effect disappears and a distinct dependence (Curve o———o in *Figure 5*) of the effect on dose appears. The course of the survival curve is slightly different from that of the adrenalectomized animals. Again irradiation of the pituitary gland alone (*in situ*) produced no effect in the six-week period of observation.

Spleen—Splenectomized animals given whole body irradiation with different doses in the independent range (3·5-day effect) behaved like animals that had not been operated; here the complete 3·5-day effect was observed. The mean survival time amounted to somewhat above 3·5 days, the difference, however, is only small. Results of these tests are shown in *Figure 5*.

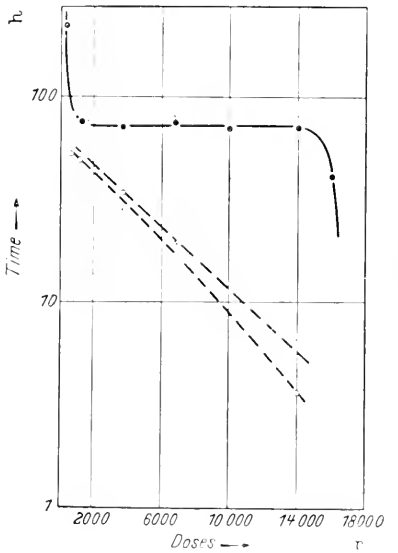


Figure 5. Survival time of white rats after irradiation in the dose range 1,000r–15,000r ●———● *irradiated controls (3·5-day effect),* +———+ *irradiated splenectomized animals,* o———o *irradiated adrenalectomized animals,* ×———× *irradiated hypophysectomized animals.*

Intestine—Irradiation of the intestine *in situ* did not have a significant effect. The survival time was long. The applied doses were within the 3·5-day dosage range.

Irradiations of more than one organ in situ—The applied doses were within the range of the 3·5-day effect. When liver, spleen, kidney, and intestine were irradiated simultaneously an effect was apparent and the survival time fell to approximately 4 days; but a constant range could not be definitely determined, because the scatter of the results was relatively great. We hope to answer this question soon when conditions of irradiation are modified. In addition, the following combinations of organs were chosen: (*a*) liver, spleen, and kidney, (*b*) liver, spleen, and intestine, (*c*) spleen, kidney, and intestine, (*d*) liver and kidney. Liver and spleen as well as liver and intestine were irradiated with 15,000r *in situ*. In all cases the survival time was reduced gradually and differently. The effect was most obvious with all those combinations in which the liver participated. The experiments are still in progress and the interpretation has to wait until all

doses in the independent range have been tried out. At present we can only say that simultaneous irradiation of two or more organs shows a clear effect on the survival time, and is therefore in contrast to irradiation of *one* isolated organ only. Participation of the liver intensifies the effects.

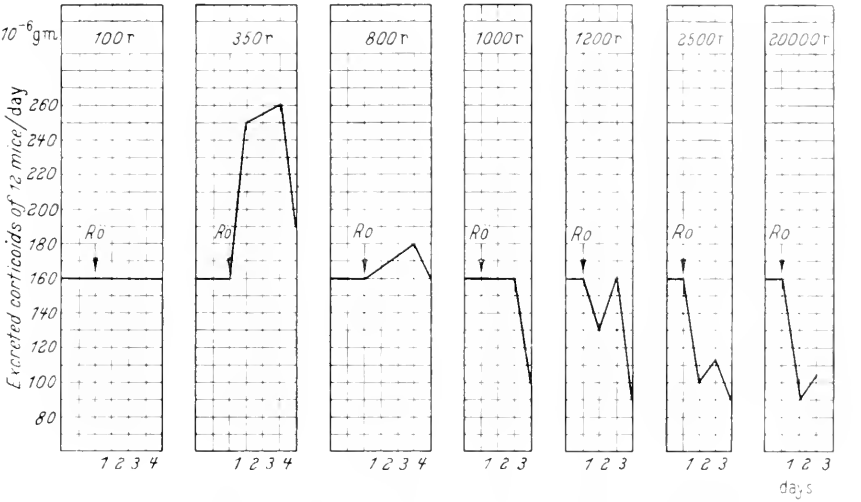


Figure 6a. Urinary excretion of corticoids of mice after whole body irradiation.

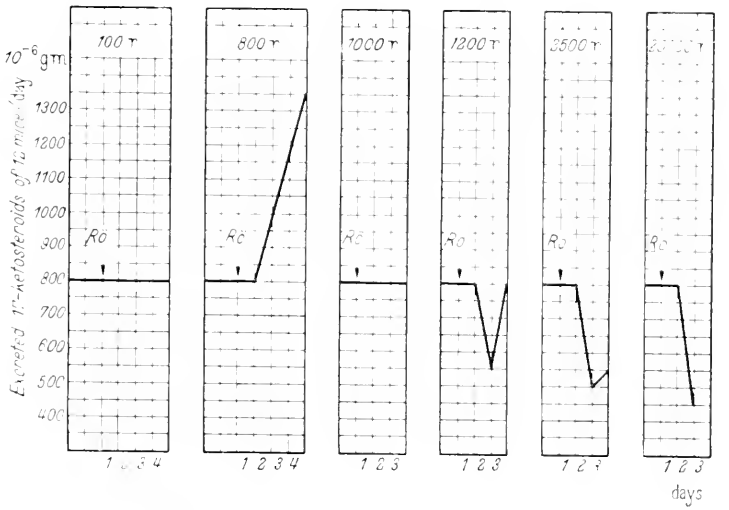


Figure 6b. Urinary excretion of 17-ketosteroids after whole body irradiation

The influence of irradiation on some hormonal systems within the 3·5-day dose range was examined by analysing the urinary excretion of the corticoids and the excretion of 17-ketosteroids of the irradiated animals[6]. With doses less than those necessary for the 3·5-day effect an increase of excretion appears in both cases. At the beginning of the independent range this increase stops relatively abruptly and is followed by a decrease of the excretion.

In the case of the excretion of 17-ketosteroids the dose of 1,000 r has no effect. Decrease of excretion starts at 1,200 r and takes place one day after irradiation. The excretion of corticoids decreases at 1,000 r, beginning immediately after irradiation. The results of these tests are illustrated in *Figure 6.*

Blood—Some changes in the blood after irradiation with large doses were examined. For clinical purposes after irradiation the changes in the 'prothrombin-time' was studied in detail. These examinations carried out with RÖMER and BELLER[7], showed a clear effect on the blood of *in vitro* irradiation (*see Figure 7*).

Investigations of metabolism—The metabolism of tissues irradiated *in vitro* was measured by the method of Warburg[10, 11]. These investigations are still

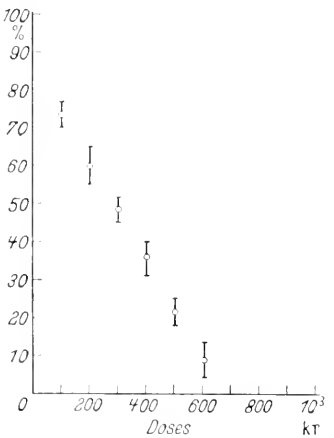


Figure 7. Decrease of 'prothrombin time' after irradiation of blood in vitro

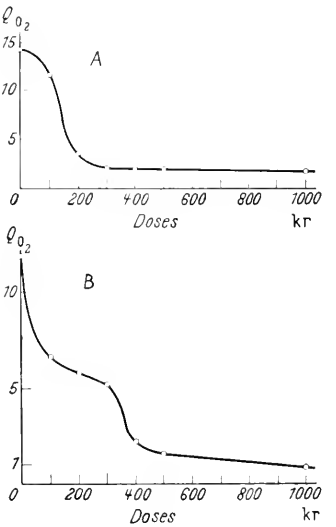


Figure 8. Tissue respiration after irradiation in vitro *of (A) liver and (B) spleen of white mice*

in progress. *Figure 8* shows two examples of the results obtained, *i.e.* respiration of liver and spleen tissue slides after irradiation with high doses. Especially the 'residual' respiration which is preserved after doses of 1,000,000 r had been applied, should be noted.

The curves are analogous to those curves obtained earlier by RAJEWSKY and INOUYE[8] by tissue irradiation with alpha-particles.

FRACTIONATION OF DOSE

To clarify the 3·5-day effect we investigated the time intensity factor in this range. As already mentioned, change of irradiation time from 2·5 seconds to 10 hours gave no changes of the 3·5-day effect. We therefore studied, in co-operation with AURAND[9], the effect of dose fractionation. I shall illustrate our results with only one example. In *Figure 9* the curves for the dose effect are represented for a single and for fractionated irradiation,

respectively with a total dose of 1,200r and a time interval of 24 hours between the two part-doses of 600r each.

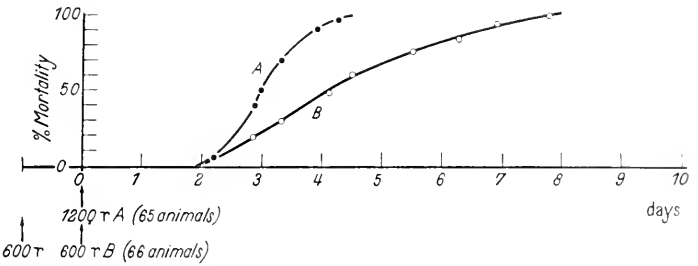


Figure 9. Percentage of animals dead at different times after single and fractionated whole body irradiation (total dose 1,200r*). A single irradiation, B fractionated irradiation with two* 600r *doses at 24-hours interval*

It is not the mean survival time that is shown in this diagram, but the percentage of animals which died at different days after irradiation. A clear effect of fractionating of the dose is apparent.

SUMMARY

The dose-mortality curve for whole body irradiation of white mice has been determined over the range from 250r to 200,000r. The dose mortality curve obtained can be divided into several dose ranges and may be connected with different mechanisms of damage. Irradiation of rats and guinea-pigs showed that the dose effect curves were similar to that found for mice. With very high doses the survival time is decreased to times which correspond practically to instant death.

The mortality curve in the dose range of approximately 1,000r to 15,000r shows a plateau where life span of the irradiated animals remains independent of the dose. It could be shown by irradiation with lead-shielding of part of the body only that this dose range contains three different constant survival times, corresponding perhaps to three different mechanisms of action : (*a*) 3·5-day effect (whole body irradiation and trunk irradiation), (*b*) 5-day effect (irradiation of the central trunk region), (*c*) 7-day effect (irradiation of the head).

By irradiation of different parts of the body through a slit we could localize the effects mentioned above, in certain regions of the body. Since irradiation of certain localized strips of 5mm width were sufficient to cause these effects without irradiation of the other parts of the body.

The irradiation of animals from which single organs had been removed before irradiation and irradiation of some organs *in situ* have shown that the adrenal and the pituitary glands are of decisive importance in regard to the development of the 3·5-day and the 7-day effect respectively. In the cases of the adrenalectomized and hypophysectomized animals no dose-independent ranges were found and survival time was strongly dose dependent. Irradiations of these organs alone (exteriorized or *in situ*) did not decrease survival time significantly. Apparently not only injury of the organ itself but also disturbances of the functional systems in which it participates are necessary for the lethal effects to occur. Treatment with cortisone of the

adrenalectomized animal restored the 3·5-day effect. The irradiation of other organs (single and combined : liver, spleen, kidney, intestine) showed a special role for the liver. The irradiation of the splenectomized animals did not result in a change in the 3·5-day effect. Treatment with penicillin and streptomycin also did not influence the 3·5-day effect. After *in vitro* irradiation with high doses, examinations of blood ('prothrombin time') and measurements of tissue respiration were carried out.

To clarify the 3·5-day effect the irradiation intensity was changed or the dose was fractionated. While change in dose rate showed no effect, a significant difference was found if the same dose was divided in two parts.

REFERENCES

[1] Rajewsky, B. 'Naturforschung und Medizin in Deutschland 1939–1946 (Fiat-Review of German Science 1939–1946)' Bd. 21 *Biophysik* Teil I. Dieterich'sche Verlagsbuchhandlung, Wiesbaden, 1948.

[2] Heuse, O. *Z. angew. Physik*, 1953, **5** 361.

[3] Ph. Miller, C., Hammond, C. W., Tompkins, M. and Shorter, G. *J. Lab. clin. Med.* 1951, **38** 331, and 1952, **39** 462.

[4] Rajewsky, B., Heuse, O. and Aurand, K. *Z. Naturf.* 1953, **8b** 157.

[5] Rajewsky, B. and co-workers. *Z. Naturf.*, in press ; *Strahlentherapie*, in press.

[6] Wilhelm, G. *Strahlentherapie*, in press.

[7] Römer, H. and Beller, F. K. 7. International Congress of Radiology, Copenhagen 1953.

[8] Rajewsky, B. and Inouye, K. *Naturwissenschaften*, 1937, **25** 540.

[9] Aurand, K. *Strahlentherapie*, in press.

[10] Aurand, K. and Panly, H. *Z. Naturf.* 1954, **9b** 506.

[11] Panly, H. *Strahlentherapie*, in press.

DISCUSSION

M. V. Haigh : In work with Dr. Edith Paterson at the Christie Hospital, Manchester, on whole body irradiation of Rhesus monkeys, we also find evidence for a so-called 'protective effect' of an initial dose of X-ray followed by a second dose at varying intervals later. In this time factor work, our L.D. 50 (30 days) is determined by the probit method. For single session irradiation a series of animals were irradiated at 450 r, 500 r, 550 r, 600 r, and 650 r respectively and the L.D. 50 was found to lie between 500 r and 550 r. 20 animals being used in these latter two groups.

In the 'time factor' experiments the initial dose of X-ray was fixed at 260 r (approximately $\frac{1}{2}$ L.D. 50 for the single session). One group of monkeys then received a second dose of X-rays seven days later and the total dose of X-rays was then found to lie between 700 r and 750 r at L.D. 50 levels. Thus the complementary dose to the initial 260 r at 7-day interval is lower than the L.D. 50 dose for single session. This shows that at 7 days the animals had not recovered from the initial 260 r, and it has not shown a 'protective' effect. A second group of monkeys given an initial dose of 260 r were irradiated to L.D. 50 levels after an interval of 20 days. The L.D. 50 was found to lie between 850 r and 900 r (on the probit graph at 880 r) and here the complementary dose of X-rays is 880 r — 260 r = 620 r which exceeds the L.D. 50 level for single session. Here the animals have recovered from their initial 260 r and are apparently protected from a higher dose than they would have survived in single session. This has also been shown to be true in mice* The work has not been extended beyond the 20-day interval so that we have no evidence as to whether after 30 days the protective effect of the initial dose is not any longer apparent.

* Paterson, Gilbert, Matthews. *Brit. J. Rad.* XXV No. 296, Aug. 1952.

ACTION OF IONIZING RADIATIONS ON CELL CONSTITUENTS

Maurice Errera

Laboratoire de Morphologie animale, Université libre de Bruxelles

In order to understand the mechanisms of the physical effects of radiations, it is important to distinguish between primary and secondary effects. From a biochemical or biological standpoint, these must not be confused with *initial* (or *immediate*) and *late* effects which occur through a chain of yet unknown events. Effects observable in a biological system after very short delays may be tentatively classified as *initial*. Amongst them, retardation and inhibition of mitosis, chromosome breakages, mutations, have been observed very shortly after irradiation, but often these effects become more conspicuous if some time or several cell generations elapse before observation : on the other hand unless very high dosages are used killing effects are usually observable only after several cell divisions. Whether these fundamental effects are related to one another is still an open question and the answer will depend on whether it is possible to ascribe them to some common initial biochemical step. The search for cytological damage will help to identify the cellular components liable to be altered initially : both damage to the nucleus (chromosome breakage or stickiness, altered morphology of the nucleolus) or to the cytoplasm (swelling, vacuolization, change in staining of mitochondria) have been observed.

Attacking the problem from the other end, physical chemists and biochemists have usually looked for radiation effects on purified cellular components treated *in vitro* (effects on lipids, proteins, polysaccharides or even more simple molecules). Unfortunately nothing proves that cell components behave in a similar way outside and inside the cell, where they often belong to complex structures and where they are in close contact with numerous protecting agents.

In our opinion, it is of fundamental importance to look for effects on cellular components which can be demonstrated *immediately* after irradiation : the longer one waits, even if smaller dosages are used, the more numerous will be the compounds liable to be altered by secondary biochemical reactions—small initial biochemical alterations (which as we have seen may be direct or indirect for the physicist) may lead to important secondary effects and it will become more and more difficult to trace the chain of reactions induced by the radiations. We do not want to minimize the importance of these secondary effects, which may have considerable biological consequences, but for the biophysicist or the biochemist they will hardly help to solve the fundamental mechanisms one is looking for.

It is *a priori* difficult to predict which cellular compounds will be altered when irradiated *in vivo*, and which will be the biological consequence of such alterations : our knowledge of ' key ' constituents is still very limited. So

far radiobiological work has been of great importance to analyse the possible mechanisms of action of radiations and one is just beginning to uncover the possible mechanisms of primary steps. Unfortunately this has led to very little information concerning the cellular constituents whose alterations produce biological effects.

(1) *Effects on proteins irradiated* in vivo

Physical chemists may study the action of radiations on various chemical or physical properties of proteins, if these are treated pure or in solution. This is not possible when the same constituents are irradiated *in vivo* : methods of purification are not yet adequate and biochemical properties of proteins must be relied on : enzymes may fortunately be studied a very short time after irradiation of whole organisms in rapidly prepared extracts or homogenates : we believe that effects on enzymes found immediately after the irradiation of a variety of different cells or organisms would be of fundamental importance.

Barron and his co-workers studied the effects of X-rays on several enzymes in solution and were able to distinguish between two classes of differently susceptible enzymes : those whose activity depends on reduced —SH groups and which are inactivated with high ionic yields (adenosine triphosphatase, hexokinase, succinic dehydrogenase, triose phosphate dehydrogenase) and those whose inactivation needs a higher expenditure of energy (such as *d*-amino-acid oxidase, carboxypeptidase, trypsin) and whose activity is not dependent on —SH groups[9]. This scheme is certainly not a definite one, for ribonuclease, classified as radio-resistant by Barron, has recently been shown to be dependent on —SH groups[51a]; and the relative difficulties to oxidize these groups by ionizing radiations remains to be explained.

But —SH enzymes are within the cell in close contact with reducing agents like glutathione, ascorbic acid or other types of protecting agents and their oxidation *in vivo* by ionizing radiations, although highly probable, has not been proved to be of direct importance for the cell. The importance of Barron's contribution is to have found specific chemical changes responsible for biochemical effects and to have shown that these effects may be of great biological importance[10]. Enzymes have, however, other chemical groups indispensable for their biochemical effectiveness and these may also be susceptible to radiations : this chapter of enzymology is still in its infancy and only a few active groups of a limited number of enzymes are known so far. Free carboxyl groups are needed for the activity of insulin and lyzozyme, some free phenol or indole groups as well as amide or guanidyl groups are needed for both these enzymes and also for trypsin ; S—S groups are required for insulin whilst free amino groups are needed for lyzozyme[32]. Most of these groups are known to be susceptible to chemical effects of ionizing radiations and it is probable that if radiation effects on specified protein side groups were better understood one might be able to deduce, as Barron did for —SH groups, which ones of these other groups are most important. Research of this type might lead to the discovery of other protective mechanisms than those already found.

We have summarized in *Table I* what is known of the effect of ionizing radiations on different individual enzymes. On examination of these data,

Table I.—Effects of ionizing radiation on individual enzymes

Enzyme	*Dosage* r	*Tissue*	*Effect observed*	*Author-reference*
Adenosine triphosphatase	640	spleen	increase	Ashwell & Hickman[5]
	100–800	rat thymus	negligible	Thomson *et al*[72]
	—	kidney, spleen, liver	none	Fisher *et al*[29]
Arginase	500	rat, liver	none	Ludewig & Chanutin[54]
		embryonic tissue	slight inhibition	Holmes[41]
Carboxylase	72,000	yeasts	none	Aldous & Steward[2]
Catalase	500	rat liver	none	Ludewig & Chanutin[54]
	800	mouse liver	inhibition of activity begins 2 mins. after irradiation	Feinstein *et al*[28]
	100–500	mouse liver	inhibition possibly due to serum catalase inhibitor	Kazuo Mori *et al*[46]
	72,000	yeasts	none	Aldous & Steward[2]
	48,000	growing barley coleoptyle	slight rise (irradiated *in vitro*)	Forssberg[31]
	55,000	Brown Pierce carcinoma	slight rise (irradiated *in vitro*)	
	83,000	rat liver, Jensen sarcoma	slight rise (irradiated *in vitro*)	
Cathepsin	L_{50}	rat, mouse liver	increase (perhaps due to destruction of inhibitor)	Ballin & Feinstein[7]
Choline oxidase	200–800	rat liver	none	Kunkel & Phillips[49]
	600		none	Dinning *et al*[18]
				Roth *et al*[63]
Dehydrogenase :				
alcoholic	72,000	yeast	none	Aldous & Steward[2]
lactic	—	rat kidney	none	Fisher *et al*[29]
		liver-spleen	none	
malic	800	rat thymus	negligible	Thomson *et al*[72]
succinic	800		negligible	
	400–800	rat liver	none	Le May[52]
	20,000	sea urchin sperm	inhibition	Barron *et al*[10]
Succinoxidase	—	rat liver, kidney	none	Fisher *et al*[29]
	—	rat spleen	inhibition reversed by SH	
Triosephosphate	640	spleen homogenate	none	Hickman & Ashwell[40]
Deoxyribonuclease	500	rat liver	slight inhibition	Douglas *et al*[19]
		spleen	increase from 4 to 24 hours	
	500,000	tetrahymena	none (in homogen. immediately after irradiation)	Eichel & Roth[24]
Esterase	500	rat liver	none	Ludewig & Chanutin[50]
Hexokinase	72,000	yeast	slight inhibition	Aldous & Steward[2]
	500	spleen, liver kidney	slight increase	

Table I. Effects of ionizing radiation on individual enzymes—continued

Enzyme	*Dosage* r	*Tissue*	*Effect observed*	*Author-reference*
Phosphatase (alkaline)	500	rat, thymus, liver	increase during first day	Ludewig & Chanutin[50]
		rat liver	none	Ludewig & Chanutin[50]
	600	rat liver	temporary decrease 6 first hours	Petrakis *et al*[59]
	600	rat intestine	increase in nuclei and nucleoli of Lieberkuhn crypts	Ross & Ely[62]
		bone marrow	permanent inhibition for dosages >1500 r	Woodard & Spiers[76]
Ribonuclease	500,000	tetrahymena	none	Eichel & Roth[24]
	600	rat liver	initial rise followed by drop	Roth *et al*[63a]
Rhodanese	500	rat liver	none	Ludewig & Chanutin[51]
Transaminase	1,500	rat heart	none	Brin[16]
		rat duodenum	inhibition after a few days	Brin[16]
		rat liver	rise after 2–5 days	Brin[16]
		rat spleen	inhibition	Brin[16]
Tryptophane peroxidase	1,000	rat liver	rise after a few hours (not if adrenalectomized)	Thomson & Mikuta[73]
Xanthine oxidase	600	rat liver	no initial action followed by rise	Roth *et al*[63a]
Zymase	72,000	yeast	slight drop	Aldous & Steward[2]

one immediately sees that, when irradiated *in vivo*, most proteins studied so far are very radio-resistant. A same enzyme, in different tissues, may show a variety of responses to irradiation according to the experiment and even —SH enzymes like adenosine triphosphatase, hexokinase and ribonuclease do not appear to be inactivated by dosages which initiate profound biological damage.

(2) *Energy sources*

If instead of individual enzymes, complete chain reactions like glycolysis, respiration or oxidative phosphorylations are investigated, more promising results are obtained. Classical experiments of Crabtree and Gray[17] have shown that irradiation of rat retinal tissue with 1,000 r inhibits the formation of lactic acid without altering respiration. Holmes[41] discovered that lactic dehydrogenase was more radio-sensitive in tissues of young embryos than in more mature organisms. Hickman and Ashwell obtained similar inhibitions in mouse spleen homogenates after total body irradiation[40]; but triose phosphate dehydrogenase (an —SH enzyme) was not affected. The block seems to result from the alteration of an adenine nucleotide phosphorous acceptor and such inhibitions of glycolysis may be related to the fact that liver glycogen increases after irradiation, even in fasting animals (McKee[58], Ross and Ely[63]).

Respiration also responds in some instances to irradiation. The most systematic studies were performed by Barron who showed a decrease in the respiration of irradiated tissue slices and grasshopper eggs. This effect was attributed to the radio-oxidation of the —SH groups of succinic dehydrogenase (Barron, Gasvoda and Flood[10].) Similar results were obtained on liver respiration by Marsili and Paleotti[57]. However Le May[52] did not find any inhibition of succinoxidase, succinic-dehydrogenase or cytochrome oxidase, and Dubois also found no inhibition[20]. In some cases, as reported by Billen, Stapleton and Hollaender, respiration of *E. coli* is even increased after irradiation[14].

An effect of the Krebs cycle seems nevertheless apparent after *in vivo* irradiation of mice injected with radioactive glucose : there is a marked decrease in the radioactive CO_2 produced (Hevesy and Forssberg[38]). However all tissues do not respond similarly : $C^{14}O_2$ production is increased immediately after irradiation in the bone marrow (Altman, Richmond and Salomon[3]). Dubois and Kochran[20] have, on the other hand, shown an inhibition of citric acid synthesis in bone marrow. These results indicate that, at least in some tissues, the large cytoplasmic granules (mitochondria) have been affected ; this has also been shown cytochemically by Janus green staining[64]. This is also supported by the numerous instances of inhibition of phosphorylation. Ashwell and Hickman[5] showed that spleen homogenates of irradiated animals synthesize less energy rich *P* bonds, although their succinoxidase is not affected. This inhibition increases during the first 3 days after irradiation, but may in some instances be demonstrated as early as one hour after irradiation (Potter and Bethell[60]). Van Bekkum obtained similar results in spleen[74], but in *E. coli* no inhibition of phosphorylations seems to occur, although one does find a leakage of ATP into the medium[14] and Florsheim found no inhibition of phosphorylations in mouse brain[30].

These inhibitions may be the result of the inactivation of a single enzyme of the chain of reactions if it exists in limiting amounts and if no protecting agent is available at the time of irradiation, as has already been pointed out by Boell[15]. Thus most of these complex systems may be, but are not always (it may depend on the tissue or experimental conditions), inhibited by irradiation.

Very stimulating experiments by Sherman[65, 66] may throw some light on these discrepancies. If yeasts are grown on a medium rich in nitrogen, one finds no effects of X-rays on glycolysis ; but if these organisms are grown on a synthetic medium poor in nitrogen, dosages of about 30.10^3 r will be inhibitory. This may mean that competitive constituents have been eliminated by the low nitrogen diet and the enzymes of glycolysis become more exposed.

(3) Synthesis of lipids, proteins and nucleic acids

As we have just seen, radiation effects on isolated proteins or on energy yielding systems do not appear to be of constant importance : in some cells the activity of these systems may even be increased after irradiation. It was therefore necessary to look for effects on systems of a greater complexity still.

Inhibition of protein synthesis or of incorporation of amino acids into proteins does not appear to be of crucial importance either : many instances where these are not immediately affected have been described as can be

Table II. Effects of ionizing radiations on lipid and protein metabolism

	Dosage r	*Effect observed*	*Author-reference*
I PROTEIN METABOLISM			
Nitrogen fixation in Azotobacter		inhibition	Whelden *et al*[75]
Incorporation of glycine-^{14}C (rat, rabbit)	500–800	no effect in spleen and intestine	Abrams [1]
Incorporation of alanine-^{14}C		inhibition after 2 hours increase after 4 days	Hempelman *et al*[35]
Synthesis of haemoglobin (rabbit)	800	increased in spleen in bone marrow, initial rise in hemin followed by drop ; no alteration of globin metabolism	Richmond, Altman & Salomon[61]
	—	increased in reticulocytes	Bacq *et al*[6]
Incorporation of ^{14}C-acetate (mice)	880	increased in brain, liver, plasma	Hevesy & Dreyfus[39]
Antigen formation—			
anti red cell antibody (rabbits)	700	inhibition from first day	Taliaferro *et al*[70]
incorporation of ^{35}S in antibodies	—	inhibition	Stevens *et al*[69]
antibody formation (rabbit)	—	inhibition, not as marked if spleen and appendix are shielded	Jacobson[43]
Adaptive enzymes—			
lactase of *E. coli*	20,000	no effect	Yanovsky[77]
maltozymase and galactozymase (yeast)	365,000	no inhibition but slight increase in the lag period	Baron *et al*[8]
hydrogenlyase (resting *E. coli*)	60,000	complete inhibition (no effect on preformed enzyme)	Billen & Lichstein[13]
galactozymase	48,500	no inhibition	Brandt, Freeman & Swenson[16a]
II LIPID METABOLISM			
Fatty acid synthesis bone marrow (rabbit)	800	immediate 2 – 3-fold increase	Altman, Richmond & Salomon[3]
Acetate incorporation (mice)	880	increased	Hevesy & Dreyfus[39]
^{32}P incorporation in liver slices (rat)	1,000	increased	Entenman & Weinman[25]

seen from *Table II*. Synthesis of adaptive enzymes has been inhibited only in a few cases by X-rays, although such an affect is usual with ultraviolet rays. Synthesis of adaptive enzymes is probably related to the presence of

cytoplasmic cell particles. If the cells are made to divide in the absence of the adaptive substrate SPIEGELMAN[68] has calculated that these particles would be diluted between the daughter cells and eventually lost. It would be of interest to investigate if the synthesis of adaptive enzymes is more sensitive to irradiation after a culture of micro-organisms has gone through rapid cell divisions : it would furthermore give an experimental check to Spiegelman's attractive hypothesis.

Concerning fatty acids synthesis, one has not found any inhibition by radiation. As this biochemical activity is performed by mitochondria, one must conclude that their sites, responsible for fatty acid synthesis, are not radio-sensitive. It would be of interest, as we have seen that oxidative phosphorylation which is also a mitochondrial activity, may be inhibited, to study different enzymes bound to mitochondria and see if the ones presumed to be bound to the same class of particles undergo inactivation simultaneously or not.

Experiments on the synthesis of nucleic acids have given more encouraging results and an inhibition of the synthesis of deoxyribonucleic acid (DNA) has constantly been found in irradiated cells, whereas inhibition of ribonucleic acid synthesis or turnover is somewhat less marked or synthesis may even be increased. This is apparent from the work of HEVESY[37], HOLMES[42], ABRAMS[1], FORSSBERG[31a], KELLY[47, 48], BACQ[6] and others and from some of the work performed on bacterial viruses (LATARJET[50]).

As we have pointed out earlier, the inhibition of DNA synthesis may be the result of one of the following causes :

(*a*) Inhibition of the synthesis of DNA precursors : this mechanism seems improbable because the synthesis of mononucleotides in yeast or liver cells is not inhibited (SHERMAN and FORSSBERG[67]). Similar results obtained with *E. coli* with ultra-violet light point to the same conclusion : various low molecular weight derivatives of DNA begin to accumulate immediately after irradiation when the synthesis of DNA is blocked (KANAZIR[44]). These data indicate that the block in DNA synthesis probably operates at later stages of this chain reaction.

(*b*) DNA metabolism could become abnormal as a consequence of the incorporation into the normal chain of reactions of an unusual precursor which could be formed by the effects of irradiation on some building block. Many instances of the incorporation of chemical analogues of nitrogen bases have been described in the case of RNA metabolism in mice, bacteria or viruses, and one knows also that abnormal ribosides may be formed by exchange enzymatic reactions (FRIEDKIN[33]).

(*c*) Several authors have looked for immediate physical or chemical effects of radiations on DNA irradiated *in vivo* or *in situ*. The earliest of these attempts was made on nucleated erythrocytes : the gels obtained with the nuclei of irradiated cells are less rigid than those of controls and one knows that the rigidity of these gels depends upon the presence of ' intact ' DNA[21, 22]. More recently LIMPEROS and MOSHER[53] found that the DNA extracted from thymus of irradiated mice, immediately after irradiation, contained a decreased purine to pyrimidine ratio, ' depolymerization ' being observable only 24 hours later. HARRINGTON and KOZA[34] found that the affinity for methyl green of the nuclei of irradiated embryos was diminished

after 24 hours. These experiments indicate a possible effect on DNA or on the nucleoproteins.

However EULER and HAHN[26] found no alteration of physical-chemical properties of deoxyribonucleoproteins extracted from irradiated thymus nuclei ; but unfortunately no quantitative recovery was attempted ; also at the time of the experiment the methods of extraction were perhaps not so gentle as they are now. ANDERSON[4] has recently shown that the high radio-sensitivity of DNA was a test for its degree of depolymerization, which decreases very rapidly in homogenates. More recently, KAUFMAN[45] found no decrease in the affinity for methyl green of the nuclei of irradiated onion root tips but there is an increase in affinity for fast green and the author thinks this is probably the reason for the smaller degree of swelling of irradiated cell nuclei when they are placed in trypsin solution.

It must be emphasized that in all these data, tending to show that DNA or nucleoproteins may be affected a very short time after irradiation, most of the evidence so far obtained is very indirect. One method which might give interesting results would be to compare the radio-sensitivity (based on chemical or physical tests) of DNA irradiated dry and at low temperature where indirect effects are reduced to a minimum, *in vitro* and in organisms like bacteria. One must not forget that at least for DNA, biological tests are much more sensitive to minute alterations of the molecule than physical-chemical ones. This appears clearly from the work of ZAMENHOF[18] on the stability of the transforming factor ; the study of the transforming activity of DNA extracted immediately after irradiation of the bacteria would indicate perhaps if an immediate alteration of DNA is to be found. Actually the results presented in this symposium by Ephrussi-Taylor and Latarjet indicate that such an immediate alteration does occur.

Some more direct evidence can be extrapolated from work done with bacteriophage. The inhibition of phage multiplication when infected bacteria are irradiated does seem to indicate that the phage itself is altered because normal phage is capable of multiplying in heavily irradiated bacteria. That unmodified DNA is necessary for phage multiplication is proved by an experiment of HERSHEY where phage is inactivated by the transmutation of ^{32}P atoms of its nucleotides[36].

Biological effects become apparent only where some cellular constituents are ' limiting ' : biochemical mutations in micro-organisms can be observed only when some important metabolic pathway is interrupted ; this occurs if some building block, normally synthesized by the enzyme lost as a consequence of the mutation, is not available for the cell. In more complex organisms it is possible that a chain of reactions may be blocked by radiations in one area but that the products continue to be supplied from another site which is less radio-sensitive. In this case no radiation effect may be observed. To produce observably biological, biochemical, or biophysical lesions the conditions must be such that the factor studied is ' limiting '. The experiments on glycolysis of Sherman, mentioned previously, support this view.

The influence of the ' ploidy ' of a cell on its radio-sensitivity is another illustration of this concept. LATARJET and EPHRUSSI[51] have observed that haploid yeasts are killed exponentially whereas if they are diploid the killing curve becomes sigmoid. A similar effect was found by ZIRKLE and TOBIAS[79] :

In the case of the haploid strain, the alteration of a single genetically indispensable ' site ' is sufficient to obtain a killing effect ; two such ' sites ' must be destroyed in the case of a diploid strain. The same explanation might be given to the radio-resistance of the B/r strain of *E. coli* as compared to the wild B type.

CONCLUSION

As a conclusion to this discussion, the only clearly apparent immediate effect of ionizing radiation appears to be on DNA metabolism, perhaps through some alterations of the structure of DNA or nucleoprotein itself.

This does not necessarily mean that the DNA-containing cell constituents are more sensitive to ionizing radiations than others, but effects on DNA constituents may perhaps be more conspicuous because of their controlling role on cellular processes and perhaps their greater specificity.

Conditions on which other cell constituents can be made limiting are not yet well understood. We mentioned the hypothesis of Spiegelman that micro-organisms grown in the absence of adaptive substrate lose their capacity of forming adaptive enzymes, perhaps through the loss of some specialized cell particle. Similarly, Lwoff observed that rapidly dividing *Euglena* finally lose their chloroplasts. It becomes conceivable that under certain conditions (starvation, rapid division, *etc.*) limiting conditions might be obtained and could help to explain some of the conflicting results discussed in this paper (effects on protein synthesis or respiration).

REFERENCES

[1] Abrams, R. *Arch. Biochem. Biophys.* 1951, **30** 90.
[2] Aldous, J. G. and Steward K. B. *Rev. canad. Biol.* 1952, **11** 49.
[3] Altman, K. I., Richmond, J. E. and Salomon, K. *Biochim. biophys. Acta*, 1951, **7** 460.
[4] Anderson, N. G. *Fed. Proc.* 1954, **13** 3.
[5] Ashwell, C. and Hickman, J. *J. biol. Chem.* 1953, **201** 651.
[6] Bacq, Z., personal communication.
[7] Ballin, J. C. and Feinstein, R. N. *Fed. Proc.* 1952, **11** 184.
[8] Baron, L. S., Spiegelman, S. and Quastler, H. *J. gen. Physiol.* 1953, **36** 631.
[9] Barron, E. S. G., Dickman, S., Munk, J. A. and Singer, T. S. *J. gen. Physiol.*, 1949, **32** 537.
[10] Barron, E. S. G., Gasvoda, B. and Flood, V. *Biol. Bull., Wood's Hole*, 1949, **97** 144.
[11] Barron, E. S. G. and Seki, S. L. *J. gen. Physiol.* 1952, **35** 865.
[12] Barron, E. S. G. and Johnson, Ph. *Arch. Biochem. Biophys.* 1954, **48** 149.
[13] Billen, D. and Lichstein, H. C. *J. Bact.* 1952, **63** 533.
[14] Billen, D., Stapleton, G. E. and Hollaender, A. *J. Bact.*, 1953, **65** 131.
[15] Boell, E. J. *J. cell. comp. Physiol.* 1952, *Suppl.* **2** 19.
[15a] Brandt, C. G., Freeman, P. J. and Swenson, P. A. *Science*, 1951, **113** 383.
[16] Brin, M., *Fed. Proc.* 1952, **11** 190.
[17] Crabtree, H. G. and Gray, L. H. *Brit. J. Radiol.* 1939, *N.S.* **12** 39.
[17a] Crabtree, H. G. *Biochem. J.* 1951, **29** 2334.
[18] Dinning, J. S., Meschian, I., Keith, C. K. and Day, P. L. *Proc. Soc. exp. Biol.* 1950, **74** 776.
[19] Douglass, C. D., Fellas, V. M., Meschian, I. and Day, P. L. *Fed. Proc.* 1954, **13** 201.
[20] Dubois, K. P. and Cochran, K. W. *Proc. Soc. exp. Biol.* 1951, **76** 422.

[21] Errera, M. *Cold Spr. Harb. Symp. quant. Biol.* 1947, **12** 60.
[22] Errera, M., *Ann. Soc. Sc. méd. nat. Brux.* 1951, **5** 65.
[23] Errera, M. et Herve, A. *Mécanisme de l'Action biologique des Radiations.* 1951, Liege, Desoer ; Paris, Masson.
[24] Eichel, H. J. and Roth, J. S. *Biol. Bull., Wood's Hole*, 1953, **104** 351.
[25] Entenman, C. and Weinman, E. O. *Fed. Proc.* 1952, **11** 44.
[26] Euler, H. v. and Hevesy, G. *Kgl. dansk. Vid. Selsk. Biol. Med.* 1942, **17** 3.
[27] Euler, H. v. and Hahn, L. *Acta radiol. Stockh.* 1946, **27** 269
[28] Feinstein, R. N., Butler, C. L. and Hendley, D. D. *Science*, 1950, **111** 149.
[29] Fisher, M. A., Purvis Coulter, E. and Costello, M. J. *Proc. Soc. exp. Biol.* 1953, **83** 266.
[30] Florsheim, W. H., Doerbach, C. and Morton, M. E. *Proc. Soc. exp. Biol.* 1952, **81** 121.
[31] Forssberg, A. *Ark. Kemi*, 1945, **21A** no. 7.
[32] Fraenkel Conrat, J. and H. *Biochim. biophys. Acta*, 1950, **5** 89.
[32a] Fraenkel Conrat, H. *Arch. Biochem. Biophys.* 1950, **27** 109.
[33] Friedkin, M. *Fed. Proc.* 1952, **11** 216.
[34] Harrington, N. J. and Koza, R. W. *Biol. Bull., Wood's Hole*, 1951, **101** 138.
[35] Hempelman, L. H., Carr, S., Franz, I. D., Masters, R. and Lamdin, E. *Fed. Proc.* 1950, **9** 183.
[36] Hershey, A. D., Kamen, M. D., Kennedy, J. W. and Gest, H. *J. gen. Physiol.* 1951, **34** 305.
[37] Hevesy, G. *Advanc. Enzymol.* 1947, **7** 111.
[38] Hevesy, G. and Forssberg, A. *Nature, Lond.* 1951, **168** 692.
[39] Hevesy, G. and Dreyfus, D. *Ark. Kemi*, 1952, **4** 337.
[40] Hickman, J. and Ashwell, G. *J. biol. Chem.* 1953, **205** 651.
[41] Holmes, B. E. *Biochem. J.* 1935, **29** 2285.
[42] Holmes, B. E. *Brit. J. Radiol. N.S.* 1949, **22** 487.
[43] Jacobson, L. O., Robson, M. J., Marks, E. K. *Proc. Soc. exp. Biol.* 1950, **75** 145.
[44] Kanazir, D. and Errera, M. *Biochim. biophys. Acta*, 1954, **14** 62.
[45] Kaufman, B. P., McDonald, M. R., Gay, H., Rowan, M. E. and Moore, E. C. *Yearb. Carnegie Instn.* 1951, **50** 203.
[46] Kazuo Mori, Seiko Momoki and Hisashi Ito. *Igaku to Seibutzaku*, 1951, **18** 303 ; *Chem. Abstr.* 1951, **45** 9580.
[47] Kelly, L. and Jones, H. B. *Proc. Soc. exp. Biol.* 1950, **74** 493.
[48] Kelly, L. and Payne, A. *Fed. Proc.* 1952, **11** 256.
[48a] Klein, G. and Forssberg, A. *Exp. Cell. Res.* 1954, **6** 211.
[49] Kunkel, H. O. and Phillips, P. H. *Chem. Abstr.* 1951, **45** 9580 ; *Arch. Biochem. Biophys.* 1952, **37** 366.
[50] Latarjet, R. *J. gen. Physiol.* 1948, **31** 529.
[51] Latarjet, R. and Ephrussi, B. *C.R. Acad. Sci., Paris*, 1949, **229** 306.
[51a] Ledoux, L. *Biochim. biophys. Acta*, 1953, **11** 517.
[52] Le May, M. *Proc. Soc. exp. Biol.* 1951, **77** 337.
[53] Limperos, G. and Mosher, W. A. *Amer. J. Roentgenol.* 1950, **63** 681.
[54] Ludewig, S. and Chanutin, A. *Arch. Biochem. Biophys.* 1950, **29** 441.
[55] Ludewig, S., Chanutin, A. and Leutz, E. A. *Amer. J. Physiol.* 1950, **163** 648.
[56] Lwoff, A. C.N.R.S., colloques internat., *Unités biologiques douées de continuité génetique.* 1948, Hermann, Paris.
[57] Marsili, G. and Paleotti, M. *Bol. Soc. ital. biol. sperim.* 1952, **28** 1269.
[58] McKee, R. W. *Fed. Proc.* 1952, **11** 256.
[59] Petrakis, N. L., Ashler, F. M., Ferkel, R. L., *Naval Radiol. Dep. Lab. Rep.* AD 126 B ; *Chem. Abstr.* 1950, **44** 2581.
[60] Potter, R. L. and Bethel, F. H. *Fed. Proc.* 1952, **11** 270.
[61] Richmond, J. E., Altman, K. I. and Salomon, K. *J. biol. Chem.* 1951, **190** 817.

[62] Ross, M. H. and Ely, J. O. *Amer. J. Roentgenol.* 1949, **62** 723.
[63] Ross, M. H. and Ely, J. O. *J. cell. comp. Physiol.* 1951. **37** 163.
[63a] Roth, J. S., Eichel, H. J., Wase, A. W., Alper, C. and Boyd, B. J. *Arch. Biochem. Biophys.* 1953, **44** 95.
[64] Scherer, E. and Ringelb, D. *Strahlentherapie*, 1953, **90** 34.
[65] Sherman, F. G. *Fed. Proc.* 1951, **10** 125.
[66] Sherman, F. G. *Experientia*, 1952, **8** 429.
[67] Sherman, F. G. and Forssberg, A. *Arch. Biochem. Biophys.* 1954, **48** 293.
[68] Spiegelman, S., De Lorenzo, W. F., Campbell, A. M. *Proc. nat. Acad. Sci. Wash.* 1951, **37** 513.
[69] Stevens, K. M., Gray, I., Schwartz, M. S., Lichter, R. J., Highland, G. P. and Dubois, K. G. *Amer. J. Physiol.* 1953. **175** 141.
[70] Taliaferro, W. H., Taliaferro, L. G. and Janssen, E. F. *Science*, 1952, **115** 487.
[71] Thompson, C. B. *Amer. J. Physiol.* 1952, **170** 731.
[72] Thomson, J. F., Tourtelotte, W. W. and Cattar, M. S. *Proc. Soc. exp. Biol.* 1952, **80** 268.
[73] Thompson, J. F. and Mikuta, E. T. *Proc. Soc. exp. Biol.* 1954, **85** 29.
[74] Van Bekkum, D. W., Jongepier, H. J., Nieuwekerk, H. T. M. and Cohen, J. A. *Brit. J. Radiol.* 1954, *N.S.* **27** 127.
[75] Whelden, R. M., Enzman, E. V. and Haskins, C. P. *J. gen. Physiol.* 1941, **24** 789.
[76] Woodard, H. Q. and Spiers, F. W. *Brit. J. Radiol.* 1953. *N.S.* **26** 38.
[77] Yanofsky, Ch. *J. Bact.* 1953, **65** 383.
[78] Zamenhof, S., Griboff, G. and Marullo, N. *Biochim. biophys. Acta*, 1954, **13** 459.
[79] Zirkle, R. E. and Tobias, C. A. *Arch. Biochem. Biophys.* 1953, **47** 282.

RELATIVE BIOLOGICAL EFFICIENCY OF FAST NEUTRONS AND GAMMA RADIATION FOR CHRONIC IRRADIATION OF MICE

R. H. Mole, R. J. Munson and G. J. Neary

Radiobiological Research Unit, Medical Research Council, Harwell

A graphite pile at A.E.R.E. Harwell has been used for daily irradiation of mice with fast neutrons substantially free from gamma radiation. Sterilization of male and of female mice and a reduction in the weight of the testis have provided sensitive indicators of radiation damage. Dose-response curves for fast neutrons and cobalt gamma radiation have been found to be very similar : there has been no evidence so far of any qualitative difference. Relative biological efficiency factors lay between 3 and 8 for exposures ranging from 24 hours to 5 months : there are definite differences between different strains of mouse. An experiment to determine the relative biological efficiency for lethal effects of low daily irradiation has been started but no results are yet available.

DISCUSSION

L. Ehrenberg : The authors found the relative biological efficiency of neutrons to vary between different mouse races, and the reason for this has been attributed to different sensitivities to gamma rays. I want to point out that this result is similar to those obtained in plant material : several physiological factors have an influence on the sensitivity to sparsely ionizing radiations, but not on the sensitivity to fast neutrons. This difference between the two types of radiations is probably due to a different mechanism of action.

REMARKS CONCERNING SULPHYDRYL PROTECTION AGAINST MAMMALIAN RADIATION INJURY

HARVEY M. PATT

Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois

THE modification of radiation effects by chemical agents has been and continues to be an important focus in radiobiological investigation. We have learned that a number of substances have the capacity for modifying a variety of responses to ionizing radiations. Viewed in an historical perspective, a number of landmarks appear along the way, but it is perhaps sufficient for our purpose simply to recall the early interest and accomplishment in this area. Armed with some understanding of the radiolysis of water and cognisant of its potential contribution to the development of radiation injury in plants and animals, present investigators have greatly extended the possibilities for modification of radiation effects. The list of chemicals that protect against one or another radiation effect ranges from sugar to alcohol, from the familiar epinephrine to the esoteric para-aminopropiophenone. It includes sodium nitrite, cyanide, and pitressin as well as cysteine, glutathione, cysteinamine and other amines. These substances are by no means equally effective and, no doubt, differ somewhat in their mode of action. The more recent developments have been reviewed elsewhere[1]. We may note in passing, however, the first definitive experiments on the oxygen effect by THODAY and READ[2,3] and the chemical protective effects in animals described by our own group[4, 5] and by CHAPMAN[6, 7] BACQ[8, 9] and their collaborators.

While certain of the facts of chemical modification are reasonably well established, the mechanisms underlying the facts are frequently obscure or at best only circumstantial. We may define chemical protection for our purpose as the reduction of a given response by administration of a suitable agent prior to irradiation. One is inclined generally to interpret protective phenomena of this sort in radiochemical rather than in biological terms. This implies, as in the case of anoxia, that the protection is due, for the most part, to a decrease in the biological effectiveness of the radiation rather than to a basic change in the sensitivity of the responding system. We will be concerned here mainly with the effects of cysteine which, in certain respects at least, may be thought of as a characteristic example of this type of protection. Other pharmacological agents that are related to oxygen availability in one form or another probably also fall into this classification. This may obtain even for the paradoxical cyanide, which protects some species but not others, potentiates effects on isolated tissues and is evidently neutral to suspensions of isolated cells[1].

For the sake of completeness it should be noted that there is another category of modifying factor, one that is concerned apparently with the events responsible for injury to specific physiological systems or to recovery from such injury. Tissue shielding and transplants, pretreatments with phenylhydrazine, foreign protein, oestrogens and other hormones are examples of these rather specific procedures.

There is reason to believe that protection by suitable sulphydryl substances, *e.g.* cysteine, is a fairly general phenomenon and that both destructive and regenerative processes may be modified. This is apparent particularly when protective effects can be quantitated in terms of radiation dose–biological response parameters. Although the desirability of such evaluation is perhaps obvious, it is worthy of emphasis in view of inferences that are sometimes drawn from single dose determinations or semi-quantitative measurements. Under appropriate experimental conditions, cysteine and other sulphydryl substances have been shown to diminish many of the effects of gamma and X-irradiation, *e.g.* lethality of bacteria, isolated cells and animals, chromosome breakage in plant cells, mitotic block in corneal epithelium, decreased nucleic acid turnover, enzymic changes in spleen and thymus, lenticular opacities, epilation, splenic atrophy, leucopenia and anaemia[1, 10]. Cysteine affords a rather uniform protective action against lethality, splenic involution, lymphopenia and granulocytopenia in the intact animal[5] and the killing of thymocytes and ascites tumour cells *in vitro*[11].

It is worth noting that d and l cysteine are equally effective in mice but that all sulphydryl substances are not protective to animals, perhaps because of differences in their biological fate. The latter is a complicating factor, not infrequently overlooked, which must enter into interpretation of *in vivo* findings. The action of cysteine and β-mercaptoethylamine in mice appears to be similar[12]. The latter is more efficient but is also more toxic than the former. It is of interest that maximally tolerated doses of each give equivalent protection against acute radiation lethality ; suboptimal amounts seem to be completely additive. Further indication of their similarity of action is apparent also from enzyme protection studies. As shown by Petersen and DuBois[13] prior administration of cysteine or β-mercaptoethylamine in roughly equimolar amounts has a comparable effect on the radiation-induced increase in adenosine triphosphatase and 5-nucleotidase activities of spleen and thymus.

The degree of protection is a function of cysteine dose and is independent of radiation dose, at least within certain limits. The effect is manifest, therefore, by a change in slope of the radiation dose-response curve. Results obtained in mice with a split-exposure procedure, in which a standard dosage of cysteine was given between two radiation fractions or a proportional amount preceded each fraction, suggest that (*1*) irradiation just prior to cysteine administration does not influence the protection against subsequent exposure ; (*2*) the lethal potential of small dosages of radiation cannot be reversed by cysteine ; and (*3*) the protective action of two suitably spaced cysteine injections is additive[5]. Essentially similar relationships have been found for the oxygen effect in plants[14]. Unlike the picture with animals, there is a definite, although lesser, protective effect when cysteine

is added to a thymus lymphocyte suspension immediately after X-irradiation[15]. This can be attributed only in part to the persistence of toxic substances in the medium. Differences in the time course of protection of various systems may be related perhaps to differences in the kinetics of reactions with cysteine and in the time constants for development of irreversible injury. Thymus cells suspended in the test tube represent a fairly sluggish system in which oxygen is rate limiting for the disappearance of cysteine. On the other hand, the systems most responsible for acute lethality of the living animal are those with a rapid turnover in which the chances for intersection of a chain of events are limited accordingly. The possibility exists that some *in vivo* responses may be amenable to prompt post-irradiation modification by chemicals of this type.

Of considerable interest is the fact that cysteine protection in mice is an inverse function of ionization density, being less for fast neutrons than for gamma rays or X-rays[16]. This finding parallels the oxygen effect in irradiated systems and is supported by other data[11]. It may be stated that cysteine does not protect thymus lymphocytes in the absence of oxygen[15]. The resistance of packed thymocytes and the failure of cysteine to protect them may be attributed to their hypoxic state. Cells equilibrated with oxygen before packing by centrifugation appear to be as sensitive as cells in suspension and are readily protected. This is interpreted as evidence for a cellular site of action which is implicated also from studies with tumour fragments. It should be noted that there is a rapid uptake of oxygen and an increase in lactic acid when cysteine is added to the thymic cell suspension. These data suggest perhaps that its action is related to, although not necessarily the direct consequence of, the availability of intracellular oxygen. Potentiation of the protection of the intact animal by suitable dosages of dinitrophenol or sodium nitrite or by 10 per cent oxygen[17], its inhibition by high oxygen tensions[18], and the finding of an increase in the arteriovenous oxygen difference after cysteine injection[19] may also be thought of as supportive evidence for this interpretation. It should be remarked that the oxygen content of mixed venous blood does not necessarily reflect the situation in various loci : hence the failure to detect a change with β-mercaptoethylamine[20] does not exclude a possible role of oxygen in the protective mechanism. It may be noted also that the dosages used were considerably below those required for protective effects in other species.

Certain parallels have been drawn between oxygen poisoning and X-irradiation and may signify a common primary mechanism of action through oxidizing free radical formation as suggested by GERSCHMAN *et al*[21]. It may be noted in particular that some substances that are protective against X-ray injury are also effective against oxygen poisoning. It is well to recall that the time course of the two syndromes differs widely unless many thousands of roentgens are used. This may reflect differences in concentration and distribution of the toxic intermediates produced in the two situations, although other interpretations are possible and perhaps more likely.

It appears that mechanisms other than the immediate oxidative reactions induced by activated water may also be involved in this form of protection against X-radiation. It has been observed, for example, that protection of thymocytes in suspension by cysteine is dependent upon temperature during

the first 30 to 60 minutes after irradiation[11]. A brief period of chilling at 2°C immediately after X-ray exposure completely reverses the effect. Temperature dependence of cysteine action before irradiation has also been shown. The latter may be thought of perhaps in terms of a decreased rate of reaction with oxygen in the cold. An explanation of the post-irradiation temperature dependence for cysteine protection of thymus lymphocytes is not readily apparent, but it is clear that we must also inquire about the role of delayed and of chain reactions in the interpretation of these phenomena. The pathways for reaction or degradation of organic radicals formed during irradiation may depend upon oxygen availability in addition to other factors such as pH, temperature and the redox potential[22]. It is possible that decreased oxidation of cysteine during a critical post-irradiation period frees oxygen for reaction with chemical intermediates which might otherwise be directed into other channels. The results with thymocytes may be contrasted with chemical protective effects in bacteria. Several modes of action apparently prevail in the protection of the various systems, owing perhaps to differences in the nature of the chemical intermediates and their reactions, the time required for development of irreversible injury, and the relative contribution of the intra- and extra-cellular phases to the over-all effect.

Uniformity of the protection (dose reduction) by cysteine against a number of radiation sequelae in the intact animal suggests that the decisive action occurs at an early stage in the chain of events. It does not necessarily imply protection against all radiation changes since different primary mechanisms may be involved in their development. It should be emphasized that there is scant information concerning protection against the more chronic sequelae of irradiation, although one might suppose *a priori* that some effectiveness would also be manifest here. For the present, these modifying effects may be interpreted best in terms of the more immediate physicochemical ramifications of energy transfer. Protective chemicals of this sort may alter the effective radical concentration (*e.g.* of OH or HO_2) either directly or indirectly by relative depletion of oxygen. Effects on chain reactions initiated by the primary interactions may also be involved. The experimental findings in general are consistent with such assumptions and there is little basis at the moment for postulating a more selective *in vivo* protection of one or another biochemical entity or physiological mechanism by this class of chemicals.

Reference has been made from time to time to the possibility of a primary attack of ionizing radiation on sulphydryl groups generally[23]. More recently, it has been suggested that inactivation of Coenzyme A may be of importance in the initiation of cellular damage by irradiation[24]. There is now ample evidence that sulphydryl enzyme inactivation is neither a selective nor a uniform process *in vivo*[1, 10]. The possibility that effects of this sort may occur in areas where —SH groups are few and their inactivation is critical cannot be ruled out, however. It should be noted that there is no evidence for an over-all destruction of Coenzyme A by whole body X-irradiation. As shown by THOMSON and MIKUTA[25], rats given 1000r retain their capacity to acetylate p-aminobenzoic acid and sulphanilamide for at least 3 days after exposure. While it is true that CoA levels in the

lens are decreased as cataract develops, this is a delayed and not an immediate effect of X-irradiation[21]. Evidence for a post-irradiation effect of β-mercaptoethylamine in the liver shielded animal, perhaps suggestive of a biochemical locus, is, at best, conflicting[12, 26]. No doubt, various biochemical and physiological states can profoundly affect radio-sensitivity. Protection of mammalian systems with chemicals such as cysteine and β-mercaptoethylamine appears, however, from the preponderance of circumstantial evidence, to be related mainly to an effect on energy transfer mechanisms rather than to a specific effect on one or another biological determinant of injury or recovery.

REFERENCES

[1] Patt, H. M. *Physiol. Rev.* 1953, **33** 35.
[2] Thoday, J. M. and Read, J. *Nature, Lond.* 1947, **160** 608.
[3] Thoday, J. M. and Read, J. *ibid*, 1949, **163** 133.
[4] Patt, H. M., Tyree, E. B., Straube, R. L. and Smith, D. E. *Science*, 1949, **110** 213.
[5] Patt, H. M., Mayer, S. H., Straube, R. L. and Jackson, E. M. *J. cell. comp. Physiol.* 1953, **42** 327.
[6] Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Cronkite, E. P. and Chambers, F. W., Jr. *Radiology*, 1950, **55** 865.
[7] Cronkite, E. P., Brecher, G. and Chapman, W. H. *Milit. Surg.* 1951, **109** 294.
[8] Bacq, Z. M., Herve, A., Lecomte, J. and Fischer, P. *Science*, 1950, **111** 356.
[9] Bacq, Z. M. and Herve, A. *Bull. Acad. roy. Med. Belg.* 1952, **17** 13.
[10] Patt, H. M. *Annu. Rev. Physiol.* 1954, **16** 51.
[11] Patt, H. M. *Ann. N. Y. Acad. Sci.* 1954, in press.
[12] Straube, R. L. and Patt, H. M. *Proc. Soc. exp. Biol. Med.* 1953, **84** 702.
[13] Petersen, D. F. and DuBois, K. P. *Fed. Proc.* 1954, **13** 394.
[14] Read, J. *Brit. J. Radiol.* 1952, **25** 336.
[15] Patt, H. M., Blackford, M. E. and Straube, R. L. *Proc. Soc. exp. Biol. Med.* 1952, **80** 92.
[16] Patt, H. M., Clark, J. W. and Vogel, H. H., Jr. *ibid*, 1953, **84** 189.
[17] Mayer, S. H. and Patt, H. M. *Fed. Proc.* 1953, **12** 94.
[18] Salerno, P. R. and Friedell, H. L. *ibid*, 1953, **12** 364.
[19] Salerno, P. R. and Friedell, H. L. *Radiation Res.* 1954, in press.
[20] Charlier, R. *Proc. Soc. exp. Biol. Med.* 1954, **86** 290.
[21] Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P. and Fenn, W. O. *Science*, 1954, **119** 623.
[22] Selected papers: 'The Chemistry of Biological After-Effects of Ultraviolet and Ionizing Radiations.' *Brit. J. Radiol.* 1954, **27** 36, 117.
[23] Barron, E. S. G. *Symposium on Radiobiology*, p. 216 Ed. by J. J. Nickson. Wiley, New York, 1952.
[24] Bacq, Z. M., Dechamps, G., Fischer, P., Herve, A., Le Bihan, H., LeComte, J., Pirotte, M. and Rayet, P. *Science*, 1953, **117** 633.
[25] Thomson, J. F. and Mikuta, E. T. *Proc. Soc. exp. Biol. Med.* 1954, **86** 487.
[26] Maisin, J. H., Lambert, G., Mandart, M. and Maisin, H. *Nature, Lond.* 1953, **171** 971.

LE MÉTABOLISME DE LA CYSTÉAMINE

W. G. Verly*, G. Koch et S. Grégoire

Laboratoires de Pathologie Générale et de Biochimie ; Université de Liége

La β-mercaptoéthylamine ou cystéamine est une des molécules qui forment le coenzyme A, le catalyseur des acylations biologiques et de la réaction de condensation du groupe acétyle activé avec l'oxaloacétate pour former du citrate, première étape du cycle de Krebs. La cystéamine ne semble pas être utilisée pour la biosynthèse de la pantéthéine ou du coenzyme A ; elle apparaît, *à l'état combiné*, au cours de la biosynthèse de ces substances par décarboxylation d'un résidu cystéine. De la cystéamine libre pourrait se former dans les tissus par hydrolyse du coenzyme A ou de la pantéthéine ; Gregory et Lipmann ont trouvé un enzyme qui catalyse cette réaction d'hydrolyse dans le foie de certains oiseaux.

Bacq a montré que 3 mg de cystéamine, injectés dans le péritoine, protègent des souris de 20 g contre 700 r de rayons X, dose mortelle pour 100 pour cent des animaux non protégés.

De la cystéamine marquée avec du ^{35}S a été préparée par Rayet et Urbain.

3 mg de cette cystéamine ont été injectés dans le péritoine de souris de 20 g et l'on a étudié la persistance du soufre radioactif dans le corps de la souris en fonction du temps après l'injection, et la distribution du ^{35}S entre les organes.

Le ^{35}S, par gramme de tissu, est plus élevé dans le foie, les reins et l'intestin (+ pancréas) que dans le reste du corps[1].

24 heures après l'injection, 34 pour cent du ^{35}S se trouvent encore dans le corps de la souris[1]. La protection contre le rayonnement X observée après l'administration de la *même* quantité de cystéamine à une souris de *même* poids est de très courte durée ; la plus grande partie de la radioactivité présente dans l'organisme 24 heures après l'injection ne se trouve vraisemblablement plus dans des molécules de cystéamine.

On a dosé le ^{35}S présent dans des molécules de cystéamine et de cystamine (β : β'-diaminodiéthyl-disulfure) dans les tissus de la souris après injection de la même dose de ^{35}S-cystéamine[2]. 24 heures après l'injection, 2 pour cent seulement de la cystéamine se trouvent intacts—ou sous forme de cystamine—dans le corps de l'animal. La moitié de la cystéamine marquée disparaît des tissus en 40 minutes environ.

La presque totalité du ^{35}S présent dans l'organisme 15 minutes après l'injection de 3 mg de cystéamine marquée à une souris de 20 g, se trouve dans des molécules de cystéamine ou de cystamine ; 24 heures après l'injection, on trouve encore 34 pour cent du soufre radioactif injecté, mais 2 pour cent seulement se trouvent dans des molécules de cystéamine et de cystamine. Il semble que, pendant les premières minutes, la disparition de la ^{35}S-cystéamine soit due principalement à l'excrétion urinaire de ^{35}S-cystéamine-

* Associé du Fonds National Belge de la Recherche Scientifique.

cystamine ; dans la suite, la perte de cystéamine marquée est due davantage à un catabolisme dans les tissus ; le rein élimine à ce moment un mélange de ^{35}S-cystéamine-cystamine et de catabolites marqués.

Dans le but d'identifier les produits du catabolisme de la cystéamine, on a injecté dans la veine fémorale d'un chien de 7 kg, 104 mg de cystéamine marquée avec du soufre radioactif[3]. En 8 heures, 16 pour cent du ^{35}S injecté ont été excrétés dans les urines ; 4 pour cent se trouvent dans des molécules de cystéamine ou de cystamine. La cystéamine-cystamine radioactive est surtout excrétée pendant les deux premières heures ; le quatrième échantillon d'urine analysé (6–8 heures après l'injection) n'en contient pratiquement plus. On peut conclure que, des 104 mg de cystéamine injectés à ce chien de 7 kg, 4 à 5 pour cent seulement ont été excrétés sous cette même forme dans l'urine.

Une fraction importante du ^{35}S urinaire se trouve dans des molécules de sulfates ; on observe également une excrétion de taurine marquée. Eldjarn a aussi observé la transformation en taurine et sulfates de la cystamine donnée au rat, au lapin et à l'homme.

Cystéamine-cystamine, taurine et sulfates ne suffisent pas pour rendre compte de la totalité du ^{35}S excrété dans l'urine ; il existe d'autres métabolites marqués qui n'ont pas encore été identifiés.

REFERENCES

[1] Verly, W. G., Bacq, Z. M., Rayet, P. et Urbain, M. F. *Biochem. biophys. Acta*, 1954, **13** 233.

[2] Verly, W. G., Grégoire, S., Rayet, P. et Urbain, M. F. *Biochem. J.*, 1954, **58** 660.

[3] Verly, W. G. et Koch, G. *ibid*, 1954, **58** 663.

STUDIES ON THE MECHANISM OF RADIATION PROTECTION AND RECOVERY WITH CYSTEAMINE AND β-MERCAPTOETHANOL*

ALEXANDER HOLLAENDER and C. O. DOUDNEY

Biology Division, Oak Ridge National Laboratory, Tennessee

IN an effort to elucidate the mechanism involved in the protection of living material from X- and gamma radiation, a comparison was made of the protective efficiency of cysteamine (β-mercaptoethylamine) and β-mercaptoethanol on *Escherichia coli* B/r. The latter compound was chosen for study

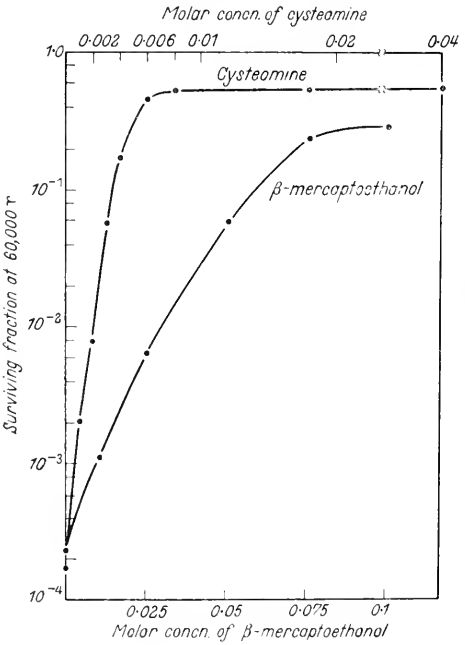


Figure 1. The effect of cysteamine and β-mercaptoethanol on the survival of E. coli *B/r during exposure to* 60 kr *of* 250-kVp *X-rays*

because it resembles cysteamine very closely in structure and in chemical properties.

The effect of concentration on protection against X-ray damage to *E. coli* B/r at 60 kr for these two compounds is depicted in *Figure 1* (upper scale, cysteamine ; lower scale, mercaptoethanol). Cysteamine reaches its maximum protective level at 0·006 M, and the plateau for mercaptoethanol is about 0·1 M.

* Work performed under Contract No. W-7405-eng-26 for the U.S. Atomic Energy Commission.

Figure 2 shows a comparison of the protective ability of the two compounds on *E. coli* irradiated in a balanced salt solution at 2° C. More than 99·9 per cent of the irradiated bacteria are killed at 60 kr, but in cysteamine at the same dose, only about half of the bacteria are killed.

With increasing energy in excess of 60 kr the protective ability of cysteamine decreases markedly and reaches another level of protection at about 90 kr. At submaximally protective concentrations of cysteamine the decline in protective ability comes at correspondingly lower energy levels. However,

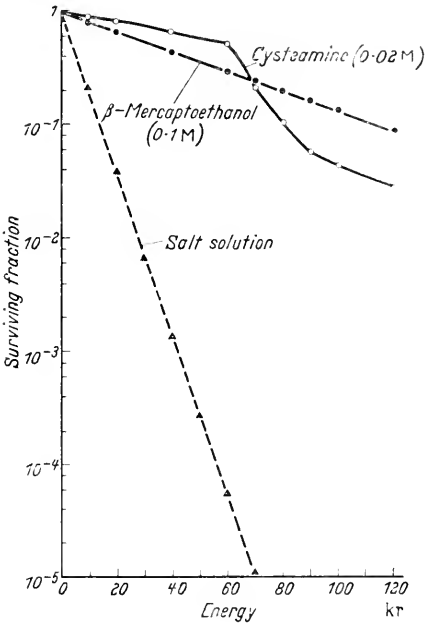


Figure 2. The effect of cysteamine and β-mercaptoethanol on survival of X-irradiated E. coli *B/r*

even at comparatively low concentrations of cysteamine (0·002 M), a very high initial level of protection is maintained. In contrast to the peculiar survival curve obtained with cysteamine, mercaptoethanol protects linearly with increasing energy and fails to show a change in its protective ability at higher energy levels. Of all the compounds tested, cysteamine is the only one which produces this peculiar response to radiation. Up to 60 kr, the dose reduction factor (DRF) for cysteamine is about 12, falling to about 6 at 90 kr. Mercaptoethanol shows a DRF of about 8. Up to 60 kr, the addition of mercaptoethanol or any other protective compound to cysteamine either at maximally or submaximally protective concentrations does not add to the level of protection obtained. This applies also to oxygen removal. In excess of 60 kr, mercaptoethanol, as well as other protective compounds, is additive to cysteamine protection. Also, at these energy levels oxygen removal adds to the protective effect of cysteamine.

Cysteamine in the presence of phosphate ions loses much of its protective ability, the DRF being changed from 12 to about 6 in its presence. We believe that phosphate interferes in some way with the mechanism by which

cysteamine protects the bacterial cells since increasing the concentration of cysteamine many times over that of phosphate within the maximally protective concentration range of cysteamine (0·006 to 0·02 M) does not increase protection. A number of interesting aspects of the interference of phosphate with cysteamine protection are under investigation.

Studies in our laboratory have shown that *E. coli* B/r grown aerobically in nutrient broth will recover from X-ray damage to a considerable degree if plated out on agar containing either spleen, beef, or yeast extract[1]. Although time is too limited to discuss all the comparative studies carried out in our laboratory on the nutritional requirements for recovery of *E. coli*, we wish to point out that a synthetic medium containing—in addition to inorganic salts and glucose—glutamine, uracil, and guanine will support recovery of broth-grown *E. coli* equivalent to that obtained with natural extracts. This medium is given in *Table I.* We have found that cysteamine protection

Table I.—Composition of medium II

Component	*Amount*
L-Glutamic acid or glutamine or aspartic acid	150μg
Guanine	30μg
Uracil	30μg
Salts A (K_2HPO_4, 25g ; KH_2PO_4, 25g ; H_2O to make 250ml)	1 ml
Salts B ($MgSO_4.7H_2O$, 10g ; NaCl, $FeSO_4.7H_2O$, and $MnSO_2.4H_2O$, 0·5g ; H_2O to make 250 ml)	1 ml
Glucose	2g
Agar	3·4g
Water	200cc

(From Stapleton, Sbarra and Hollaender[1])

is limited if X-irradiated, broth-grown bacteria are not supplied, after irradiation, with some nutrient medium. Here again beef, yeast, or spleen extract will give a high level of survival, whereas, on minimal medium, survival is markedly reduced. The difference in survival on the two types of media is about one thousandfold at 90kr. If cysteamine-protected bacteria are plated on minimal agar the DRF is changed from 12 to about 2. *E. coli* protected by mercaptoethanol do not show this striking effect. Here the DRF is reduced only from 8 to 6 if the bacteria are plated on minimal plates instead of nutrient plates. We have shown that marked recovery of cysteamine-protected cells is not dependent on added nutritional factors of growth at suboptimal temperature. Maximum recovery occurs at 18° C. This compares[2] with recovery at suboptimal temperatures of unprotected *E. coli* B/r.

Our first thought in trying to explain the peculiar protection curve demonstrated with cysteamine, was that radiation must affect the cysteamine either directly or indirectly. This was tested by irradiating the cysteamine before it was added to the bacterial suspension. There was practically no effect of X-radiation up to 120kr in modifying the curve obtained for a given concentration of cysteamine. This, of course, does not rule out some effect of X-rays on the compound in interaction with the bacterial cell to explain the peculiar curves obtained.

A number of other conditions involving cysteamine protection of *E. coli* have been tested. Cysteamine-protected, irradiated *E. coli* suspended in yeast extract for only 15 minutes, then removed by centrifugation, washed carefully, and plated out on minimal agar plates recover as if they were plated on yeast extract plates. In other words, the stimulus to recovery from yeast extract is transferred to the bacteria within a few minutes. Another test was made to see whether the cysteamine in the suspending medium is important or whether the cysteamine goes into the bacterium and exerts its protective effect inside the cell. For this purpose, bacteria were kept in cysteamine for 30 minutes, then washed off with salt solution and resuspended before irradiation. The data show that the cysteamine must have exerted its effect inside the bacterial cell since, in spite of careful washing, the cells remain highly resistant to the lethal effects of radiation as compared to the control.

The problem of mechanism of protection has also been approached by studying the effectiveness of cysteamine modified structurally in various ways[3]. When both the thiol and amine groups were covered with other groups, it was found that the data obtained with bacteria in regard to derivatives of cysteamine do not necessarily apply to the protection of mice. For instance, S,β-aminoethylisothiouronium bromide HBr protects mice exceedingly well against the lethal effects of irradiation. As a matter of fact, this compound has many advantages over cysteamine for protection of mice. However, this compound actually increases the killing effect of radiation on bacteria. For example, at 60 kr only about 1/100 as many cells survive in the presence of this compound as in its absence. One could speculate that actively metabolizing mammalian tissue could break this compound down into a form which is protective. *E. coli* in the resting-cell state, as we study them (*i.e.* in a salt solution at 2° C), are not able to do this. Perhaps this compound in its intact form competes in some manner with compounds or for sites responsible for part of the innate radiation resistance of the cell. In contrast, the acetylated derivatives of cysteamine offer small protection for mice. However, both the S-acetyl and N-acetyl derivatives of cysteamine will protect *E. coli* to a very high level. There is no obvious reason why these latter two compounds should not protect mice as well as the thiouronium compound. The N,S-diacetyl derivative of cysteamine will protect *E. coli* only if incubated with the organisms at 37° C for 30 minutes prior to irradiation. It is apparent that we cannot conclude from experiments with bacteria that the results are directly applicable to mammals. We believe, however, that a fuller understanding of the details of the mechanism of cysteamine protection in bacteria will aid in defining both the pre- and post-irradiation conditions which may lead to more successful control of radiation damage to mammals.

REFERENCES

[1] Stapleton, G. E., Sbarra, A. J. and Hollaender, A. 1955, to be published.

[2] Stapleton, G. E., Billen, D. and Hollaender, A. *J. Cellular Comp. Physiol.* 1953, **41** 345.

[3] Doherty, D. G. and Burnett, W. T., Jr. *Abstr. of Papers, 126th Natl. Meet. Amer. Chem. Soc.* 1954, p. 9C.

DISCUSSION ON PAPERS BY PATT, VERLY AND HOLLAENDER

Z. M. Bacq : I fully agree with Hollaender that the metabolic actions of cysteamine and cystamine are very important. The differences between cysteamine and cysteine on which we have often insisted are the following :

Cysteamine, just like all amines, cannot be directly incorporated in proteins or in coenzyme A. Cysteamine depletes the liver of its glycogen[1] and the suprarenals of their ascorbic acid[2] ; it liberates in the blood several reducing substances of which only ascorbic acid has been identified[3]. Cysteine has none of those actions.

I am not surprised that, as Hollaender showed, any change in the structure of cysteamine decreases its protective power. We have established both in mice[4] and in a synthetic polymer *in vitro*[5] that the structure which favours chelation also favours protection against ionizing radiation. If one substitutes anything to one or several hydrogens in cysteamine, one decreases the chelating power of the molecule. We have observed that S-methyl, S- or N-mono or diacetyl cysteamine are inactive or less active[5] ; the acetyl derivatives are still active because they are hydrolized in

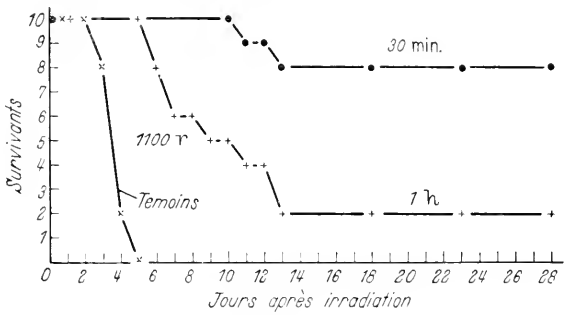


Figure 1

the body and liberate cysteamine. Even the physiological N-pantothenyl cysteamine (=pantetheine, a growth factor for micro-organisms and a fragment of CoA) is inactive[5, 6].

We think [5] that the general mechanism of action of the radioprotection in aqueous systems is that of a competition for free radicals rather than an energy transfer, but this is a theoretical discussion. We do agree with Patt, that the protection occurs at a very early stage, and is quantitatively the same. The observed differences can be accounted by the fact that cysteamine when injected to mammals does not distribute itself equally in all tissues. The spleen, the bone marrow concentrate it (Eldjarn*) ; the liver destroys it actively (Verly), the testes on the contrary are at a much lower level than the blood (Eldjarn). We also believe that anoxia is not a possible component of the protection by cysteamine, because cysteamine protects dog's reticulocytes *in vitro*[7], *i.e.* cells containing haemoglobin saturated with O_2, and

* L. Eldjarn presented a paper the details of which will be published elsewhere :

(1) Eldjarn, L. and Nygaard, O. 'Cysteamine-cystamine : Intestinal absorption, distribution among various organs and excretion.' *Arch. internat. de Physiol.*, in press.

(2) Nygaard, O. and Eldjarn, L. 'Cysteamine-cystamine : Effect on uptake and turnover of radioactive iodine by the thyroid gland in rat.' (To be published.)

(3) Shapiro, B. and Eldjarn, L. 'The effects of ionizing radiation on aqueous solutions of cysteamine and cystamine.' (To be published.)

(4) Shapiro, B. and Eldjarn, L. 'The possible mechanism for the decomposition of cysteamine and cystamine by ionizing radiation.' (To be published.)

that as large amounts as possible of cysteamine injected in dogs do not decrease the O_2 saturation of mixed venous blood taken in the right heart[8].

If as demonstrated by Patt, anoxia decreases the protective effect of cysteine, it is, in our interpretation, because cysteine competes for those free radicals (mainly HO_2) which are formed only in the presence of oxygen.

So far as the practical aspect is concerned, it seems that, at the present time, the best compromise between toxicity, oral activity and cost is cystamine[9]. This molecule $(CH_2—CH_2—S)_2$ has over cysteamine the advantage of chemical stability and oral activity. It protects 20 g mice even against 1,100 r (*Figure 1*) when irradiation begins 30 minutes after *ingestion* of 7 mg. The protective action is already marked 15 minutes after ingestion, reaches a maximum at 30 minutes and slowly declines : it has not completely disappeared 4 hours after ingestion. It is well tolerated by man (500 mg).

Harvey M. Patt : The data of Charlier, to which Bacq has referred, do not exclude a possible role of oxygen in the protection by β-mercaptoethylamine. In the first place, the dosage employed in the dog was considerably below the dosage requirement for a protective effect in mice. It is not known whether this dose will, in fact, protect dogs against acute radiation lethality. Secondly, it should be noted that the oxygen content of mixed venous blood is a gross over-all index and does not necessarily reflect the situation in various loci. In this connection, it is important to recall the rather selective distribution of β-mercaptoethylamine reported by Eldjarn.

Z. M. Bacq to Patt : On a weight basis dogs are much more sensitive to cysteamine than are mice ; this behaviour is not exceptional ; all big mammals are on weight basis more sensitive to drugs than small ones. Charlier was satisfied to confirm when taking blood on the right heart, that high doses of cysteine decrease markedly the oxygen content of venous blood while equiprotective doses of cysteamine (about the maximal tolerated dose) have no such effect. Admittedly we assume that the protective power of cysteamine compared to that of cysteine is the same in the dog as in the rat or mouse.

The introduction of cysteamine in blood in presence of O_2 does not change its colour, does not alter the O_2 content of red cells *in vitro*, but protects the reticulocytes against X-rays.

H. Betz to Bacq : Bacq has shown us that the injection of cysteamine produces a decrease of the ascorbic acid content of the adrenal cortex. I wonder whether there is a simultaneous drop of the cholesterol content. We have shown, with Van Cauwenberge[10] that in the case of the injection of sodium salicylate, the drop of cholesterol is a more sensitive test than the drop of ascorbic acid. If the injection of cysteamine produces a kind of alarm reaction, one would expect a decrease of both ascorbic acid and cholesterol. A drop of the vitamin C alone would rather suggest that cysteamine acts on the metabolism of the ascorbic acid.

Z. M. Bacq to Betz : We have observed[2] that cysteamine *increases* the cholesterol content of the suprarenals and does not change the number of circulating eosinophiles. Thus, we consider that cysteamine does not produce an alarm reaction but acts on the distribution of ascorbic acid in the body.

P. Mandel : A la suite des travaux du Bacq et suivant ses indications concernant les doses et conditions d'injections de la cystéamine, nous avons étudié[11] l'effet protecteur de ce composé contre une irradiation de l'animal entier de 700 r (*C.R. Soc. Biol.* 1953, **236** 2010). Nous avons constaté que la réduction de l'acide ribo et désoxyribonucléique de la moelle osseuse et de la rate est beaucoup plus atténuée chez les animaux ayant reçu avant l'irradiation de la cystéamine.

Mouton : Suite à la remarque de Bacq que le pouvoir protecteur des dérivés genre B'ecaptan disparaît lorsque le pouvoir de chelation disparaît, peut-on dire de façon générale que la protection *consiste* en une chelation des ions métalliques bivalents (p. ex. ; Cu^{++}) du milieu irradié ?

Z. M. Bacq : D'après les expériences du Alexander[5] sur la dégradation du polyméthacrylate par le rayonnement X en milieu oxygéné, l'on obtient les mêmes effets radiochimiques lorsque la teneur en cuivre du milieu est extrêmement réduite. Ce que l'on peut dire en tous cas, c'est que les groupements chimiques responsables du pouvoir protecteur sont aussi les groupements chimiques intéressant la chélation. En effet, si on ajoute assez d'ions Cu^{++} pour saturer le chélateur, on fait disparaître en même temps le pouvoir radioprotecteur.

J. A. V. Butler : When a radical reacts with an organic compound, it turns it into a radical, *e.g.*

$$RH + OH^{\oplus} \longrightarrow R^{\oplus} + H_2O ;$$

and reaction with another radical has to occur before the radical is abolished, *e.g.*

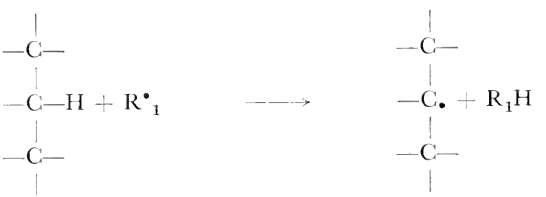
$$R^{\oplus} + OH^{\oplus} \longrightarrow ROH.$$

It would appear that effective protective compounds should be those which facilitate a double reaction involving two radicals. Chelating compounds which, as Bacq, Alexander and Fox pointed out, are very effective are those with two functional groups in close proximity and these are favourably constituted for such a double reaction to occur, *e.g.*

The advantage of a mixed amino-sulphydryl compound may be in the greater size of the S atom, which permits a closer contact of the two functional groups.

M. Magat : Je voudrais donner le point de vue d'un physico-chimiste sur le mode d'action des agents protecteurs du type de la cysteamine dans le cas d'action indirecte. Je pense que, si mon analyse est correcte, elle permettra de prévoir la nature des composés chimiques que l'on pourrait envisager comme des protecteurs possibles.

La plupart des réactions d'oxydation, de cracking *etc*, débutent de la manière suivante : un radical primaire $R^{\bullet}_1$ formé par un processus quelconque (dans notre cas par le rayonnement ionisant) arrache un atome, en général un atome d'hydrogène à la molécule M, en la transformant en un radical, selon le schéma :



Ce nouveau radical, disons un macroradical si nous pensons au DNA, peut subir une transformation, soit en se décomposant avec formation d'une liaison double ou d'un radical, soit par addition d'oxygène en formant un radical peroxydyle *etc*.

Dans d'autres cas, le radical primaire pourra attaquer une double liaison en formant un nouveau radical et s'y additionner conduisant ainsi à une polymérisation.

Le rôle du protecteur P consiste à empêcher le système de dépasser le stade de formation du radical $M^{\bullet}$. Ce but peut être atteint soit en réagissant préférentiellement à M avec le radical initial $R^{\bullet}$ selon la réaction :

$$R^{\bullet} + PA \longrightarrow RA + P^{\bullet}$$

à condition que $P^{\bullet}$ soit un radical relativement inerte, incapable de réagir avec M en lui enlevant un atome ou en ouvrant une liaison double.

Pour que la réaction $MH + P^{\bullet} \longrightarrow M^{\bullet} + PH$ ne se produise pas, il faut qu'elle soit endothermique, c'est à dire qu'il faut que l'énergie de liaison E(P—H)

soit plus faible que l'énergie de liaison E(M—H). Cette condition est assez facile à satisfaire, beaucoup plus facile que la condition que la réaction d'ouverture de double liaison soit endothermique. Il se trouve que pour les mercaptans, $E_{S-H} < E_{C-H}$, c'est à dire que les mercaptans cèdent facilement des atomes d'hydrogène à des radicaux possédant une valence libre sur un atome de carbone. Le radical RS$^{\bullet}$ est incapable d'arracher à son tour un atome d'hydrogène. Il peut par contre ouvrir une double liaison vinylique et amorcer une chaîne de polymérisation. Cette propriété des mercaptans est largement utilisée dans l'industrie du caoutchouc synthétique où l'on peut ainsi régler à volonté le poids moléculaire des chaînes. Si la cellule, ou la partie de la cellule qu'il s'agit de protéger ne possède pas de composés avec des liaisons vinylique, ce qui je crois est une hypothèse qu'il est permis de faire, le radical R'S$^{\bullet}$ ne pourra que réagir avec un autre radical, par exemple en donnant R'S—SR' ou R'SR.

Les mercaptans ne sont pas les seules substances satisfaisant à nos conditions, mais ils ont l'avantage de reconstituer la molécule M sous forme initiale, certains d'entre eux donnant des sous produits non toxiques (cystine par exemple).

Qu'est-ce qui détermine maintenant le choix de certains mercaptans, de préférence à d'autres ? Plusieurs critères interviennent probablement :

(*a*) le solubilité dans le milieu entourant les entités à protéger

(*b*) leur stabilité vis à vis du métabolisme

(*c*) la non toxicité des sous produits.

Bref, toute une série de critères que j'appèlerais biologiques.

En outre, certaines substitutions peuvent abaisser l'energie de liaison S—H et rendre l'hydrogène plus ' mobile '.

Evidemment, et je tiens à insister là-dessus, le mode de protection esquissé ci-dessus n'est probablement pas le seul. Je ne rappellerai que la protection par les thiocarbamates dont il a été question dans l'exposé de Fox. Dans ce cas on pourrait penser, entre autre, à une ' réparation ' de la molécule coupée, le soufre jouant le rôle d'une ' colle ' et formant un pont entre les deux fragments. En effet, dans l'industrie du caoutchouc, on utilise de tels composés comme accélérateurs de vulcanisation ou même comme agents vulcanisants ? Toutefois, nos connaissances sont actuellement trop limitées pour que cette suggestion soit autre chose qu'une hypothèse très hasardeuse.

P. Alexander : In our joint investigation with Bacq concerning the mode of action of substance which protects mice against the lethal effects of ionizing radiations we reached the conclusion that these substances function by competing for HO_2 radicals. We were very conscious of the fact that out of the hundred or more substances studied the active SH compounds could act as transfer agents in the way in which Magat describes. However, this property is not shared by all the protective substances and in a detailed series of experiments, which are summarized in the paper in this symposium on page 49, Fox and I showed conclusively that their biological action cannot be ascribed to their ability to act as transfer agents. It is obviously dangerous to pick on one property of SH— compounds, which are capable of undergoing many different types of chemical reaction, without considering the other active compounds. This is why we have always insisted that activity in model experiments can only provide information concerning biological mechanisms if the parallelism extends over many active compounds.

In my extensive studies on the effects of ionizing radiations on polymers with my colleagues Fox and Charlesby, we have found a number of systems where protection occurs by a transfer mechanism. In these systems different compounds are placed in a different order of effectiveness from that found *in vivo* or in the synthetic systems where protection occurs by competition for free radicals.

There is one other point in Magat's discussion on which I wish to comment and that is his suggestion that there is a general mechanism *via* peroxide formation by

which ionizing radiations degrade polymers. We set out hoping to find such a mechanism and after having studied the effect of X-rays on aqueous solutions of about eight chemically different polymers, we find that almost every one shows a different behaviour. Some degrade only in the presence of oxygen while others degrade equally readily in its absence ; still others crosslink to give gels. The whole picture is very complex and it is not possible to deduce a general mechanism involving oxygen from experiments with one material.

In particular the suggestion of Magat that DNA degrades according to this general pattern seems to be unfounded since the initial X-ray induced reaction is independent of oxygen. Further, both polystyrene in chloroform and DNA in water continue to degrade after the irradiation with X-rays is complete (after-effect) but the addition of an —SH compound immediately after irradiation hastens the decomposition of polystyrene (see paper by Fox, page 61) while Errera has shown that it reduces the ' after-effect ' of DNA. The same simple peroxide mechanism can obviously not apply to both systems.

H. B. Newcombe : In Magat's discussion, one of the points made by Patt appears to have been overlooked, namely that an effect can sometimes be demonstrated even when the chemical is added just after irradiation (*e.g.* in the case of cysteine and thymus leucocyte suspensions).

At this time, the free radicals would have disappeared and it must be supposed that the protection must occur either through reaction with the induced peroxides or through an effect on the post-irradiation metabolism of the cells.

R. Latarjet : L'effet final de la cystéamine est la résultante de divers processus dont les importances relatives dépendent des conditions expérimentales. En particulier, à l'action protectrice *précoce* peut succéder une action *tardive* en sens contraire. En effet, la cystéamine peut favoriser l'action radiomimétique des peroxydes radioformés. L'expérience suivante en fait foi, effectuée par Brinton dans mon laboratoire, conformément à une prévision théorique du Fox.

Des bactéries (E. coli, souche B) sont mises, pendant 10 min., en présence d'hydroperoxyde de cumène à la concentration de $1{,}5\ 10^{-4}$ à 37° C. On les soumet alors à une brève et violente illumination qui augmente beaucoup l'action toxique du peroxyde (preuve que celui-ci agit par ses produits de décomposition). On élimine alors le peroxyde par dilution, et on étale les bactéries sur gélose en présence de diverses substances. Après incubation, on dénombre les colonies bactériennes. Voici les résultats d'une expérience :

Substance ajoutée après le traitement par le peroxyde	*Nombre de colonies*	*Remarque*
Néant (témoin)	361 389	
Catalase	880 838	Restauration par action peroxydasique
Diethyldithiocarbamate	602 638	Restauration par stabilisation des peroxydes
Cystéamine	1 4	augmentation de l'effet toxique par décomposition des peroxydes

Cette expérience montre que, loin de protéger, la cystéamine peut agir, *après* l'irradiation, par un processus qui augmente très notablement l'action cytotoxique de certains intermédiaires chimiques produits dans les tissus par l'irradiation.

REFERENCES

[1] Bacq, Z. M. et Fischer, P. *Arch. Internat. Physiol.* 1953, **61** 417.
[2] Van Cauwenberge *et al.* *Arch. Internat. Physiol.* 1953, **61** 124.
[3] Fischer, P. et Pirotte, M. *Arch. Internat. Physiol.* 1954, **62** 76.
[4] Bacq, Z. M., Herve, A. et Fischer, A. *Bull. Acad. Méd. Belg.* 1953—VIe série t.XVIII, 226.
[5] Alexander, P., Bacq, Z. M. *et al.* *Radiation Res.*, in press.
[6] Mitchell, J. S. Personal communication. 1954.
[7] Bacq, Z. M., Nizet, A. et Herve, A. *Arch. Internat. Physiol.* 1952, **60** 449.
[8] Charlier, R. *Proc. Soc. Exper. Biol. Med.* 1954, **86** 290.
[9] Bacq, Z. M. *Bull. Acad. Méd. Belg.* 1953, VIe série, t. XVIII, 426.
[10] Van Cauwenberge and Betz, H. *Lancet*, 1952, **i**, 1083.
[11] Mandel, P. *et al.* *C.R. soc. Biol.* 1953, **236** 2010

OBSERVATIONS ON THE EFFECT OF SPLEEN-SHIELDING AND THE INJECTION OF CELL SUSPENSIONS ON SURVIVAL FOLLOWING IRRADIATION *

LEON O. JACOBSON, EDNA K. MARKS and EVELYN O. GASTON

Department of Medicine and the Argonne Cancer Research Hospital, The University of Chicago, Chicago, Illinois

OUR finding that spleen-shielding and spleen or embryo transplants enhance the survival of mice exposed to a lethal amount of total-body X-radiation (1025 r) led to some further experiments in which we demonstrated that a suspension of spleen cells injected intraperitoneally also increases the survival of mice exposed to 800 r[1, 2]. This latter observation was confirmed by COLE *et al*[3]. LORENZ[4] found that the survival of mice exposed to 900 r was significantly increased if the animals were given intravenous injections of bone marrow suspensions after X-irradiation. These findings together with the observations that liver-shielding or the injection of mashed embryo suspensions protected against radiation-induced mortality prompted us to investigate the effectiveness of suspensions of cells from the liver of adult, baby, and embryonic mice. In addition, since the results from some of the experiments varied because the suspensions of cells from the several sources (spleen, bone marrow, and liver) were not standardized, it seemed timely to determine the optimum number of cells from each of these tissues that would provide maximum survival and the minimum number of cells necessary to effect survival following lethal irradiation. Such information was needed to compare the 'potential' of cells from various tissues and to serve as a baseline for 'cell-type' studies of the various suspensions.

This report gives the observations that we have made thus far.

Observations

Bone marrow—LORENZ[1] aspirated bone marrow from four long bones (femora and radii) of mice by means of a 26-gauge hypodermic needle. The marrow was then suspended in buffered saline solution and given intravenously immediately after preparation to an irradiated mouse. We employed this technique in our earlier studies. The amount of marrow obtained under these conditions was found to vary considerably and thus the results were not consistent.

In the studies reported here, mice that were to receive bone marrow suspensions were exposed to 900 r total-body X-radiation. Bone marrow for the suspensions was obtained from 10- to 12-week-old mice. Saline or Locke's solution was used as the diluent. Cell counts were made according

* The contents of this paper have also been presented at the meetings of the 5th International Congress of Hematology, Paris, France, September 6–12, 1954.

Table I. A comparison of the effect of intravenous injections of various concentrations of bone marrow cell suspensions from 10- to 12-week-old *mice on the survival of mice exposed to* 900 r

Total No. of cells ($\times 10^6$)	*Mice* (*No.*)	*Time of death after X-rays* (days)																											*Survivors*	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	(*No.*)	(%)
20·0–40·0	20						1	2	1	1	2	1			1			1		1	1									8	40·0
15·0–19·9	43							8	1	4	1		1		1	1		1		1		1	3		1			2		17	39·5
10·0–14·9	133				2	5	6	15	5	5	3	2	4	1	4	2	2	2	2	1	1	1	1		3		3	3	1	3	56	42·9
5·0–9·9	182		1			2	2	8	8	3	3		6	3	5	3	2	1	1	4	2	6	2	3	2	2	5	3	3	2	100	54·9
1·0–4·9	111					1	8	12	12	6	4	3	7	3	2	2	2	2	1	1	1		1		2		2	1	1		37	33·3
0·137–0·975	60					1	6	13	11	4	3	1	3	3	2	3					2							1			7	11·6

Table II. Effect of bone marrow cell suspensions from 4- to 6-week-old *mice on the survival of mice exposed to* 900 r *whole-body X-radiation*

Total No. of cells ($\times 10^6$)	*Mice* (*No.*)	*Time of death after X-rays* (days)															*Survival*	
		5	6	7	8	9	10	11	12	13	14	15	16	17–24	25	26	27	28	(*No.*)	(%)
10–26	13		2		1			1				1			2		1		5	38·0
7–8	33		2			1	1	1							1		1		26	78·7
3–5	15	1			2	1	1		1				1						8	53·0

to routine laboratory methods to determine the number of nucleated cells per mm^3. Since the suspensions taken from individual mice varied from a maximum of 10×10^6 cells in $0{\cdot}5\,cm^3$ of diluent to a minimum of $0{\cdot}07 \times 10^6$ cells in $0{\cdot}5\,cm^3$ diluent, pooled marrow aspirations were made and the suspensions were standardized so that $0{\cdot}5\,cm^3$ contained the number of cells* we wished to administer intravenously to each irradiated mouse. The total number of nucleated cells injected per mouse used in these studies varied from $39{\cdot}0 \times 10^6$ to $0{\cdot}13 \times 10^6$.

Although survival was found to be enhanced with as few as $0{\cdot}5 \times 10^6$ cells per mouse, a suspension that provided each mouse with 5 to 10×10^6 cells proved to be the most effective (54·9 per cent survival) in decreasing mortality (*Table I*). With a suspension of 1 to $5{\cdot}0 \times 10^6$ cells per mouse, 33·3 per cent of the animals survived the 28-day period of observation. As the number of cells was decreased, the per cent survival decreased correspondingly ; only 11·6 per cent survived when less than 1×10^6 cells were given to each mouse, and none survived when less than 250,000 cells were injected. It must be emphasized that suspensions containing more than 10×10^6 cells in $0{\cdot}5\,cm^3$ were no more effective in enhancing survival than those containing 1 to 5×10^6 cells. Of 196 mice that received more than 10×10^6 cells, 81 or 41 per cent survived the 28-day period of observation. The incapacity of these concentrations to provide the protection afforded by lesser amounts is being studied.

The results described above were obtained with marrow from adult (10- to 12-week) mice. When young mice (4- to 6-week) were used as donors, the results were more gratifying since fewer cells were necessary to obtain comparable results. There was 78·7 per cent survival when 7 to 8×10^6 cells were given (*Table II*). Studies are now in progress in which mice that had been irradiated with lethal amounts of X-rays were given less than 3×10^6 cells and as few as 50,000 nucleated cells. The data suggest that survival is effectively enhanced even when the total number of nucleated cells falls below 1×10^6.

Effect of spleen-cell injections on survival of irradiated mice

Studies of the effect of cells from the spleen on the survival of irradiated mice were also carried out in a way that was similar to that described for the bone marrow suspensions. In the original experiments, the spleen cells were obtained by inflating the extirpated mouse spleen with saline or Locke's solution. The cells that were thus extruded were injected intravenously into mice that had been subjected to 900 r.

In later experiments, the spleens from adult mice were mashed gently in a mortar, or cut and pushed through a syringe, and suspended in Locke's solution. All of the cells that could be removed conveniently with a 26-gauge needle were used to make the suspension. In some instances, the cells were centrifuged and washed several times with Locke's solution before the final suspension was made. Since the spleens from baby mice are very fragile, it is necessary only to push them through a syringe to release the cells desired for injection, and centrifugation is not necessary.

* Cells refers throughout the paper to the nucleated cells counted in an acetic acid (2 per cent) dilution.

Table III. A comparison of the effect of intravenous injections of various concentrations of spleen cell suspensions from 2- to 5-day-old *mice on the survival of mice exposed to* 900 r

Total No. cells (× 10^6)	*Mice* (*No.*)	*Time of death after X-rays* (*days*)																				*Survival*	
		4	5	6	7	8	9	10	11	12	13	14	15–18	19	20	21	22	23	24	25	26	27	28	(*No.*)	(%)
8·5–10·9	10													1								2		7	70·0
5·0–6·6	4			1	1																			2	50·0
3·1–4·9	12				1		1	1								1		1						7	58·3
2·25–2·8	11																			1		1	1	8	72·7
0·5–0·75	30	1		2				1	2	1		1				1								21	70·0

Table IV. Comparative effect of spleen cell suspension from 2-day-old, 4- to 6-week-old, *and* 10- to 12-week-old *mice on the* 28-day *survival of mice exposed to* 900 r

Total No. of cells (× 10^6)	*Cells from* 2-day *donors*			*Cells from* 4- to 6-week *donors*			*Cells from* 10- to 12-week-old *donors*															
							Cells washed and heparin			*Cells washed no heparin*			*Unwashed cells no heparin*			*Cells extruded from spleen* †		
	Recipients (*No.*)	*Survivors* (*No.*)	(%)	*Recipients* (*No.*)	*Survivors* (*No.*)	(%)	*Recipients* (*No.*)	*Survivors* (*No.*)	(%)	*Recipients* (*No.*)	*Survivors* (*No.*)	(%)	*Recipients* *No.*	*Survivors* (*No.*)	(%)	*Recipients* (*No.*)	*Survivors* (*No.*)	(%)
56–63							40	5	12·5	15	4	26	2 *	0	0			
30–44				26	8	30	7	4	57	4	1	25	4 *	0	0			
19–30				22	10	45	15	0	0	10	1	10	4 *	0	0			
11–17				14	10	22	8	0	0	6	1	16				19	0	0
8–11	10	7	70	39	10	25				12	2	16				33	6	18
5–8				58	23	39				18	1	5				58	7	12
3–5	12	7	58	8	3	37				16	0	0	6	0	0	22	0	0
2–3	11	8	72·7	6	2	33				11	2	18				10	0	0
0·5–0·75	30	21	70															

* Died within few minutes after receiving injection.

† Cells obtained by inflating spleen with Locke's solution or saline

In the original work by JACOBSON *et al*[5] in which splenic implants were found to enhance survival, four spleens from baby mice were required to effect increased survival of adult irradiated mice. When spleen-cell suspensions were injected intravenously, however, the cells from only one spleen were sufficient to enhance survival in several irradiated mice. To correlate the results of these studies with those from bone marrow experiments, cell counts were made on the spleen-cell suspensions. When 8 to 11 $\times$ 10^6 nucleated cells from the spleens of 2-day mice were given intravenously, 70 per cent of the recipients survived the 28-day period of observation, with no deaths occurring before the 18th day after irradiation ; with 3 to 5 $\times$ 10^6 cells, 7 of 12 or 58 per cent survived ; with 2 to 3 $\times$ 10^6 nucleated cells, 8 of 11 or 71 per cent survived ; and with as few as 0·5 to 0·75 $\times$ 10^6 cells, 70 per cent survived. As indicated in *Table III*, survival was generally in the range of 50 to 70 per cent regardless of the total number of cells injected.

Cells obtained from adult spleen have thus far proved to be less effective than cells from the spleens of younger mice. Instantaneous deaths were frequent after the intravenous administration of cell suspensions containing 10 $\times$ 10^6 or more cells*. This toxic action can be overcome to a great extent if the adult spleen cells are washed thoroughly by centrifugation in Locke's solution and if a few drops of heparin are added to the suspension made from the washed cells just prior to injection. The data in *Table IV* provide a comparison of the effectiveness of spleen cells obtained from mice aged 2 days, 4 to 6 weeks, and 10 to 12 weeks. In the latter group, the effect of washing the cells and the addition of heparin are also shown.

Although spleen cells from adult mice had little, if any, effect on the survival of mice when 8 to 11 $\times$ 10^6 cells were given, 26 per cent of the irradiated recipients survived when 50 to 60 $\times$ 10^6 cells were injected intravenously. The addition of heparin did not influence the 28-day survival.

The effect of embryo cells on survival of mice—Since earlier studies showed that 35 per cent of mice survived when embryo mash was given intraperitoneally following a lethal exposure to X-radiation (1025 r) and since less than 1 $\times$ 10^6 nucleated cells from 2-day mouse spleens were necessary to enhance survival, an effort has been made to determine the number of embryo cells that are necessary to bring about this protective action.

Suspensions that were made from cells obtained by pressing the entire embryo through a tissue press or by grinding it in a mortar inevitably proved fatal to the irradiated recipient, which was injected intravenously. Portions of the soft tissue of the embryo (mostly liver) were tested. Suspensions from this tissue were made with Locke's solution and were given intravenously after irradiation.

With 10 to 88 $\times$ 10^6 cells, 46·8 per cent of the mice survived 900 r. Fifty per cent survived with as few as 1 to 3 $\times$ 10^6 cells, and 26 per cent survived with 0·3 to 1·0 $\times$ 10^6 cells. With such a small amount as 0·1 to 0·3 $\times$ 10^6 embryo (liver) cells, 25 per cent survived 900 r (*Table V*).

The comparative effectiveness of various tissue-cell suspensions is shown in *Table VI*. The data indicate that 5·0 to 10·0 $\times$ 10^6 cells per mouse are

* Considerable difficulty is encountered when heparin is added to the suspension before the cells are counted since clumping of the white cells under these conditions is marked and accurate counts cannot be made.

Table V. A comparison of the effect of intravenous injections of various concentrations of embryo (liver) cell suspensions on the survival of mice exposed to 900r

Total No. of cells ($\times 10^6$)	*Mice* (*No.*)	*Time of death after X-rays* (days)																							*Survival*	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	(*No.*)	(%)
10–88	32			4	1		4	1	1	2									1	1	1	1					15	46·8
6–8	20						1			1	3																15	75
3–6	48	1	1	3	4	1	2		1	1	2	1				1							1			1	28	58
1–3	79	1	4	10	9		2	3		3		1						1	1	1	2					1	40	50
0·3–1·0	38			3	4	5	3	2	3	1	2		1	2				1				1					10	26
0·1–0·3	40	1	1		1	3	6	7	5	2			1					1		2	1						9	25
0·050 (50,000)	20		1			1	6	7	4																		1	5

Table VII. Effect of intravenous injections of cells from liver of 2-day-old *mice on survival of mice exposed to* 900r

Total No. of cells ($\times 10^6$)	*Mice* (*No.*)	*Time of death after X-rays* (days)																							*Survival*	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19–21	22	23	24–25	26	27	28		(*No.*)	(%)
14–21	17			3	1													2	1		1	1			8	47
4·3–5·1	10						1	1				1							1						6	60
2·0–3·0	22			1	1	1	1	2	1		1											1			13	59
1·3–1·6	28	1			1	1	3	2	1	2			2		1								3		13	46
0·75–0·77	20		2		3	1				1			2	1	1	2		1							4	20

as effective as higher concentrations. This is probably not, however, a true comparison since the bone marrow represents the effect of adult (10- to 12-week) tissue, whereas the liver and spleen were obtained from embryos or 2-day-old mice.

The effect of cells from the liver on the survival of irradiated mice—In previous experiments, it was found that cells obtained from embryonic mouse liver effectively enhanced the survival of mice exposed to 900r. Therefore, studies were made on cells obtained from the livers of 2-day-old mice to determine whether such cells are equally effective when the liver is no longer an embryonic organ.

The mice were killed by cervical fracture, and the liver was removed and mashed by pushing it several times through a syringe, using Locke's solution as the diluent. The cells were removed with a 26-gauge needle and cell counts were made. The suspension was then injected intravenously into the irradiated mice.

Table VI. A comparison of the effect of intravenous injections of bone marrow, spleen, embryo and liver cell suspensions on the survival of mice exposed to 900r

Total No. of cells ($\times 10^6$)	*Survival* (%)			
	Bone marrow (10- to 12-week)	*Spleen cells* (2-to 5-day)	*Embryo cells* (16- to 20-day)	*Liver cells* (2-day)
0·137–0·925	11·6	70·0	24·0	20·0
1·0–4·9	33·3	65·2	41·8	54·0
5·0–9·9	54·9	64·2	63·2	—
10·0–14·9	42·9	—	46·8	—
15·0–19·9	39·5	—	—	47·0

In a preliminary experiment, the cells were obtained from 2-day-old CF No. 1 mice and were given intravenously to LAF_1 females exposed previously to 900r. No deaths occurred during the first 21 days after irradiation ; however, 3 of 8 died before the 28-day period of observation was completed. In subsequent experiments, the CF No. 1 mice were used as both recipients and donors. Of the 97 mice that have been injected with cells from the livers of 2-day mice, 44 (43 per cent) survived. Mice that received 2 to 3 $\times 10^6$ nucleated cells appeared to survive equally as well as those that were given 4·3 to 5·1 cells (*Table VII*). With total nucleated cells of the order of 0·75 $\times 10^6$, there was 20 per cent survival. A suspension of cells obtained from the liver has been photomicrographed (*Figure 1*, page 129) to show the various cell types that are present in the injection solution.

The effect of injections of irradiated bone marrow cell suspensions on the survival of mice—In some experiments, mice were exposed to 600r total-body X-radiation. In others, exposures ranging from 100 to 500r were given. At intervals following exposure, marrow from the irradiated mice was removed and given intravenously to mice exposed previously to 900r. An attempt was made to prepare a suspension that contained the same number of cells that would produce the maximum protection provided by normal non-irradiated marrow.

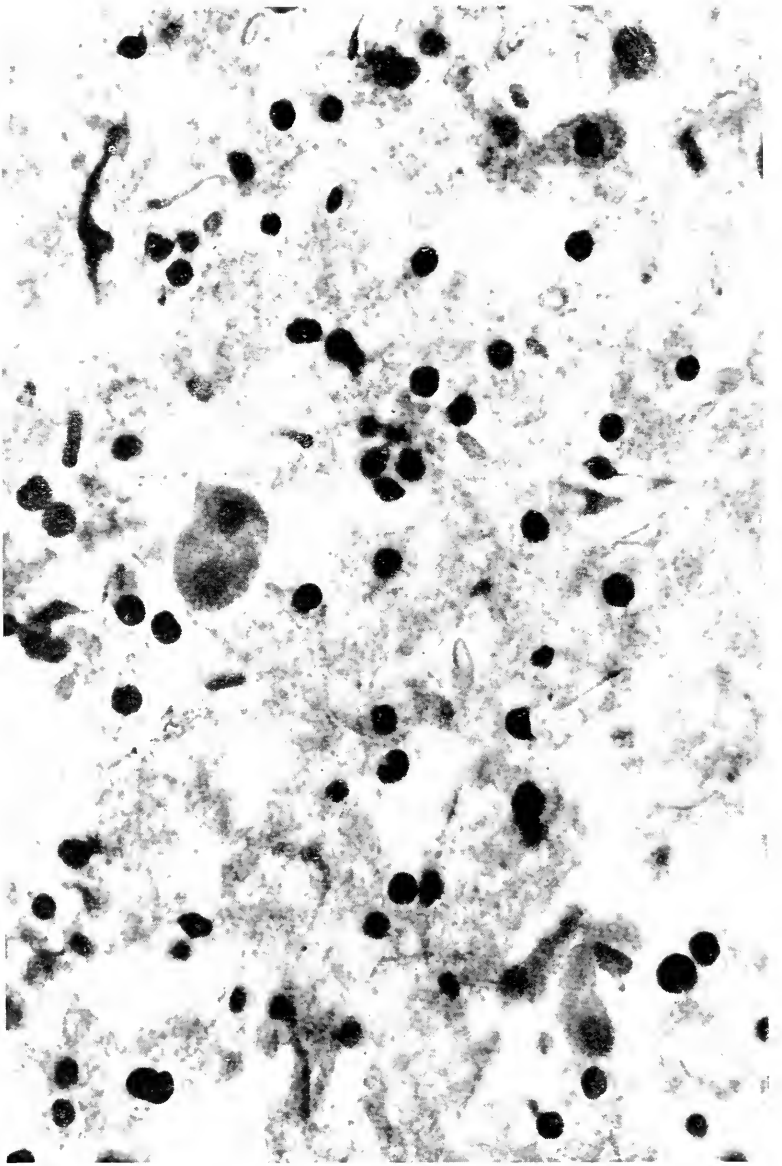


Figure 1. A photomicrograph of a suspension of cells from the liver of 2-day mice. The cells were suspended in Locke's solution

Suspensions of marrow made from mice at 0, 1, 3, and 6 hours after exposure to 600r had no effect on survival when they were injected intravenously into mice exposed previously to 900r even though the number of cells obtained was comparable to that from normal non-irradiated mice.

By 18 hours after total-body exposure to 600r, the marrow of the donor mice had become so aplastic that 24 mice were required to provide enough cells to make up the desired concentration for injection into 6 irradiated mice. An equivalent number of donor mice was required to provide marrow 24 hours after 600r. Not only had the factor in the marrow that is effective in enhancing survival been destroyed or inhibited, but some alteration in the marrow had also taken place that caused it to have a toxic effect on the recipient mice. Ten of 18 mice died within a few minutes following intravenous administration of this suspension, and none survived beyond the 10th day. The manifestation of this 'toxic' substance was not apparent during the first 18 hours after irradiation, and no studies have been made to determine how long it persists.

A striking contrast, however, exists between marrow cells taken from a mouse 1 day after it has been irradiated and those taken 8 days after irradiation. Although it was necessary to sacrifice 36 or more animals to obtain enough cells to inject 4 animals with a suspension containing 9×10^6 cells, all 4 survived the 28-day period of observation and no immediate deaths occurred. Later experiments revealed that survival, following the administration of 8-day (600r) marrow, paralleled that of mice injected with normal marrow. With a suspension containing about $1{\cdot}0 \times 10^6$ cells from either normal or 8-day marrow, 25 per cent of the irradiated recipients survived. Only 10 per cent survived when less than $1{\cdot}0 \times 10^6$ cells were given.

Suspensions of bone marrow cells removed from mice 6 days after 600r were injected into mice that had been exposed previously to 900 r. Indications are that at 6 days some mechanism is already affecting survival. However, the difficulty encountered in obtaining a sufficient number of cells from such aplastic marrow for a suspension containing about 8×10^6 cells has made the extension of this study impractical.

Other studies were made in which the donor mice received smaller amounts of radiation (*Table VIII*, page 131). The marrow from donor mice exposed to 100 or 200 r was as effective as normal marrow in enhancing the survival of mice exposed to lethal amounts of radiation. Marrow removed as early as 1 hour after irradiation was as effective as that removed 3 days later. It appears that 300r depresses but does not inhibit the activity of the 'factor' when the marrow is given 1 day or less after total-body irradiation.

Exposures of 400 to 500r destroy or inhibit the factor when the marrow cells are removed 1 day after X-radiation. However, these exposures, like 600r, require many donors and give a 'toxic' effect that is expressed in a fairly high frequency of deaths immediately after the intravenous administration of the suspension.

It has been demonstrated recently that the 'toxic' effect is present in the supernatant material * of the bone marrow suspensions. Centrifugation

* Centrifuged at 2,500rpm for 5 minutes. Supernatant was not completely cell-free.

Table VIII. A comparison of the effect of normal marrow and marrow from mice exposed to 100 to 600r *one day before they were used as donors*

X-ray exposure to donors (r)	*Total No. of cells* ($\times 10^6$)	*Recipient Mice* (*No.*)	*Time of death after X-rays* (days)														*Survivors*	
			4	5	6	7	8	9	10	11	12	13	14	15	16	17–28	(*No.*)	(%)
600	6–9	10					5	2	3								0	0
400	6–8	6						2	1	1	1	1					0	0
300	6–8	18		1	1	3	2	3	4				2		1		1	5·5
200	8–9	13			1	3								1			8	61·5
100	9–12	12				1	2	1								1	7	58
none	6–8	118	2	1	3	6	3	1		5	2	2	2	1	1	23	66	55
none	9–12	136	3	3	10	7	3	4	1	2	2	5	3	2	1	26	64	47

and re-suspension of the bone marrow cells in saline or Locke's solution considerably reduce the deleterious effect.

To determine the length of time during which radiation damage is reversible, non-irradiated bone marrow suspensions were injected intravenously into irradiated mice from 1 to 5 days after 900 r. Some beneficial effect was still obtained when the bone marrow was injected as late as 5 days after X-irradiation.

Effect of cell suspensions on survival of mice exposed to 750 r—Experiments similar to those described above are in progress. These differ only in that the recipients are being exposed to 750 r. The mice are irradiated without anaesthesia in plastic tubes. The target distance is 79 cm and the dosage rate, approximately 60 r per min. The animals that were exposed to 900 r were anaesthetized with Nembutal during irradiation ; the target distance was 57 cm and the dosage rate, 69 r per min.

Preliminary data indicate that 50 per cent survival is obtained when as few as $0{\cdot}5 \times 10^6$ nucleated cells are injected intravenously after the mice have been exposed to 750 r. The survival rate is the same with cells obtained from 2-day liver or embryo liver or spleen and with cells from the bone marrow of 4- to 5-week mice.

In a preliminary experiment, 6 of 10 mice survived with $0{\cdot}075 \times 10^6$ (75,000) embryo cells ; 22 of 32 survived with $0{\cdot}150$ to $0{\cdot}2 \times 10^6$ (150,000–200,000) embryo cells ; 4 of 10 with 0·175 (175,000) cells from 2-day spleens ; 6 of 16 with $0{\cdot}225$ to $0{\cdot}3$ (225,000–300,000) cells from 2-day liver ; and 6 of 8 have survived 28 days that received 150,000 bone marrow cells obtained from 4- to 5-week-old mice.

Comments

It is obvious from our data that (1) a relatively small total number of cells is sufficient to insure significant survival when these are injected into mice that have been irradiated with 900 r, and (2) embryo liver cells and baby mouse spleen or marrow cells are more effective than adult liver, spleen, or marrow cells. As one reduces the total-body exposure to the recipient mice (750 r), an even smaller number of cells is required to significantly enhance survival. In view of the observation that cells from the liver are effective, it is perhaps important to determine whether the vital cells are parenchymal ectopic primitive blood cells or some other cellular constituent of liver, *e.g.* the Küpffer cells. It should be possible to obtain information on the cell type or types involved in the elaboration of the factor(s) responsible for the recovery of irradiated animals by such techniques as differential centrifugation and by a study of their morphologic characteristics.

Cole and his co-workers[3] claim to have prepared splenic suspensions from the mouse in which only the nuclei of the cells were intact. According to their report, these preparations administered intraperitoneally to mice that have been exposed to 750 r routinely increase survival. If their suspensions contained as many as 50,000 morphologically intact cells or if the induced damage was reversible, the experiments must be extended to include larger numbers of animals and the absence of intact cells (less than 100,000) must be established before Cole's interpretation of his results can be accepted.

We have tried to repeat his work but have been unable to obtain significant survival when morphologically intact cells were absent from the injection preparation. In fact, we have been unable to produce a preparation, using Cole's technique, that was entirely cell-free. Approaching the problem from another point of view, we have attempted to learn whether Cole's technique would destroy or limit the capacity of leukaemic cells to induce leukaemia in normal or irradiated mice[6]. Thus far, we have found that these preparations produce leukaemia in the recipient mice. Many morphologically intact cells were found present in the injection material and their leukaemia-inducing capacity was obviously not affected.

SUMMARY AND CONCLUSIONS

Nucleated cells derived from various tissues of the haematopoietic system of normal adult, baby, and embryonic mice and suspended in saline or Locke's solution have been prepared and injected into irradiated mice. These preparations were standardized in terms of the number of nucleated cells per 0·5 cm^3 of suspension fluid (range, $0{\cdot}05 \times 10^6$ to 88×10^6) in order to make comparisons of the effects of the number of cells and their source on the enhancement of survival of irradiated recipients. The data indicate that fewer cells are required in suspensions made from young mice than from adult donors. The sources from which the cells were derived were unimportant. Sixty-three per cent of the mice irradiated with 900 r survived following the injection of about 3×10^6 cells from young donors as compared with 23 per cent survival when the recipients were injected with the same number of bone marrow cells from adult donors. With 750 r, only 150,000 marrow cells from 4- to 5-week old mice were required to enhance survival. Bone marrow obtained from donor mice irradiated with 100 or 200 r one day before removal of the bone marrow cells is effective ; whereas that from mice exposed to 400 r or more is ineffective in enhancing the survival of mice exposed to 900 r.

REFERENCES

[1] Jacobson, L. O., Marks, E. K., Gaston, E. O., Robson, M. J. and Zirkle, R. E. *Proc. Soc. Expt. Biol. Med.* 1949, **70** 740.

[2] Jacobson, L. O. *Cancer Res.* 1952, **12** 315.

[3] Cole, L. J., Fishler, M. C., Ellis, M. E. and Bond, V. P. *Proc. Soc. Expt. Biol. Med.* 1952, **80** 112.

[4] Lorenz, E., Congdon, C. C. and Uphoff, D. *Radiology*, 1952, **58** 863.

[5] Jacobson, L. O., Simmons, E. L., Marks, E. K. and Eldredge, J. H. *Science*, 1951, **113** 510.

[6] Simmons, E. L., Goldwasser, E. and Jacobson, L. O. Manuscript in preparation.

DISCUSSION

L. J. Cole : I think it should be pointed out, relative to the data of Jacobson *et al*, that their spleen cell suspensions were administered by intravenous injection ; in our own studies, the spleen nuclei fraction preparations were routinely administered intraperitoneally. The former route of administration is far less efficient than the latter, *e.g.* less material is required for the same level of protection. Thus, we have found that the intravenous injection of spleen nuclei fraction, derived from the equivalent of 0·75 spleen, will provide the same degree of protection against 750 r (whole-body X-irradiation) as the nuclei fraction equivalent to 5 spleens, when injected intraperitoneally.

SPLEEN PROTECTION: THE CELLULAR HYPOTHESIS

D. W. H. Barnes and J. F. Loutit
M.R.C. Radiobiological Research Unit, A.E.R.E., Harwell, Didcot, Berkshire

It must now be incontestable that there is in spleen and bone marrow a 'recovery factor', the administration of which to otherwise lethally X-irradiated mice permits their survival. Jacobson, who originally made the observation, and most other workers in this field favour the hypothesis that the factor is a chemical agent or hormone.

We have not been fully convinced by their arguments and from our own experimental work cannot exclude that the active principle is the transfer

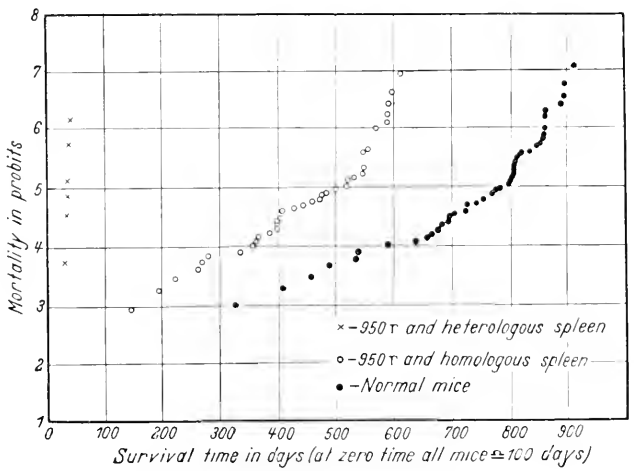


Figure I. Survival of CBA male mice

of living cells which act for the time being at least as a tissue graft. We have been concerned (*1*) with a dose of radiation (X-rays) which is normally 100 per cent lethal to our CBA strain—950 r. Thus any survivor of treatment is significant, and (*2*) with survival not only for a conventional 21 or 30 days but for an appreciable period thereafter.

Under these circumstances unlike other workers we have not been able to obtain recovery with heterospecific material—spleen or bone marrow from rabbits and guinea pigs[1].

Latterly we have reported our results with homospecific but heterologous mouse spleen[2]. Spleen of strain A mice given to our standard test animals of the CBA strain gave a significant survival rate (9/16) at 30 days, but this early success was not maintained and all survivors were dead by 100-odd days. This is to be contrasted with the administration of homologous mouse spleen from donors of strain CBA. Here the median life expectation

of mice surviving 30 days was 500 days. The data are summarized in *Figure 1* which shows in addition the times to death of unirradiated normal mice of the same colony.

Furthermore, whilst spleen of mouse strain A has some temporary recuperative effect, even this temporary benefit was lost if the CBA recipient had been previously immunized with strain A spleen. All these results are more easily explicable in terms of the active principle acting as a tissue graft than being a chemical growth hormone.

The active agent whatever its nature is extremely labile. In our hands it becomes inactivated by a few hours' storage at room temperature. It cannot be kept chilled in the refrigerator at 4° C or frozen at −15° C for 48 hours[2]. One might have anticipated that the frozen state at least would have preserved a hypothetical hormone but not an intact cell or tissue. On the other hand PARKES and his collaborators[3] have demonstrated that many tissues can be preserved at −79° C (alcohol—dry ice) after imbibing glycerol to prevent formation of ice crystals. We have adopted the Smith–Parkes technique and preserved finely chopped CBA spleen for 48 hours. On homogenizing this after quick thawing and injecting the homogenate intravenously we have just obtained the following results (*Table I*).

Table I.—30-day survival CBA mice given 950r X-rays

Saline control	*Fresh CBA spleen* ≡1 spleen	≡1/5 spleen	*Stored CBA spleen* −79° C ≡1 spleen	≡1·5 spleen
0/5	4/4	3/5	4/5	0/2
0/5	4/5	5/5	1/5	0/5

While the initial results suggest some loss on storage, they are at least promising in that some activity has been preserved.

Nevertheless the unequivocal answer to the main problem—is the spleen factor a living cell or a chemical agent ?—will come from one of two experiments, the demonstration either of positive survival after treatment with a proven cell-free material, or of the persistence in the surviving host of the donated cells or their descendants.

Our first essay at the latter experiment was by genetic tagging of the donated spleen. In this case the donor spleen had of necessity to be heterologous. It proved to be one of the less effective heterologous materials. The median survival time of the 10 recipients was increased only from 8 days to 23 days and no tagged descendants of these cells were identified.

REFERENCES

[1] BARNES, D. W. H. and LOUTIT, J. F. *Proc. R. Soc. Med.* 1953, **46** 251.
[2] BARNES, D. W. H. and LOUTIT, J. F. *Nucleonics*, 1954, **12** No. 5, 68.
[3] SMITH, A. U. *Proc. R. Soc. Med.* 1954, **47** 57.

THE MODIFICATION OF THE RADIATION RESPONSE BY SHIELDING PROCEDURES

L. F. Lamerton and E. H. Belcher

Physics Dept., Institute of Cancer Research, Royal Cancer Hospital, London

The purpose of the present communication is to give a very brief review of progress of work carried out in the Physics Department of the Institute of Cancer Research on the radiation response of the young growing rat, with and without various shielding procedures. Throughout the work the endeavour has been to use doses of radiation less than the lethal dose, so

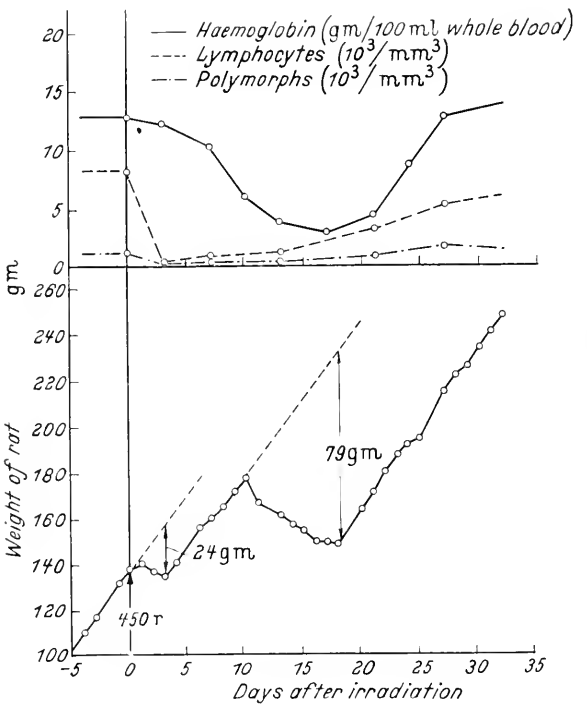


Figure 1. Changes in growth rate and blood picture following whole-body X-irradiation (450r) to young rat (albino strain)

that the irradiated animal can be studied in the recovery period as well as in the phase of development of damage. We regard this as an important aim if one is concerned with the problem of the mitigation of the effects of large-volume exposure, since there are undoubtedly a number of mechanisms concerned in the radiation response, and it is important to know at which stage a particular procedure is effective.

The young growing rat proves to be, from a number of points of view, a suitable material for studying the various phases of the radiation response

(LAMERTON, ELSON, HARRISS and CHRISTENSEN[1]). *Figure 1* is a typical example of weight and blood changes in a young rat (colony-bred albino strain) given 450r whole body X-irradiation. The weight drop immediately following irradiation gives a measure of the severity of the initial phase of the radiation response, while the second or ' acute ' phase of the response is characterized by an anaemia and a second weight drop. Whether or not a second weight drop occurs, and its severity when it occurs, is correlated with the severity of the post-irradiation anaemia. In the case of the young growing rat, we have found the anaemia to be uncomplicated by the infection that a number of workers have reported in various species. Cultures have been made of heart blood from a number of rats in the second phase of the radiation response, and have been negative.

It is probable that the initial weight drop of the rat is a manifestation of the ' radiation sickness ' which is observed clinically, of which the origin is so much in doubt. The work with rats suggests that important factors in the production of the initial weight drop are the products of damaged tissue (probably lymphoid tissue), together with direct radiation damage to the gut, but the type of study described by Rajewsky in the present Conference may throw much more light on the processes at work.

So far as the second phase of the radiation response is concerned, the important factor appears to be the severity of the post-irradiation anaemia, which is not the result of gross local bleeding, but of a generalized haemorrhage characterized by a considerable leakage of red cells into the lymph. We have attempted to distinguish the two factors controlling the severity of the radiation-induced anaemia—the mechanisms responsible for the bleeding, and the capacity of the animal for compensatory erythropoiesis.

A series of studies have been made on platelet changes following irradiation (LAMERTON and BAXTER[2]), and the findings are in agreement with those of Cronkite and other workers that the platelet fall is a very important and possibly the essential factor in initiating the severe bleeding. There is evidence from these studies, though not conclusive, that there is a critical platelet count of about 20,000 to 30,000 per mm^3, below which it is necessary for the blood platelet level to fall before a very severe bleeding and anaemia is produced.

With regard to the capacity of the animal for compensatory erythropoiesis, measurement of the radioactive iron turnover in various tissues has proved to be a valuable technique for a quantitative determination of the pattern of erythropoietic activity in the animal (LAMERTON, BELCHER and HARRISS[3]; BAXTER, BELCHER, HARRISS and LAMERTON[4]; BELCHER, GILBERT and LAMERTON[5]). An example of an application of this technique is shown below.

THE EFFECT OF SHIELDING PROCEDURES

At the 1953 Radiobiology Conference in Aarhus we reported on some results of head shielding during radiation exposure. Such a procedure does not affect appreciably the extent of the initial weight loss, but reduces very considerably the severity of the radiation-induced anaemia. These investigations have been continued (Lamerton and Baxter[1]) and it has been found that hind-leg shielding is just as effective as head shielding. Even

shielding of a small portion of the femur will reduce very considerably the severity of the anaemia, whereas shielding of a skin tube or skin flap will not.

It appears that these effects are due to shielding of a small part of active bone-marrow. Platelet changes in the shielded animals have been studied and it has been found that one effect of shielding is to reduce very considerably the platelet fall, by virtue of megakaryocyte activity in the shielded part. It is therefore possible that reduction in the platelet fall accounts for the effectiveness of the shielding procedure, but it is also necessary to determine to what extent the shielding procedure modifies the pattern of

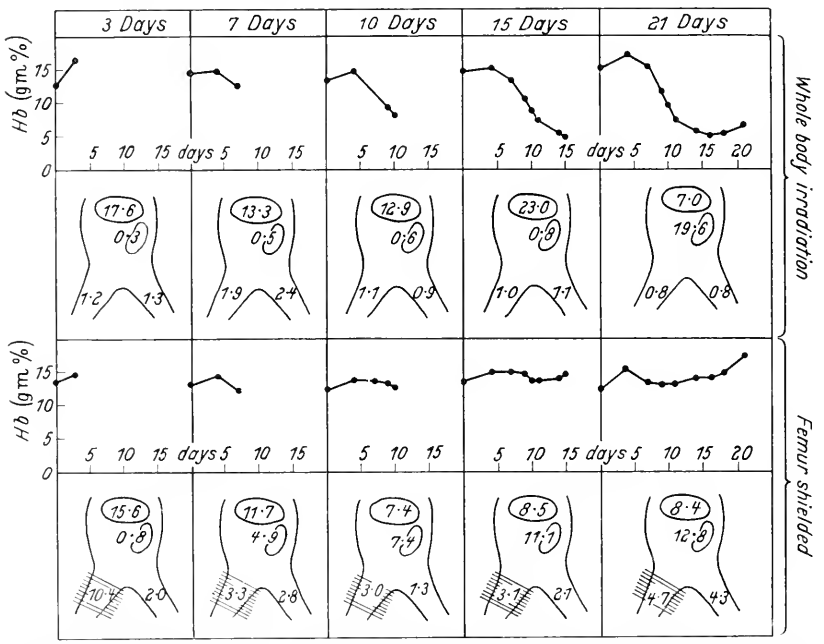


Figure 2(a). Uptake of ^{59}Fe, *expressed as percentage of injected dose, in liver, spleen and hind limbs of irradiated rats (albino strain) 4–5 hours after subcutaneous injection*

erythropoietic activity in the various tissues of the animal. This has been studied by a radioactive iron technique as follows.

Investigation of radioactive iron turnover in shielded and unshielded rats

In the experiment described here, two groups of rats were irradiated, one group given 450r whole body irradiation and the other group 450r with one hind leg shielded. The rats were sacrificed in pairs at various times after irradiation (3, 7, 10, 15 and 21 days). At a time of 4–5 hours before sacrifice the rats were injected subcutaneously with 1 μc ^{59}Fe as ferric chloride in citrate buffer (carrier iron content < 1 μg). Immediately before killing, the animals were exsanguinated under anaesthetic by withdrawing blood by cardiac puncture at the same time as Ringer solution was injected into a lateral tail vein. By this means 90 per cent or more of the blood can be removed. Various tissues were then dissected out and their

activity assayed in a well-type scintillation counter. The results are given in *Figure 2(a)*. The serial haemoglobin values show the efficiency of the shielding procedure. The percentage of the injected dose of radioactive iron which appears in the spleen, hind limbs and liver, at the time of 4–5 hours after injection, is shown. Mean values from control animals of the same age are shown in *Figure 2(b)*.

In the unshielded animals it can be seen that the uptake in the hind limbs is depressed below normal values even up to 21 days. At 21 days, however, when the haemoglobin curve has begun to rise, the spleen is seen to have an extremely high uptake of the radioactive iron. It appears therefore that following whole-body irradiation the spleen recovers erythropoietic function earlier than bone-marrow. This is confirmed histologically.

The pattern of erythropoiesis is seen to be modified considerably by the shielding of one hind leg. First, the shielded limb has a very high uptake of radioactive iron at 3 days. Secondly, splenic erythropoiesis develops at a much earlier stage than in the case of the unshielded animals.

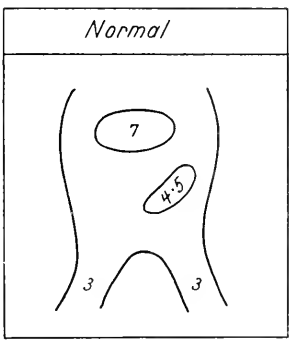


Figure 2(b). Mean uptake of ^{59}Fe, *expressed as percentage of injected dose, in liver, spleen and hind limbs of unirradiated control rats (albino strain) 4–5 hours after subcutaneous injection*

It is possible that these effects are the result of specific humoral factors produced in the shielded limb (Jacobson[6]). On the other hand, the pattern of erythropoietic activity may be determined by general physiological changes resulting from the irradiation, perhaps related to the severity of the anaemia produced, or possibly by nervous mechanisms. Further work is being carried out with different doses of radiation, with different strains of rat, and also with splenectomized rats, in an endeavour to elucidate some of the problems involved.

SUMMARY

A short progress report is given on the studies of the radiation response of the young growing rat, and the modification produced by procedures such as hind-leg shielding. Platelet changes in irradiated animals have been investigated, and a radioactive iron technique has been used to compare the changes in the pattern of erythropoietic activity in various tissues in rats irradiated with and without shielding procedures.

ACKNOWLEDGEMENTS

Our thanks are due to a number of colleagues who are collaborating in these investigations, and in particular to Miss E. B. Harriss, Dr. C. F. Baxter, Miss K. Adams

and Miss M. Winsborough. We are very indebted to Professor W. V. Mayneord for his constant support and encouragement.

The financial help of the Medical Research Council and of the British Empire Cancer Campaign is gratefully acknowledged.

REFERENCES

[1] Lamerton, L. F., Elson, L. A., Harriss, Eileen B. and Christensen, W. R. *Brit. J. Radiol.* 1953, **26** 510 and 568.

[2] Lamerton, L. F. and Baxter, C. F. 'An Experimental Study of Radiation-induced Anaemia with Reference to Shielding Procedures and Platelet Changes,' *ibid.*, in press.

[3] Lamerton, L. F., Belcher, E. H. and Harriss, Eileen B. *Radioisotope Conference, 1954*, Volume I. London, Butterworths, 1954.

[4] Baxter, C. F., Belcher, E. H., Harriss, Eileen B. and Lamerton, L. F. 'Anaemia and Erythropoiesis in the Irradiated Rat. An Experimental Study with particular reference to Techniques involving Radioactive Iron,' *Brit. J. Haematol.*, in press.

[5] Belcher, E. H., Gilbert, I. G. F. and Lamerton, L. F. *Brit. J. Radiol*, 1954, **27** 387.

[6] Jacobson, L. O. *Cancer Res.* 1952, **12** 315.

DISCUSSION

B. Jolles : In connection with the findings described by Lamerton and with what has been said it is worth recording that Swift, Taketa and Bond* in their experiments with regionally fractionated X-irradiation (divided exposure) have shown among other things that the degree of protection obtained in rats appeared similar, irrespective of the order in which the two portions of the body were irradiated or of the time interval (10 or 120 minutes) allowed between exposures. The matter is complicated further by the fact that there are several recovery factors with different mechanisms of action and time lags. In investigations on the skin radiation reaction recovery factors I have noted that only some of the reaction phases are influenced by the amount of normal surrounding tissue.

* Swift, Taketa and Bond. *Radiation Res.* 1954, **1** 241.

ON THE NATURE OF THE SPLEEN-BONE MARROW RADIATION RECOVERY FACTOR*

LEONARD J. COLE and MARIE E. ELLIS

Biological and Medical Sciences Division U.S. Naval Radiological Defense Laboratory, San Francisco 24, California

FOR the past few years our research efforts have been directed towards the problem of the nature of the radiation recovery factor(s) present in the spleen and/or bone marrow of certain animal species, as first shown by means of spleen shielding and spleen implant studies by JACOBSON *et al*[1], and by LORENZ *et al*[2] with bone marrow injection. In the present paper, we will discuss briefly the properties of the recovery factor in mouse spleen ; take up the question of the presence of viable cells in protective spleen preparations ; compare some properties of bone marrow and splenic homogenates with respect to protective activity, and discuss some implications thereof.

In following up the initial work of Jacobson *et al* we were able to demonstrate the post-protective efficacy of Potter-Elvejhm homogenates of spleens from immature LAf_1 mice, when administered in a single intraperitoneal injection to otherwise lethally X-irradiated adult LAf_1 mice[3]. Under these conditions, the LD_{50} of spleen homogenate-treated mice was increased by approximately 200r. What can we now say about the properties of this recovery factor ?

(*1*) The factor appears to be particulate in nature ; and is insoluble in aqueous saline or phosphate buffer media. Thus far, it has been impossible to obtain the active principle in solution[4].

(*2*) It is localized in the nuclear fraction, obtained by differential centrifugation of sucrose-salt homogenates of spleen, and is not present in the microsome or mitochondria particulate fractions[5].

(*3*) It is thermally labile—being inactivated when kept at 60°C for 20 minutes[6].

(*4*) It is inactivated by relatively low doses of X-irradiation *in vitro* (725r) ; and is also inactivated by exposure to ultrasonic vibrations.

(*5*) It is inactivated by treatment with the enzyme deoxyribonuclease (DNA-ase), or with trypsin, but not by ribonuclease[7].

(*6*) Thus far, we have been unable to obtain active preparations by lyophilization of saline or phosphate buffer spleen homogenates.

(*7*) It does not dialyse through cellophane membranes. After 3 hours of dialysis at 5°C, the protective activity of spleen homogenates is retained in the non-dialysable fraction.

(*8*) The factor exhibits properties of species and strain specificity.

* These studies have been supported in part by funds from The Bureau of Medicine and Surgery, U.S. Navy Department. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official, or reflecting the views of the Navy Department.

The tentative conclusion drawn from the above experimental data is that the radiation recovery factor, as present in immature LAf_1 mouse spleen, is a particulate, subcellular macromolecular deoxyribonucleoprotein complex.

Most spleen nuclear preparations are not entirely devoid of cellular bodies—so far as one can judge by microscopic examination. It becomes particularly relevant, therefore, to attempt to ascertain whether or not the protective activity of such spleen nuclear fractions can be ascribed to the presence of presumably intact, viable cells, which could possibly 'seed' the depleted haematopoietic system of the irradiated animal and thereby elicit renewed haematopoiesis, as a consequence of functional repopulation. Furthermore, the conclusions from the aforementioned enzymatic analysis studies rest in part on the supposition that viable, living cells are, in general, resistant to the enzymatic action of externally-added DNA-ase or of trypsin, whereas dead cells and subcellular fractions are susceptible to the action of these enzymes. Evidence for the validity of this general supposition has been provided by the studies of NORTHRUP[8] and of BOHUS JENSEN[9] with trypsin ; and of ELY and ROSS[10] with DNA-ase. In the present study we have attempted to obtain further experimental data on this aspect of the problem—specifically to determine whether fresh intact spleen cells are substrates for enzymatic action of DNA-ase. Release from the cells of soluble material absorbing at 260 mμ was employed as the criterion for enzyme action, since it is known that DNA-ase liberates DNA from its protein-bound state in the cell.

EXPERIMENTAL

Fresh spleen cells were obtained by gently washing minced mouse spleen fragments with 0·14 M NaCl solution containing 1 mg dextrose per ml. As observed microscopically, the cell suspension was clean and homogeneous ; no disrupted cells could be seen. Five millilitres of the suspension, containing $5{\cdot}1 \times 10^6$ cells per ml, and added Mg^{++} (0·005 M), was incubated with crystalline deoxyribonuclease (DNA-ase) (50 μ per ml) at 25° C for 30 minutes. The tubes were then chilled in ice and centrifuged at 5° C. An aliquot of the original cell suspension incubated without added enzyme, served as a control. The resulting supernatant fluids were carefully removed, and their ultraviolet absorption spectra determined with a Beckman quartz spectrophotometer. The absorption data are shown in *Figure 1.* It is evident that little, if any, material with specific absorption at 260 mμ was released from the cells by the action of DNA-ase under the conditions of these experiments. Peak absorption in the supernatant occurred at 278 mμ. and not at 260 mμ. The actual amount of DNA liberated under these conditions was calculated as follows : According to MIZEN and PETERMAN[11] one milligramme (wet weight) of spleen tissue contains $22{\cdot}9 \times 10^5$ cells. From previously reported analytical data obtained in this laboratory, the concentration of DNA in the spleen of immature LAf_1 mice is 19·7 μg DNA per mg[12]. On this basis the calculated cellular DNA content is $8{\cdot}6 \times 10^{-9}$ mg. per cell ; therefore $5{\cdot}1 \times 10^6$ cells contain a total of 43·9 μg DNA. The observed net change in optical density at 260 mμ (*i.e.* 0·018) in the present experiment, corresponds to a value of 0·7 μg DNA per ml, as determined from a DNA standardization curve. The net liberation of DNA by the

action of DNA-ase under these conditions was, therefore, 2 per cent of the total cellular DNA.

In the next experiment, 5 ml of fresh spleen cell suspension (containing $5{\cdot}2 \times 10^6$ cells per ml) was centrifuged at 2,500 rpm for 10 minutes, the supernatant was discarded, and the packed centrifugal residue resuspended, by shaking, in 5 ml of saline. The suspension was centrifuged once again, the supernatant discarded, and the residue again resuspended by shaking. The suspension was then incubated with DNA-ase at 25° C for 30 minutes. After incubation, the tubes were chilled in ice, and centrifuged at 2,500 rpm for 10 minutes in a refrigerated centrifuge. The ultraviolet absorption spectra of the resultant supernatants are shown in *Figure 2*. The specific and large absorption at 260 mμ as a consequence of the action of DNA-ase on the disrupted (*e.g.* twice centrifuged and resuspended by shaking) cell

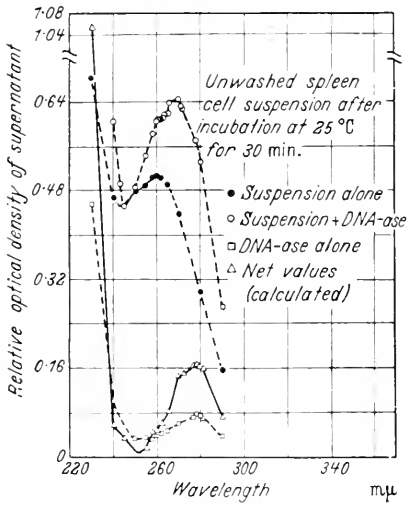


Figure 1. Effect of deoxyribonuclease treatment on unwashed mouse spleen cell suspension

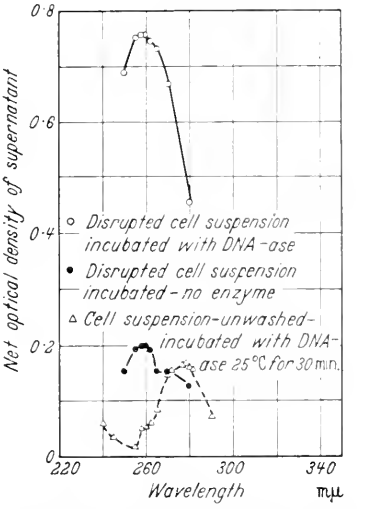


Figure 2. Effect of deoxyribonuclease treatment on disrupted spleen cell suspension

suspension, is in striking contrast to that of the unwashed cell suspension incubated with DNA-ase. The optical density at 260 mμ (*i.e.* 0·758) corresponds to a liberation of 39 per cent of the total cellular DNA in the disrupted cell suspension by the DNA-ase.

A study was next made of the effect of DNA-ase on a washed spleen homogenate preparation, the cell count of which was approximately equal to that of the cell suspension used above. The spleens obtained from one-week-old mice and weighing 280 mg were homogenized in a Potter ground-glass homogenizer, washed twice with cold 0·14 M NaCl, rehomogenized, and resuspended in 10 ml 0·14 M NaCl. Two homogenates were prepared concurrently; one contained $6{\cdot}9 \times 10^6$ cells and/or cell bodies per ml: the other $7{\cdot}9 \times 10^6$ per ml. Both homogenates were incubated at 25° C for 30 minutes, one (*A*) with added DNA-ase, the other (*B*) without enzyme. After incubation, the flasks were chilled, the homogenates centrifuged, and the clear supernatants withdrawn for analysis. The supernatant from *B*

gave a negative Dische reaction for DNA ; while the supernatant from *A* was found to contain 345 μg DNA per ml, or 3·27 mg DNA in the whole supernatant. This represents a solubilization of 43 per cent of the total DNA present in the homogenate. The ultraviolet absorption data on these supernatants, are presented in *Figure 3.* The high specific absorption at 260 mμ of the supernatant from the DNA-ase treated homogenate is again noteworthy.

The differential effect of DNA-ase on a fresh, non-disrupted spleen cell suspension and on a spleen homogenate containing the same number of whole cells, is evident from *Figure 4.*

The experimental data support the view that the DNA as present in fresh, intact, non-homogenized spleen cell suspension is not available as substrate

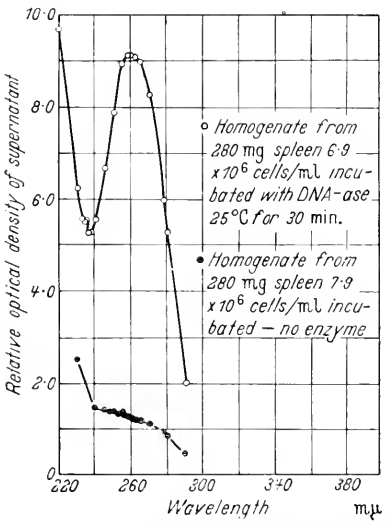


Figure 3. Liberation of soluble deoxyribonucleic acid from washed spleen homogenate by deoxyribonuclease

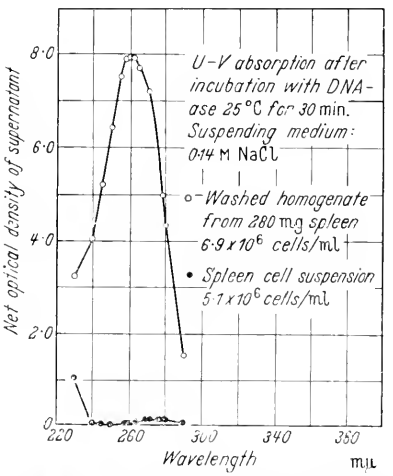


Figure 4. Comparative effect of deoxyribonuclease on spleen cell suspension versus spleen homogenate

for the enzymatic action of added DNA-ase. It follows, therefore, that if the protective activity of a given preparation of spleen nuclear fraction, or of a thoroughly homogenized and washed spleen homogenate can be inactivated by short-term incubation with DNA-ase, the active protective principle cannot be a result of the presence of intact, living cells, *per se.* The protective effect of such preparations would be referable to an infracellular biologically active substance or complex.

THE RECOVERY FACTOR IN RAT BONE MARROW

The recent finding[13] of post-protection of X-irradiated rats afforded by the intravenous injection of homologous rat bone marrow suspension, has now prompted some experiments designed to investigate the properties of the recovery factor in marrow. The pertinent experimental results are summarized in *Table I.* Rat bone marrow homogenates, prepared in sucrose-salt-ATP medium[5], containing dextrose (1 mg per ml) afford definite protection

against otherwise lethal whole-body X-irradiation (725 r). The data indicate, furthermore, that protective activity of such homogenates is associated with the nuclear fraction obtained by differential centrifugation ; the mitochondria and supernatant subfractions exhibit no protective activity, under the conditions of these experiments.

Table I.—Some properties of rat bone marrow with respect to post-radiation protection

Dose (r)	*Treatment*	*Survival at* 30 Days (No. Total)	*Mean Survival Time* (Days)
725	Rat bone marrow : homogenate (100 mg)	3/5	6·5
725	nuclei (200 mg)	5/5	—
725	mitochondria (200 mg)	0/5	9·2
725	Sucrose-salt-solution	0/5	8·8
740	Rat bone marrow : nuclei (300 mg)	5/9	10·4
740	mitochondria (300 mg)	0/9	8·2
740	microsomes (300 mg)	0/5	7·8
740	supernatant	0/6	9·7
740	Sucrose-salt-solution	0/9	7·3
725	Normal rat bone marrow (150 mg)	5/8	8·3
725	Marrow from X-irradiated rats (150 mg)*	0/8	8·8
725	Buffer-glucose-ATP	0/7	8·4
725	Normal rat bone marrow (100 mg) homogenate ..	6/10	7·2
725	Marrow irradiated *in vitro* (725 r)	0/10	9·9
725	Buffer-glucose-ATP	0/8	10·6

* Marrow removed 2 days after 750 r.

The data in *Table I* also show the high radiosensitivity of the recovery factor in rat marrow. Thus, the ability of rat bone marrow homogenates to afford protection against acute radiation death is annulled by *in vitro* irradiation of such homogenates with 725 r. (250 kvp X-rays, approximately 25 r per min). Furthermore, marrow taken from X-irradiated rats (725 r whole-body irradiation) 48 hours after exposure, no longer exhibits any protective activity. It appears therefore that the radiation recovery factor present in rat bone marrow homogenates possesses at least some properties similar to that of the protective principle in mouse spleen homogenates.

In the light of the considerable body of evidence in the literature indicating that recovery from whole-body X-irradiation injury at lethal dose levels is associated with haematopoietic regeneration, it is of interest to compare bone marrow and spleen from various animal species and strains as sources of the recovery factor, and as to haematopoietic function. It is known that homologous bone marrow is a more effective source of recovery factor than homologous spleen. This is true for the LAf_1 mouse[14] as well as for the Sprague-Dawley rat[13]. In some strains of mice, furthermore, the spleen does not provide a source of the recovery factor, whereas the bone marrow

is effective. Thus, we have found that homogenates of spleens from young C–57 black mice do not protect X-irradiated (640r) C–57 black mice against death, whereas a single injection of 8mg (wet weight) of homologous bone marrow affords protection in 80 per cent of the animals after 700r. One may infer a generalization from such results—namely, that in a given animal species or strain, the homologous post-protective factor is always present in the bone marrow ; and that in some species or strains (for example, LAf_1 mice), it is also present in the spleen—presumably associated with primitive haematopoietic function. According to CRONKITE *et al*[15] mouse spleen 'normally shows extensive extramedullary myelopoiesis, resembling myeloid metaplasia in other species'. Likewise, FRIEDELL and SALERNO[16] conclude from their internal radioisotope studies that 'the spleen is much more important in support of the haematopoietic system in the mouse than in the rat'. CRONKITE[17] states that there is no evidence that you can get protective activity with the spleen unless it is a site of myelopoiesis. It seems likely, then, that the spleen is a source of the recovery factor only in so far as it may contain certain bone marrow-like cells (presumably primitive myelopoietic cells). It follows from these considerations that the protective principle of spleen and bone marrow is derived (or is a product of) the same cell type, and is probably identical chemically. The experimental data presented above provide some support for this concept.

If the radiation recovery factor in bone marrow and that in spleen are functionally identical, and if the marrow provides the largest pool of the factor, then it may be anticipated that the surgical removal of the spleen from a mouse would not necessarily result in any increase in the sensitivity of the animal to whole-body X-irradiation. This would be especially true if a sufficient time interval between splenectomy and radiation exposure occurred—presumably permitting readjustment of bone marrow haematopoietic function to the absence of spleen. To conclude from the results of splenectomy experiments in mice that the unshielded spleen contains no radiation recovery factor[18] is unjustified on logical grounds—it appears to the authors—as well as on the basis of the experimental fact that injection of mouse spleen homogenates into otherwise lethally X-irradiated mice affords them protection against death.

SUMMARY

Since previous data indicate that the radiation recovery factor in the nuclear fraction of mouse spleen homogenates is inactivated by treatment with DNA-ase and with trypsin, it was pertinent to test the hypothesis that intact viable spleen cells are not susceptible to the action of these enzymes. Fresh spleen cells were obtained from gently minced (with fine scissors) spleen fragments, and incubated with DNA-ase at 25° C for 30 minutes. Under these conditions only 2 per cent of the total cellular DNA was liberated into the supernatant. In contrast, as much as 45 per cent of the total cellular DNA was solubilized by the action of DNA-ase on spleen homogenates containing the same cell count as the cell suspension. These data support the view that DNA, as present in intact, non-homogenized spleen cells is not available as a substrate for added DNA-ase.

Additional evidence indicating that the recovery factor in rat bone marrow possesses properties similar to that in mouse spleen, has been obtained. Thus, protective activity of rat bone marrow homogenates injected into homologous X-irradiated rats, is associated with the nuclei fraction, obtained by differential centrifugation in sucrose-salt medium and not with the mitochondria, microsome or supernatant subcellular fractions. Rat bone marrow homogenates lose their protective activity following *in vitro* X-irradiations with 725 r ; and marrow removed from irradiated rats (725 r) two days after exposure is no longer protective.

The implications of these experimental data have been discussed.

REFERENCES

[1] Jacobson, L. O., Simmons, E. J., Marks, E. K. and Eldredge, J. H. *Science*, 1951, **113** 510.
[2] Lorenz, E., Uphoff, D., Reed, T. R. and Shelton, E. J. *Nat. Cancer Inst.* 1951, **12** 197.
[3] Cole, L. J., Fishler, M. C., Ellis, M. E. and Bond, V. P. *Proc. Soc. exper. Biol. Med.* 1952, **80** 112.
[4] Cole, L. J., Ellis, M. E., Fishler, M. C. and Bond, V. P. *Federation Proc.* 1952, **11** 26.
[5] Cole, L. J., Fishler, M. C. and Bond, V. P. *Proc. Nat. Acad. Sci. (U.S.)* 1953, **39** 759.
[6] Cole, L. J., Fishler, M. C. and Ellis, M. E. *Radiology*, 1954, in press.
[7] Cole, L. J. and Ellis, M. E. *Radiation Res.* 1954, **1** 347.
[8] Northrup, J. H. *J. Gen. Physiol.* 1926, **9** 497.
[9] Bohus Jensen, A. *Exptl. Cell. Res.* 1953, **4** 60.
[10] Ely, J. O. and Ross, M. H. *Science*, 1949, **109** 367.
[11] Mizen, N. A. and Petermann, M. L. *Cancer Res.* 1952, **12** 727.
[12] Cole, L. J. and Ellis, M. E. *Cancer Res.* 1954, in press.
[13] Fishler, M. C., Cole, L. J., Bond, V. P. and Milne, W. L. *Amer. J. Physiol.* 1954, **177** 236.
[14] Lorenz, E., Congdon, D. and Uphoff, D. *Radiology*, 1952, **58** 863.
[15] Cronkite, E. P., Brecher, G. and Chapman, W. H. *Milit. Surg.* 1951, **109** 294.
[16] Friedell, H. L. and Salerno, P. R. *Radiation Res.* 1954, **1** 131. (Abstract.)
[17] Cronkite, E. P. Personal Communication.
[18] Raventos, A. *Amer. J. Physiol.* 1954, **177** 261.

DISCUSSION

R. F. Mouton : If I have correctly understood, the activity of the spleen-bone marrow recovery factor is destroyed by the process of lyophilization.

L. Cole : Yes.

R. F. Mouton : It is known that some proteins can be denatured by lyophilization, lipoproteins, for instance. This adverse effect can be in some cases avoided by addition of sucrose in the medium before lyophilization. Did you lyophilize in presence of sucrose ?

L. Cole : No.

EFFECT OF X-RAYS ON THE RESORPTION RATE OF INJECTED $NaH^{14}CO_3$ IN MICE

A. Forssberg and G. Hevesy

Institute of Radiophysics and Institute of Organic Chemistry, Stockholm

In this short version of our paper[1] we present evidence for an X-ray effect in mice, which have been given a total body irradiation of 2,000 r immediately followed by an intraperitoneal injection of $NaH^{14}CO_3$. Forty mg of bicarbonate were injected and this gave 7,000 counts/min/mouse in our instrument and about 5 per cent of the total activity was assayed. The mean life-time of the bulk of the circulating bicarbonate ions in the body of the mouse is a few minutes only, and any change in the rate of resorption will be reflected in a corresponding change in the amount of $^{14}CO_2$ exhaled within a few minutes or even seconds after the injection.

In the first set of experiments we started the collection of exhaled carbon dioxide about 10 minutes after injection of the isotope and followed this process for one hour at 10-min intervals. We ascertained that irradiated mice exhaled approximately 25 per cent more $^{14}CO_2$ than controls combined with a slight decrease (some 10 per cent) in the output of the total amount of CO_2. The latter fact had been noticed by us previously[2]. The enhanced exhalation of $^{14}CO_2$ might be expected to run parallel with a smaller amount of residual ^{14}C in the body of irradiated animals. Analyses of controls and irradiated mice about half an hour after the injection showed, however, that the activity of the homogenized and dried tissue did not vary appreciably.

The explanation seemed to be that irradiated mice exhale $^{14}CO_2$ at a markedly decreased rate for a short period immediately after the injection of the bicarbonate, leaving these animals with an increased pool of ^{14}C some 10–60 min afterwards. Experiments to that end, using a special device for collection of carbon dioxide immediately upon the injection of $NaH^{14}CO_3$, showed, that in the first 4 min of the experiment during which an appreciable percentage of the injected $^{14}CO_2$ is exhaled, the controls give off more $^{14}CO_2$ (45 per cent) than the irradiated animals (29 per cent) and more ^{14}C being preserved in the exposed animals, more $^{14}CO_2$ is exhaled in the later stage of the experiment. The activity expired during the first minute, including the 6 sec taken for the injection in these experiments, was found to be only 43 per cent of that of the control. This indicates that the X-ray effect on the process of resorption of bicarbonate and the intrusion of $^{14}CO_2$ into the alveolar space is very marked.

The slower exhalation of $^{14}CO_2$ by the exposed animals shortly after injection of the labelled bicarbonate could be due to a depressed resorption or/and disturbances of the blood circulation. To investigate how far the latter is the case we injected bicarbonate into the tail vein and collected the carbon dioxide in 2-min intervals starting immediately after injection. Only

slight differences could be noticed in the amount of $^{14}CO_2$ exhaled from irradiated and controls. It would seem, therefore, that the effect has not mainly been caused by a depressed transport rate of the injected $NaH^{14}CO_3$ through the blood plasma.

Some experiments were performed in order to elucidate the mechanism of this X-ray effect. Resorption is presumably mediated through hormonal action. This line of thought induced us to investigate the effect of the administration of various hormones. Subcutaneous injection of ACTH in amounts of 0·008–0·04 I.U. on the two days previous to the experiments and 0·04–0·2 I.U. one hour before injecting $NaH^{14}CO_3$ affected the exhalation of $^{14}CO_2$ in an opposite way to the irradiation, giving an increased output of some 30 per cent in the first minutes. When irradiating the mice after an ACTH administration it appeared that this combined treatment

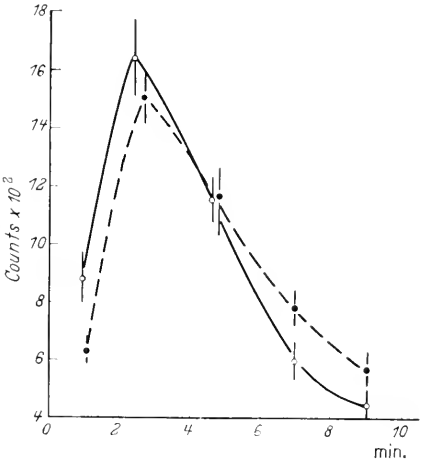


Figure 1. Exhalation of $^{14}CO_2$ from intraperitoneally injected $NaH^{14}CO_3$ in irradiated (•) and control (o) mice

cancelled out the difference between treated and control mice. It may be suggested that the diminished resorption rate observed in the exposed mouse is due to a blocking effect by the irradiation of ACTH formation. The biological half-life of the injected ACTH is about 5 min and the half-life of the ACTH secreted into the circulation may have a similar value[3]. Blocking of ACTH formation through irradiation would therefore soon manifest itself.

The combined effect of administration of hormones and irradiation is, however, rather complicated as visualized by the following experiments with adrenaline. Subcutaneous injection of 2 γ adrenaline per mouse 5–15 min before the injection of $NaH^{14}CO_3$ caused a similar initial decrease in the output of $^{14}CO_2$ as did the irradiation. Administration of adrenaline may produce vasoconstriction which depresses the rate of resorption[4]. The hypothesis that the irradiation effect on $^{14}CO_2$ exhalation is caused by an interaction of released adrenaline in the circulation could not be substantiated as the concentration of adrenaline in the urine of irradiated animals and controls was found to be similar in direct tests. This negative finding does not necessarily disprove the hypothesis as a transient increase of

adrenaline may conceivably escape notice. Combining adrenaline injection and irradiation in further experiments gave, however, not an additive depression of $^{14}CO_2$ exhalation. Instead we found an increased output of $^{14}CO_2$ amounting to about 30 per cent higher value than the controls.

Thus, it is so far established that the actual concentration of free hormones in the circulation interacts with the specific X-ray effect studied here, whereas the mechanism of this reaction is obscure. Apart from this it would appear that the effects may have some bearing on the general problem of studying the biochemical effects of the irradiation using isotope labelled compounds. Obviously the distribution of injected compounds in irradiated animals relative to controls may shift rapidly during the first minutes. This may occasionally induce misleading conclusions as to the irradiation effect studied.

A more detailed account of this work will be published elsewhere.

REFERENCES

[1] Forssberg, A., and Hevesy, G. *Amer. J. Physiol.*, in press.
[2] Hevesy, G. and Forssberg, A. *Nature, Lond.* 1951, **168** 692.
[3] Greenspan, F. S., Li, C. H. and Evans, H. M. *Endocrinology*, 1950, **46** 261.
[4] Kety, S. S. *Amer. J. Med. Soc.* 1948, **215** 352.

DISCUSSION

Z. M. Bacq : I just want to call attention to the following point.

Twenty years ago one spoke of days after irradiation. During the last years we got interested in what happens 30 minutes or one hour after irradiation. Thanks to Hevesy and Forssberg, one sees changes a few minutes after irradiation. This is indeed going in the right direction.

R. H. Mole : I wonder if Forssberg has considered whether changes in intestinal movement or circulation could be responsible for the effect of radiation in decreasing the turnover of an intraperitoneal injection. That changes do occur during or within a few minutes after irradiation was shown by Conard.

A SKIN TEST IN RADIOBIOLOGICAL STUDIES

B. Jolles

Department of Radiotherapy, General Hospital, Northampton

The following new technique has been developed for radiobiological investigations of skin recovery and of spreading factors[1], and for clinical work. When a dose of X-rays is given to circular areas of varying diameters surrounded along half their circumference at a distance of 0·5–1·0 cm by a 0·5–1·0 cm wide crescent-shaped strip (*Figure 1*), the skin reaction of the lower half of the circle, *i.e.* not having in its vicinity an irradiated strip

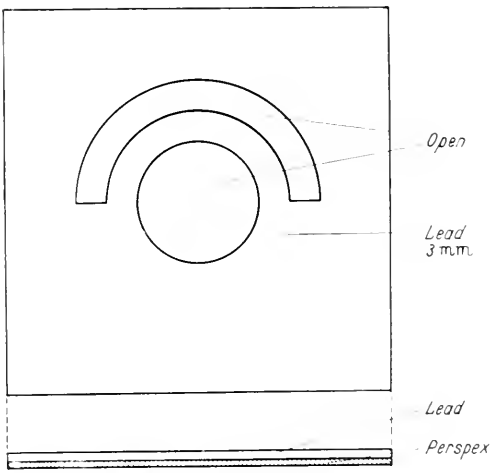


Figure 1. Perspex-backed lead applicator with the 'new moon and sixpence' pattern

of skin, shows a degree of reaction which is less than that on the upper half of the circle and on its neighbouring strip. The potentiation is due to the vicinity of irradiated areas, and the decrease in reaction is due to envelopment of normal tissues. These differences are not brought about by small variations in the dose actually delivered to different parts of the treated areas as measured by Sievert chambers.

Quantitative data can be obtained from readings of the intensity of reaction at different sectors of the irradiated areas of skin by means of a reflectance colorimeter specially constructed to the author's specification by Lovibond, Tintometer Ltd., which enables colour changes to be assessed accurately. The colorimeter is similar in shape and size to an electric hair-drier, having a pistol-type grip and being very easily placed into position. When viewing, a circular split field is presented to the eye, the left-hand segment being the colour under examination and the right-hand one being a standard white surface. Both fields are illuminated by a 12-volt lamp

inside the apparatus. A magazine holds in rotatable discs a series of the well-known Lovibond coloured glass standards of the three primaries red, yellow and blue, and by rotating the discs a suitable selection of glasses can be brought into the line of view in the right-hand side of the field to equal the colour of the object on the left. A normal chest colour would be about 1·7 red, 0·8 yellow, 0·5 blue, whereas in cases of marked erythema the values would be around 3·2 red, 1·1 yellow and 0·6 blue. The most convenient method of tabulating measurements or plotting colour changes is on a linear graph, taking the two predominant primaries and regarding the third primary as an expression of dullness. Therefore, most skin or erythema colours can be plotted as red against yellow, or each primary can be plotted separately against time or other factors.

SCREENING OF 'PROTECTIVE' SUBSTANCES

A method of testing the capacity of substances to protect the skin from X-ray damage was devised. Specimens of substances not identified except

Table I.—Effect on skin reaction of injecting substances intradermally after irradiation

Substance injected	*No. of experiments showing*	
	Protection	*No Protection*
A. β-mercaptoaethylamin	3	2
B. Glucose	5	1
C. Fructose	3	1
D. Tryptamine hydrochloride	6	1
E. Glycine	1	3
F. Thiourea	1	4
G. Urea	3	1
Synkavit	0	3
Hyaluronidase	1	1

The experiments were undertaken to test the method and not the efficacy of the chemical agents used. The identity of the substances *A* to *G* was learned after the series of experiments was concluded.

for their molar weight, and labelled *A* to *G*, were obtained through the kindness of Professor A. Haddow and Dr. P. Alexander of the Chester Beatty Research Institute. This series included substances which have no protective action as well as substances with moderate and marked activity. The strength of solutions used was 0·025 M, 0·005 M and 0·0025 M. In the first series of experiments 1 ml of 0·025 M solution of these substances was injected intradermally *immediately after* a dose of 1,000 r to 1,500 r (60 kV 10 mA filtration inherent in the tube shield only, 25 cm F.S.D., H.V.L. 1 mm Al) was given to a circular and a crescent area of skin of rabbits and the subsequent reaction observed. Some of the protective agents, when injected at 0·025 M, produce locally a more or less intense inflammatory reaction, and the injection on occasion has to be made outside the irradiated area, intradermal diffusion of the agent being relied upon. This was the case, for β-mercaptoaethylamin, but with 0·005 M and 0·0025 M solutions the inflammatory reaction was almost nil and the

decrease in the degree of skin reaction over parts of the irradiated area demonstrated the protective action of some of the substances. Better protection was obtained when 0·005 M and 0·0025 M solutions were given immediately *before* the irradiation. Results of 40 experiments in the first series (0·025 M solution given *after* irradiation) are shown in *Table I.*

Unequivocal results of a further 25 experiments with injection of weaker solutions *before* irradiation, confirmed that the method can detect protective agents and may be useful as a screening test. It offers the advantage that high local concentration of substances can be examined so as to find a protective agent for skin.

TRACING RADIOACTIVE PHOSPHORUS INJECTED INTRADERMALLY

It is difficult, and sometimes impossible, to find out by means of directional counters the whereabouts, and the concentration, of radioactive isotopes introduced intradermally. Radioactive phosphorus or zinc or gold colloid is used experimentally for direct infiltration of tumour masses as well as for intraperitoneal and intrapleural applications. The main requisite is that the injected isotope should remain *in situ* and only conjectural evidence is available that this is so. On the other hand the irregular deposition of particles of the isotope or its diffusion along lines of least resistance within the tissue framework is also known. It is believed that the phosphorus remains *in situ*, especially when suspended in oil[2]. A simple method of mapping out on the skin the whereabouts of the radioactive phosphorus injected intradermally has been devised. It consists in giving a dose of 400–800 r to the skin over the injected site. It was found that the cumulative effect of radiation due to X-rays, and radiation due to the radioactive isotope will produce a greater skin reaction over areas where both are present, than in those areas where X-rays alone are the reaction producing agent. Simple erythema and desquamative reaction on the skin often show the local distribution of isotopes. ^{32}P (orthophosphoric acid) diffuses rapidly in the tissues even in oil suspension. The circle and crescent lead applicator can be used for this purpose when the area to be X-rayed is large, thus reducing the extent of skin reaction.

REFERENCES

[1] JOLLES, B. *Brit. J. Radiol.* 1941, **14** 110 ; *Nature, Lond.* 1949, **164** 63 ; *Brit. J. Radiol.* 1950, **23** 265 ; *British Empire Cancer Campaign, 31st Annual Report*, 1954.

[2] WERFF, VAN DER, J. TH. *Radioisotope Techniques*, 1953, **1** 78. H.M. Stat. Off., London.

DU MECANISME PHYSIOPATHOLOGIQUE DE LA MORT CHEZ LES RATS IRRADIES AVEC UNE DOSE MORTELLE DE R.X.

J. Maisin, H. Maisin et A. Dunjic

Laboratoires de Recherches pour la Protection de la Population Civile, annexés à l'Institut du Cancer de Louvain, Directeur : Prof. J. Maisin

I. *Importance de la protection simultanée d'anses intestinales et de moëlle osseuse*

Jacobson et ses collaborateurs, en extériorisant la rate de souris dans une boîte de plomb, furent les premiers à montrer l'intérêt de la protection physique chez des animaux irradiés *in toto*[1]. Nous avons montré l'intérêt de la protection de la région hépatique du rat comparée à celle offerte par d'autres régions[2]. Cette supériorité de la région hépatique ne se manifeste franchement qu'à partir de doses de l'ordre de 700 r ; avec une dose de 500 r, en effet, il est impossible de distinguer l'avantage possible de la protection de la région hépatique sur la région splénique, la région thoracique, la région intestinale ou sur la protection d'une patte[3].

Gershon-Cohen et ses collaborateurs, irradiant des rats avec 600 r, n'ont pas eux non plus pu mettre en évidence l'avantage de la protection d'une région plutôt que d'une autre[4]. Nous[2] avions insisté sur le fait que la protection de la région hépatique telle que nous la comprenions, c'est-à-dire, à l'aide d'un écran de plomb de 5 mm d'épaisseur et de 12 cm², nous amenait à protéger conjointement au foie, des parties d'autres tissus et organes tels l'estomac, l'intestin, la moëlle osseuse. Nous avions envisagé l'existence d'une synergie possible entre le foie et la moëlle osseuse. Bien que la protection de la région intestinale droite à elle seule donnât une survie totale moitié moindre de celle de la région hépatique, nous n'avions pas soupçonné l'importance du rôle joué par la fraction d'intestin grêle protégé. Depuis nous avons vérifié l'excellente protection offerte par la région hépatique. Au cours d'une expérience, nous avons toutefois été frappé du fait que la protection obtenue variait dans de larges proportions suivant que l'on protégeait ou non, au moyen d'un écran de plomb de 8 cm² la zone médiane du foie[5]. Il semblait donc que l'importance de la protection variait non en fonction du volume de tissu hépatique protégé, mais bien en fonction de l'importance de la portion de moëlle osseuse ou d'intestin protégé.

Pour tenter de solutionner cette question, nous avons fait une série d'expériences en protégeant des surfaces plus petites à l'aide d'un écran de plomb de 5 mm d'épaisseur et de 4 cm² (2 cm × 2 cm) seulement. Nous avons systématiquement protégé à l'aide de ce petit écran divers organes, parties d'organes, ou des associations de plusieurs organes ou tissus de la région thoraco-abdominale du rat[10]. Nous avons irradié des rats provenant de notre stock homozygote au moyen de 700 r sous 200 kV, 15 mil, 1/4 Cu + 1 Al et 55 cm de distance F.P. Notre appareil, un Maximar 250 kV de la General Electric débite 34 r minute mesuré dans l'air à l'intégrateur

Victoreen. Les animaux, d'un poids moyen de 140 g le jour de l'irradiation, sont maintenus immobiles à l'aide d'un gabarit déjà décrit[6]. De façon à travailler dans des conditions anatomiques et physiologiques des plus semblables et aussi parce que les animaux à jeûn résistent mieux à l'irradiation[7], tous nos rats sont maintenus à jeûn, 24 heures avant et après l'irradiation. Tous les animaux ont été suivis durant une période de 30 jours à partir du jour de l'irradiation. Ils ont été pesés journellement.

Nos animaux contrôles irradiés dans ces conditions meurent tous endéans les 11 jours ; 95% d'entre eux sont diarrhéiques, et ils perdent 23,5% de leur poids moyen initial.

Si nous plaçons notre écran de plomb de 4 cm² en diverses positions allant de l'hypochondre droit à l'hypochondre gauche, immédiatement au-dessus d'une ligne virtuelle qui passe sous les derniers rebords costaux, notre pourcentage de survie après 30 jours dans 5 séries de 20 rats est faible et oscille entre 5 et 15%, la meilleure survie est obtenue par la protection d'une surface de 4 cm² correspondant à la projection à la peau de la masse du foie (région hépatique du tableau). Les animaux perdent de 16 à 21,2% de leur poids moyen du jour des R.X. endéans les 5 à 7 jours, et 80 à 95% d'entre eux présentent de la diarrhée.

Quand nous mobilisons l'écran de plomb à cheval sur la ligne sous-costale inférieure, lorsque l'écran est à droite de la colonne, les survies sont de 5/30 (16,6%), lorsqu'il est à gauche et qu'il occupe donc une position correspondant à la projection à la peau de la plus grande partie de la rate, nous n'avons aucun animal qui survit après 17 jours (0/30) ; les survies sont sensiblement meilleures quand l'écran surplombe toute la largeur de la colonne (14/40 = 35%). Ces derniers animaux ne perdent que 14,8% de leur poids moyen initial et 52,5% seulement sont diarrhéiques.

Si nous plaçons notre écran en différentes positions entre le flanc droit et le flanc gauche, en veillant à ce que son bord supérieur soit contigu à une ligne virtuelle passant 5 mm sous le rebord costal, nous obtenons 10% de survie quand nous protégeons le flanc droit ou gauche de nos rats. Par contre, si l'écran est placé à cheval sur la colonne dans une position que nous appellerons sus-ombilicale, la survie passe à 65% (26/40). Ce pourcentage de survies est étonnamment élevé pour une surface protégée aussi réduite.

Le nombre de survivants reste absolument identique (26/40) si nous glissons notre écran dans une position similaire que nous appellerons sous-ombilicale couvrant toujours la colonne mais à 3 mm au-dessus d'une ligne qui réunit les deux trochanters.

Des coupes sur animaux congelés et des radiographies d'animaux prises après administration de baryte, nous permettent de dire, qu'en protégeant les régions sus- et sous-ombilicales, nous protégeons au point de vue tube digestif d'une part, la partie terminale du duodénum, la plus grande partie des anses grêles et un fragment de colon, d'autre part la partie terminale du grêle et la majorité du colon descendant.

Quand l'écran protège un des flancs, nous protégeons quelques rares anses intestinales. Le nombre de diarrhéiques est dans ce cas de 85% et la perte moyenne maximum de poids est de 19,3% alors que celle-ci n'est respectivement que de 9,3% et de 14,5% lorsque nous protégeons ou la région sus-ombilicale ou sous-ombilicale. Chose frappante, si les pourcentages

de survies de ces deux derniers groupes sont semblables, le pourcentage de diarrhée dans le premier de ces derniers groupes est de 12,5% alors qu'il est de 45% dans l'autre.

On pourrait penser que c'est la moëlle, tout au moins en ce qui concerne la survie, qui joue un rôle prépondérant. Ceci n'est que partiellement exact. Certains d'entre nous avaient déjà limité à 700 r la dose pour laquelle la moëlle d'un membre inférieur pouvait encore être utile dans la protection contre les R.X.[8]. Répétant une expérience similaire en déposant notre écran de plomb sur la région sterno-costale, nous n'obtenons chez 20 animaux qu'une survie de 15%, la perte moyenne maximum de poids atteignant le 6me jour 23,1% et 90% d'entre eux étant diarrhéiques. La plupart de ces rats meurent plus rapidement que ceux chez qui nous avons uniquement protégé des anses intestinales (flanc gauche). Devant de tels résultats il semble donc que l'on puisse conclure à la nécessité de la protection simultanée d'anses intestinales et de moëlle.

Pour tacher de pousser l'interprétation des résultats, nous avons pensé qu'il pouvait être intéressant de procéder à l'emploi simultané de 2 ou plusieurs écrans de plomb de 2 cm × 2 cm, de les placer en différents endroits du corps de façon à permettre d'autres combinaisons protectrices et de voir si les protections obtenues étaient des sommations de résultats obtenus à l'aide des écrans employés séparément ou si elles leur étaient supérieures et pouvaient appuyer l'existence d'une synergie[10].

Quand nous plaçons au lieu d'un écran, deux écrans juxtaposés dans le sens cranio-caudal sur la région sterno-costale, nous doublons la surface protégée et nos survies ; 6 rats survivent après 30 jours, soit 30%. Nous doublons également la survie, si au lieu de protéger un flanc nous protégeons les deux flancs simultanément ; 4 animaux sur 20 survivent, soit 20% ; la plupart de ces animaux meurent néanmoins plus tardivement que ceux chez qui nous avons protégé 8 cm² de région sterno-costale. Si nous combinons la protection par un écran de la région sterno-costale à celle de la région hépatique, régions qui toutes deux donnent 15% de survie, nous obtenons chez 20 rats une survie finale de 30%, soit également une sommation.

Si nous allions la protection de la région sus-ombilicale à celle de 4 cm² de région sterno-costale ou de 4 cm² de région hépatique, nous obtenons 80% de survie dans chacun des cas, ce qui équivaut à une sommation des survies obtenues en utilisant les écrans séparément.

Si par contre, nous protégeons simultanément le flanc gauche de l'animal et la région sterno-costale d'une part, ou ce même flanc gauche et la région iléo-trochantérienne d'autre part, nous obtenons respectivement 60 et 55% de survie. Il ne s'agit pas ici d'une sommation qui aurait dû donner des résultats de l'ordre de 25% ; mais bien d'un effet multiplicatif.

Il semble qu'avec des doses de 700 r, l'effet multiplicatif soit limité à des résultats de l'ordre de 60% et qu'il ne s'agit pas d'une question de volume de tissus protégés ; en effet, la protection des régions sus- ou sous-ombilicales, qui sont chacune d'elles d'un volume moitié moindre, donne déjà une survie légèrement plus élevée (65%). Il semblerait donc exister un volume minimum d'intestin et de substance médullaire qui donne une protection qualitative importante et pour l'augmenter il faut augmenter fortement le volume des tissus protégés. Nous ne pouvons toutefois affirmer que tous

les tissus ou parties de tissus soient d'égale valeur. Nous avons, en effet, voulu déterminer quelle était pour 700r, chez nos rats, la surface minimale à protéger pour obtenir 100% de survie. Nous avons obtenu 100% de survie jusqu'au 28me jour de l'expérience en combinant chez 20 rats, la protection des régions sus- et sous-ombilicales, soit 8 cm², alors que la protection simultanée des flancs gauche et droit et d'une région thoracique, soit de 12 cm², dans un autre groupe de 20 rats ne nous donnait que 60% de survie. La protection d'un quart du corps d'un rat, que celui-ci soit inférieur ou supérieur droit ou gauche, la limite entre les 1/4 supérieurs et inférieurs étant constituée par la ligne sous-costale, ne nous donne jamais dans 4 groupes de 10 rats que 40 à 60% de survie après 30 jours. Il est toutefois important de noter que si l'on protège un des quarts inférieurs il n'y a pas de rats qui soient diarrhéiques.

La protection de la moitié inférieure du corps donne comme on est en droit de s'y attendre 100% de survie et aucun animal n'a la diarrhée. La protection de la moitié supérieure du rat donne une survie très élevée, mais tous les rats présentent pratiquement de la diarrhée.

Le *Tableau I* récapitule les taux de survie et donne une idée de la perte maximale de poids et du pourcentage de diarrhées présentées par nos divers groupes d'animaux qui comptent un minimum de 20 rats. La perte maximale de poids se produit entre le 4me et le 7me jour et correspond dans tous les groupes considérés à ce que nous appellerons par après, la première chute pondérale.

Tableau I

Nombre de rats	*Régions protégées*	*Surface protégée* cm²	*Animaux diarrhéiques* %	*Moyenne des maximum de perte de poids exprimée en % de la moyenne des poids initiaux*	*Survie après* 30 jours
20	*Hypochondre droit*	4	90	19	1 (5%)
20	*Hépatique*	..	80	21,2	3 (15%)
40	*Épigastrique*	..	95	20,8	3 (7,5%)
20	*Hypochondre gauche*	..	90	16	1 (5%)
30	*Hypochondro-flancale droite*	..	73,3	17,7	5 (16,6%)
40	*Épigastro-sus-ombilicale*	..	52,5	14,8	14 (35%)
30	*Hypochondro-flancale gauche*	..	90	16.7	0 (*après* 17 j.)
20	*Flanc gauche*	..	85	19,3	2 (10%)
40	*Sus-ombilicale*	..	12,5	9,3	26 (65%)
40	*Sous-ombilicale*	..	45	14,5	26 (65%)
20	*Sterno-costale*	..	90	23,1	3 (15%)
20	*Sterno-costale*	8	95	20,5	5 (25%)
20	*Flanc droit et gauche*	..	5	7,8	4 (20%)
20	*Sterno-costale et hépatique*	..	95	19,6	6 (30%)
20	*Sterno-costale et sus-ombilicale*	..	0	8,7	16 (80%)
20	*Hépatique et sus-ombilicale*	..	0	8,5	16 (80%)
20	*Flanc gauche et sterno-costale*	..	40	15,5	12 (60%)
20	*Flanc gauche et trochantérienne*	..	20	13,7	11 (55%)
20	*Sus- et sous-ombilicales*	..	0	9,3	19 (95%)
20	*Deux flancs et sterno-costale*	12	30	12,6	11 (55%)
40	*Contrôles*	—	95	23,5	0 (*après* 11 j.)

A la lecture de ce *Tableau I*, nous pensons pouvoir conclure qu'il existe une relation entre l'existence d'un faible pourcentage de diarrhées, une perte de poids peu importante et l'importance ou la qualité de la région intestinale protégée. En effet, les animaux qui subissent les pertes de poids les moins importantes sont ceux qui ont eu la région sus-ombilicale ou les deux flancs protégés et ceux qui subissent les pertes de poids les plus importantes sont ceux chez qui on n'a pas protégé d'anses intestinales. Pour prouver cette hypothèse, nous avons séparé dans des groupes qui comprenaient approximativement 50% de rats diarrhéiques, les animaux non diarrhéiques et diarrhéiques en deux sous-groupes distincts. Nous avons alors calculé la moyenne des maximum de perte de poids exprimée en % de la moyenne des poids initiaux dans les sous-groupes considérés, et comparé entre eux les sous-groupes ayant été irradiés dans les mêmes conditions.

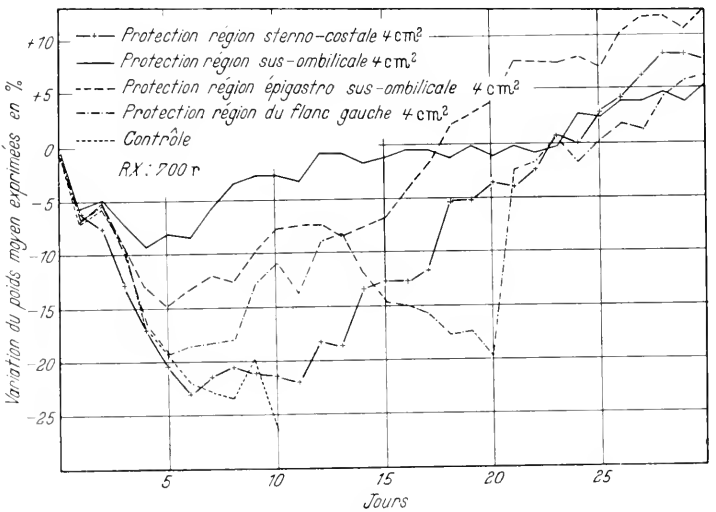


Figure 1

Nous donnons en exemple les rats chez lesquels nous avons protégé la région épigastro-sus-ombilicale et la région sous-ombilicale. Nous avons obtenu une perte de poids de 11,8% parmi les animaux non diarrhéiques dont la région épigastro-sus-ombilicale avait été protégée alors que la perte de poids des animaux du même groupe qui avaient eu la diarrhée était de 18%. Nous obtenons un écart du même ordre chez les rats dont la région sous-ombilicale est protégée, en effet, la perte de poids des rats diarrhéiques est de 20,9% alors que les animaux non diarrhéiques ne perdent que 12,6% de leur poids.

Une preuve indirecte de la relation qui existe entre la protection d'anses intestinales et le début de l'allure de courbes de poids réside dans le fait que les animaux qui n'ont eu que de la moëlle osseuse protégée ne reprennent du poids (*Figure 1*) que tardivement, soit entre le 10me et le 15me jour après l'irradiation, alors que ceux qui ont eu des anses intestinales protégées reprennent du poids dès le 6me–7me jour.

Ce faible pourcentage d'animaux diarrhéiques dans un groupe considéré va de paire avec une survie importante chaque fois qu'en même temps que

l'intestin, nous protégeons un certain volume de moëlle osseuse. D'autre part dans les divers groupes considérés nous avons pu constater que les animaux qui ont des anses intestinales protégées, mais chez qui nous n'avons pas protégé de moëlle osseuse (protection d'un ou des deux flancs par exemple), ou chez qui nous avons protégé peu de moëlle osseuse (protection d'une région hypochondro-flancale par exemple), présentaient, après avoir repris du poids à la suite de leur première chute pondérale, une deuxième chute de poids entre le 10me et le 20me jour.

La *Figure 1* ci-contre dans laquelle nous avons réuni les courbes de poids d'animaux dont le flanc gauche, la région sterno-costale, la région sus-ombilicale ou la région épigastro-sus-ombilicale a été protégée illustre bien ces diverses constatations.

Nous avons voulu pousser plus avant l'interprétation de ces résultats et voir si la protection de toute la masse des anses intestinales à elle seule ne pouvait donner une survie intéressante. La façon la plus élégante et la plus parfaite de le faire était d'extérioriser et de protéger dans une boîte de plomb les parties mobilisables de l'intestin de nos rats durant l'irradiation et d'irradier conjointement divers groupes témoins[11].

Nous avons réparti nos animaux en 4 groupes. Le groupe I est constitué d'animaux dont les intestins ont été extériorisés mais non protégés. Le groupe II comprend des animaux dont les intestins sont extériorisés et protégés. Le groupe III comprend des animaux à intestins extériorisés et non protégés dont la colonne dorso-lombaire est protégée et le groupe IV des animaux dont l'intestin extériorisé et la colonne dorso-lombaire sont tous deux protégés.

Nous anesthésions nos animaux avec du nembutal injecté par voie sous-cutanée à raison de 7,5 mg/100 mg de poids. Nous faisons une incision médiane de la paroi abdominale allant de 1 cm au-dessus du pubis jusqu'à hauteur d'une ligne tangente aux rebords costaux inférieurs. Nous sortons hors de la cavité abdominale, la masse mobile de l'intestin grêle et la région iléo-coecale, nous les entourons de gaze imprégnée de solution physiologique, les plaçons à côté du rat ou dans une boîte de plomb spécialement dessinée dont la paroi et le couvercle ont 1 cm d'épaisseur ou, dans le couvercle d'une boîte de Pétri, suivant que les intestins sont ou non protégés. Pendant l'irradiation nous recouvrons la plaie béante, de gaze imprégnée de solution physiologique. L'irradiation terminée nous remettons les anses dans la cavité abdominale et en suturons la plaie en deux plans par points séparés en ayant soin de déposer des sulfamidés en poudre sur chaque suture. Quand nous protégeons la colonne nous le faisons à l'aide d'une plaque de plomb de 5 mm d'épaisseur et de 4 cm × 2 cm de surface enveloppée de gaze imprégnée de solution physiologique. Cette plaque est mise au devant des dernières vertèbres dorsales et des vertèbres lombo-sacrées du rat dans le sens de sa longueur. Nous protégeons donc ainsi non seulement une partie de la colonne, mais un fragment de la partie inférieure des deux reins, une partie de la chaîne sympathique, les colons descendant et sigmoïde et les deux iléons jusqu'à hauteur de la ligne bitrochantérienne. Tous ces groupes d'animaux reçoivent une dose unique de 700 r dans les conditions déjà décrites.

Nos résultats sont consignés dans le *Tableau II.*

Tableau II

Nombre de rats	*Conditions expérimentales*	*Animaux diarrhéiques*	*Moyenne des maximum de perte de poids exprimée en % de la moyenne des poids initiaux*	*Survie après* 30 jours
40	*Contrôles non opérés*	95%	23,5	0 (*après* 11 j.)
19	*Gr. I (intestin extériorisés non protégés)*	17 (89,4%)	18,6	0 (*après* 8 j.)
24	*Gr. II (intestins extériorisés et protégés*	7 (29,15%)	16,8	0 (*après* 12 j.)
19	*Gr. III (intestins extériorisés non protégés et colonne dorso-lombaire protégée)*	12 (63,15%)	16,8	7 (36,8%)
19	*Gr. IV (intestins extériorisés et protégés et colonne dorso-lombaire protégée)*	3 (15,78%)	13,2	14 (73,60%)

Nous avons alors voulu dans une dernière expérience éliminer tous les tissus autres que la moëlle et l'intestin[11]. On sait, par une expérience antérieure[9] que, au niveau de la patte, les tissus autres que le tissu osseux ne jouent aucun rôle dans la protection. La dose de R.X. reçue par tous les animaux est toujours de 700r. Nous avons dès lors irradié par 700r deux nouveaux groupes d'animaux. Dans l'un (groupe V) nous avons extériorisé les anses intestinales sans les protéger, mais nous avons protégé une patte depuis le 1/3 inférieur du fémur jusqu'à l'extrémité des ongles. Dans le deuxième groupe (groupe VI) nous avons protégé à la fois l'intestin extériorisé et une patte dans les mêmes conditions que pour le groupe V.

En voici les résultats (*Tableau III*).

Tableau III

Nombre de rats	*Conditions expérimentales*	*Animaux diarrhéiques*	*Moyenne des maximum de perte de poids exprimée en % de la moyenne de poids initiaux*	*Survie après* 30 jours
19	*Gr. V (intestins extériorisés non protégés et une patte protégée)* ..	14 (73,6%)	18,6	4 (21,05%)
20	*Gr. VI (intestins extériorisés et protégés et une patte protégée)* ..	3 (15%)	17,5	13 (65%)

Les résultats obtenus dans les groupes V et VI sont superposables aux résultats obtenus respectivement dans les groupes III et IV. Les différences de survies obtenues entre les groupes III et V s'expliquent, à notre avis, par le fait que dans le groupe III on protège le colon descendant. Les courbes de poids de ces 6 groupes d'animaux sont moins bonnes que celles de groupes

non opérés ayant subi des protections d'organes ou tissus identiques. Nous attribuons cette différence à l'intervention chirurgicale. Ces courbes de poids ont néanmoins une allure semblable à celles de ces animaux non opérés. Il est donc montré que la moëlle osseuse en association avec l'intestin joue un rôle de premier plan dans le mécanisme qui assure la survie des rats irradiés par une dose sûrement mortelle de R.X. Il semble bien que l'on soit autorisé à penser qu'il existe une synergie entre intestin et moëlle osseuse. On peut se demander toutefois si le rôle de l'intestin protégé se limite à un rôle banalement nutritif permettant de continuer à digérer et à absorber les matériaux ordinaires indispensables à la vie et à la fonction médullaire ou bien si à son niveau sont élaborés des facteurs de caractères spécifiquement indispensables à la fonction médullaire normale. De façon à pousser plus avant l'interprétation du rôle joué par l'intestin nous avons injecté, par voie intra-péritonéale, des homogénats de la partie initiale du grêle comme tel ou du grêle dont la flore microbienne avait été détruite à l'aide de streptomycine. Jusqu'à ce jour nous n'avons guère obtenu de résultats positifs[17].

RÉSUMÉ

Nous avons montré dans la première partie de ce travail, l'intérêt de la protection simultanée d'un volume suffisant d'anses grêles et de moëlle osseuse chez le rat. Elle permet d'obtenir à 700 r, pour des surfaces protégées de 4 ou 8 cm², des taux de survie variant de 65 à 100% après 30 jours, alors que la protection par des surfaces identiques d'un seul de ces deux tissus ne donne que des survies de l'ordre de 15 à 30% après 30 jours. Les animaux contrôles meurent tous endéans les 11 jours. La protection d'un volume suffisant d'anses intestinales permet de réduire fortement la perte de poids initiale des animaux, celle-ci est maximale du 4me au 7me jour après l'irradiation et semble être proportionnelle aux phénomènes diarrhéiques. Nous avons également mis en évidence chez les animaux chez lesquels nous n'avons protégé que des anses intestinales ou chez lesquels nous avons protégé des anses intestinales et un volume insuffisant de moëlle osseuse, l'apparition d'une deuxième chute pondérale survenant entre le 10me et le 20me jour après l'irradiation ; celle-ci fait suite à un regain passager de poids et n'apparaît guère chez les rats dont un volume suffisant de moëlle osseuse a été protégée concomitamment aux anses intestinales. Les animaux chez qui nous ne protégeons que de la moëlle osseuse subissent une perte de poids très importante et ne regagnent du poids qu'après le 10me jour après l'irradiation.

II. *Influence de l'injection de mercaptoléthylamine (MEA) avant l'irradiation sur la survie des animaux injectés après l'irradiation d'une suspension de moëlle osseuse.*

Bacq et ses collaborateurs[12 13] ont montré l'intérêt de la MEA comme moyen de protection contre les radiations chez la souris. Maisin et ses collaborateurs[14] ont confirmé cette action chez le rat et ont mis en évidence l'intérêt de la protection mécanique de la région hépatique durant l'irradiation[2]. L'un de nous (H.M.), en collaboration avec Wolfe, Tobias et Lawrence[15], a combiné l'injection de MEA avant l'irradiation à la protection de la région hépatique par un écran de plomb de 8 cm² pendant

l'irradiation. Cette expérience réalisée sur des rats Long Evans irradiés de 850 r a donné une survie de 80% après 30 jours alors que l'on n'obtenait que 10% de survie chez les animaux injectés de MEA seule et 40% chez ceux dont la région hépatique seule était protégée.

Nous avons répété cette expérience[16] sur notre race de rats blancs homozygotes avec des doses de 700 r et 850 r sous 200 kV, 57 cm F.P. et 1/4 Cu + 1 Al. Nous avons utilisé des groupes d'animaux d'un poids moyen de 140 g. Les animaux qui ont reçu 700 r n'ont pas été soumis au jeûn. Les rats qui ont reçu 850 r ont été maintenus à jeûn 24 heures avant et après l'irradiation comme à l'accoutumée. Nous injectons les rats préalablement à l'irradiation de 10 mg de MEA par voie intra-péritonéale. Nous avons irradié des groupes de 20 rats à 700 r et des groupes de 40 rats à 850 r. Nos résultats sont reproduits dans le *Tableau IV* ci-après. Ils confirment ceux obtenus chez les rats Long Evans. Rappelons qu'en protégeant la région hépatique, nous réalisons une protection simultanée de tissus hépatique, de moëlle osseuse et de quelques anses intestinales[10]. Il était intéressant de voir si ces trois tissus devaient être protégés simultanément ou si la protection d'un ou de deux d'entre eux associée à l'injection préalable de MEA ne donnerait pas d'aussi bons résultats.

Nous avons alors associé[16] chez divers groupes de 20 animaux chacun, l'injection de 10 mg de MEA avant l'irradiation à la protection par un écran de plomb de 4 cm², (1°) de la région sterno-costale, (2°) de la région hépatique centrale ou zone épigastrique permettant la protection simultanée de moëlle osseuse et de tissu hépatique, (3°) de la région sus-ombilicale, réalisant la protection simultanée de moëlle et d'anses intestinales, (4°) du flanc gauche, région qui ne contient que quelques anses intestinales. Les repères utilisés pour délimiter ces différentes aires de protection ont été décrits par ailleurs[10]. Nous avons également irradié des groupes de 20 animaux chacun, chez lesquels nous avons protégé uniquement les régions que nous venons de signaler. Tous ces groupes d'animaux dont le poids moyen oscille entre 160 et 170 g ont reçu 850 r. Ils ont été maintenus à jeûn 24 heures avant et après l'irradiation. Le *Tableau IV* ci-après montre les divers taux de survie. On voit que chaque fois que l'on associe la protection de moëlle osseuse (région sterno-costale, épigastrique ou sus-ombilicale) à l'injection de MEA avant l'irradiation on obtient environ 75% de survie. Quand on associe uniquement une protection intestinale (flanc gauche) à l'injection de MEA, les résultats sont moins intéressants. La protection mécanique seule ne donne une survie intéressante que lorsqu'on protège la région sus-ombilicale ; ceci prouve une fois encore l'importance de la protection simultanée de moëlle et d'intestin[10, 11].

Ces divers résultats sont consignés dans le *Tableau IV* (page 163).

Dans le *Tableau IV*, il semble exister deux anomalies. Les pertes de poids des rats ayant reçu 700 r sont supérieures à celles de ceux qui ont reçu 850 r dans les mêmes conditions expérimentales. Cela s'explique par le fait que les premiers ne sont pas à jeûn ; ces animaux ont, en effet, une chute de poids plus prononcée après une irradiation aussi importante. Par ailleurs, le taux d'animaux diarrhéiques et la perte de poids des rats qui ont été protégés conjointement par l'écran de 4 cm² et une injection de MEA avant l'irradiation, sont supérieurs à ceux des rats ayant été uniquement

Tableau IV

Dose de R.X. administrée r	*Nombre de rats*	*Conditions expérimentales*	*Surface protégée* cm²	*Animaux diarrhéiques* %	*Moyenne des maximum de perte de poids exprimée en* % *de la moyenne des poids initiaux*	*Nombre de rats en vie après* 30 jours
700	20	*Rég. hépatique + inj. MEA avant* ..	8	0	10,7	18 (90 %)
..	20	*Rég. hépatique* ..	,,	25	16,3	18 (90 %)
..	20	*Inj. MEA avant* ..	—	5	12,7	5 (25 %)
..	40	*Contrôles*	—	95	23,5	0 (*après* 11 j.)
850	40	*Rég. hépatique + inj. MEA avant* ..	8	10	4,8	37 (92,5 %)
..	40	*Rég. hépatique* ..	,,	90	21,1	3 (7,5 %)
..	40	*Inj. MEA avant* ..	—	12,5	7,9	5 (12,5 %)
850	20	*Rég. sterno-costale + inj. MEA avant* ..	4	35	14,5	15 (75 %)
..	20	*Rég. épigastrique + inj. MEA avant* ..	..	35	14,25	16 (80 %)
..	20	*Rég. sus-ombilicale + inj. MEA avant* ..	..	20	9,4	14 (70 %)
..	20	*Rég. flanc gauche + inj. MEA avant* ..	..	20	12,25	6 (30 %)
..	20	*Rég. sterno-costale* ..	..	100	19,25	0 (*après* 5 j.)
..	20	*Rég. épigastrique* ..	..	90	18,6	0 (*après* 6 j.)
..	20	*Rég. sus-ombilicale* ..	..	70	18,5	7 (35 %)
..	20	*Rég. flanc gauche* ..	..	95	25,5	0 (*après* 10 j.)
..	20	*Contrôles*	—	100	33,5	0 (*après* 8 j.)

injectés de MEA avant l'irradiation. Cela s'explique par le fait que si tous ces animaux ont reçu 10 mg de MEA, les premiers pèsent plus que les seconds et ont donc reçu une dose de MEA proportionnellement moindre.

Au cours de ces recherches, nous avons pu faire deux observations importantes, pensons-nous, au point de vue de la compréhension des mécanismes d'action tant de la MEA que de la protection mécanique de l'intestin. Nous avons noté que les animaux injectés d'une dose suffisante de MEA avant l'irradiation présentaient peu de phénomènes intestinaux (diarrhées) et une perte de poids moins importante pendant les premiers jours qui suivent l'irradiation. Ce sont là, deux raisons parmi celles qui leur permettent de survivre assez longuement après l'administration de la dose mortelle de R.X. Ces animaux reprennent du poids dès le 5me jour. La majorité meurt néanmoins, après une dose de 850 r, en présentant une aplasie marquée de la moëlle osseuse. La quasi totalité de ces rats présentent une deuxième chute pondérale. Nous avions noté précédemment déjà[10] que des animaux irradiés de 700 r chez qui on protège un volume suffisant d'anses intestinales ne présentent qu'une perte de poids peu importante et peu ou pas de diarrhées, qu'ils reprennent du poids, mais que s'ils n'ont pas eu concomitamment un volume suffisant de moëlle osseuse protégée, ils présentent une deuxième chute pondérale et meurent généralement.

Nous avons représenté dans la *Figure 2* les courbes de poids d'animaux irradiés par 850r avec leur région hépatique protégée mécaniquement, avec ou sans injection préalable de MEA, celles des animaux qui n'ont subi que l'injection de MEA préalablement à l'irradiation et celles des animaux irradiés contrôles. Leur lecture permet de voir que les animaux injectés de MEA seul se comportent comme des rats irradiés par 700r, dont l'intestin aurait été protégé mécaniquement. Ils présentent, en effet, le 3ème jour après l'irradiation, une première chute de poids très peu importante qui est suivie, après une reprise nette de poids, d'une seconde chute pondérale beaucoup plus importante se situant entre le 13ème et le 17ème jour ; nous croyons que cette dernière chute pondérale est due à l'absence de protection de moëlle osseuse. De fait les animaux, qui, outre l'injection de MEA avant l'irradiation, ont eu la région hépatique protégée mécaniquement, qui ont donc eu quelques hémi-vertèbres dorsales et quelques segments

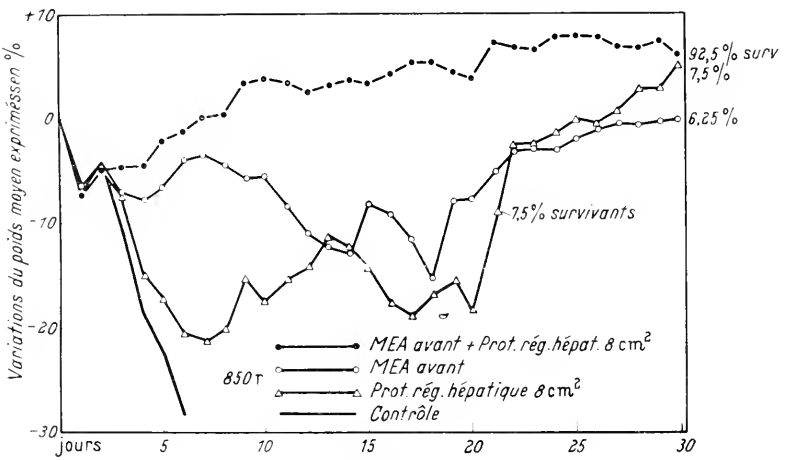


Figure 2

costaux protégés, ne présentent guère cette 2ème chute pondérale. A 850r, quoiqu'en protégeant mécaniquement la région hépatique, nous protégeons quelques anses intestinales ; nous ne réduisons pourtant guère la première chute de poids et nous n'empêchons pas une importante deuxième chute pondérale. Nous croyons que cela est dû au fait que chez des animaux ainsi protégés nous ne protégeons pas suffisamment d'intestin. En effet, quand à 850r on protège plus d'anses intestinales et un volume analogue de moëlle osseuse comme c'est le cas par exemple en protégeant la région sus-ombilicale (*Tableau IV*), on réduit forte-ment la première chute pondérale sans, toutefois, encore supprimer entièrement la seconde.

L'étude histologique des intestins des animaux injectés de MEA avant une irradiation de 700r et ceux dont l'intestin a été protégé mécaniquement pendant une irradiation de 700r permet de constater que 24 heures après l'irradiation, les lésions intestinales de ces animaux sont les mêmes que celles provoquées chez les contrôles. Mais les rats dont la région sus-ombilicale a été protégée ou qui ont été injecté de MEA présentent respectivement après la 48ème et 72ème heure une activité mitotique intense au

niveau de leurs glandes de Lieberkuhn suivie d'une réparation très rapide de leurs lésions, alors que, chez les animaux contrôles, cette activité mitotique ne débute qu'à partir du 4ème–5ème jour. Nous décrivons ces lésions en détail dans une autre communication de ce symposium[18]. Il se pourrait donc que l'absence de diarrhée chez les animaux injectés de MEA avant l'irradiation et chez ceux qui ont eu l'intestin protégé mécaniquement soit due à une régénération plus rapide de l'intestin.

Ces observations nous ont poussé à rechercher l'influence de l'injection intrapéritonéale de moëlle osseuse après l'irradiation chez des animaux injectés, avant l'irradiation, de MEA[16]. Tous les animaux utilisés ici ont un poids moyen de 140 g et ont été maintenus à jeûn de la façon habituelle. Dans ce but, nous avons injecté une suspension de moëlle osseuse dans du chlorure de sodium à 9,00/00, chez des rats, immédiatement après, 6 heures et 24 heures après l'irradiation (groupes I, V et VII du *Tableau I*). Comme contrôles, nous avons également irradié des animaux uniquement injectés de moëlle osseuse directement et 6 heures après l'irradiation (groupes IV et VI). D'autres rats injectés de MEA avant l'irradiation ont reçu de la moëlle de jeunes rats de 5 à 6 semaines qui avait été maintenue à 0°C (groupe VIII) ou à —40°C (groupe IX) pendant 18 heures. Chaque animal irradié reçoit la moëlle osseuse des fémurs, tibias et humérus d'un rat de 5-6 semaines et pesant environ 50 g. Nous avons injecté à un autre groupe d'animaux, préalablement injecté de MEA, la moëlle osseuse des fémurs, tibias et humérus de deux jeunes rats de 5 à 6 semaines (groupe II). Nous avons également injecté, à des rats préalablement injectés de MEA, la moëlle d'un fémur, d'un tibia et d'un humérus d'un rat adulte (groupe III). Finalement, une dernière série de rats injectés de MEA avant l'irradiation a été injectée de la moëlle d'un fémur, d'un tibia et d'un humérus, d'un jeune cobaye de 5–6 semaines pesant environ 150 g (groupe X). Etant donné que l'injection de MEA avant l'irradiation semble jouer le rôle d'une protection mécanique pendant l'irradiation, nous avons voulu compléter cette série d'expériences, en injectant de la moëlle osseuse de jeunes rats chez des rats chez qui nous avons protégé mécaniquement de l'intestin pendant l'irradiation. Nous l'avons réalisé en protégeant les deux flancs des rats par deux écrans de plomb de 4 cm^2 (groupe XI). Nous avons évidemment également irradié des animaux chez lesquels nous n'avions protégé que les deux flancs, comme contrôles (groupe XII).

Nos résultats sont rassemblés dans le *Tableau V*.

A la lecture de ce tableau, on peut conclure, que l'injection de moëlle osseuse directement après et 6 heures après l'irradiation permet d'obtenir des survies considérables après 30 jours pour autant que l'on injecte les animaux de MEA préalablement à l'irradiation. L'injection de moëlle à elle seule ne donne aucune survie après 8 jours et l'injection de MEA seule ne donne que 6,25 pour cent de survie après 30 jours. Nous permettons donc à un nombre intéressant d'animaux dont la plupart auraient succombé, s'il n'avait subi qu'une injection de MEA préalablement à l'irradiation, de franchir la période critique pendant laquelle s'installe l'aplasie médullaire. Rappelons qu'à 850r, la protection mécanique de moëlle osseuse seule (*Tableau II*) ne permet aucune survie. Les résultats obtenus dans le groupe II semblent montrer qu'il est inutile d'injecter des quantités de moëlle

supérieures à celles que nous avons injectées dans le groupe I. Un délai de 24 heures entre l'irradiation des animaux préalablement injectés de MEA et l'injection de moëlle osseuse semble trop long car la survie devient moins

Tableau V

Nombre de rats	*Conditions expérimentales* *Dose de R.X. administrée :* 850 r *en tous cas*	*Animaux diarrhéiques* %	*Moyenne des maximum de perte de poids exprimée en* % *de la moyenne des poids initiaux*	*Nombre de rats en vie après* 30 jours
59	Gr. I. *Inj. intrapérit. moëlle osseuse un jeune rat directement après irradiation + inj. MEA avant* ..	23,38	13,1	32 (54,2 %)
19	Gr. II. *Inj. intrapérit. moëlle osseuse deux jeunes rats directement après irradiation + inj. MEA avant* ..	10,5	11,7	9 (47,3 %)
9	Gr. III. *Inj. intrapérit. moëlle osseuse rat adulte directement après irradiation + inj. MEA avant* ..	44,4	13,9	2 (22,2 %)
10	Gr. IV. *Inj. intrapérit. moëlle osseuse jeune rat, directement après irradiation*	100	24,4	0 (*après* 6 j.)
29	Gr. V. *Inj. intrapérit. moëlle osseuse jeune rat, 6 heures après irradiation + inj. MEA avant*	25,1	13,6	12 (41,4 %)
10	Gr. VI. *Inj. intrapérit moëlle osseuse jeune rat, 6 heures après irradiation*	100	31,2	0 (*après* 8 j.)
20	Gr. VII. *Inj. intrapérit. moëlle osseuse jeune rat 24 heures après irradiation + inj. MEA avant* ..	40	14	6 (30 %)
20	Gr. VIII. *Inj. intrapérit. moëlle osseuse jeune rat maintenu à* 0° C *durant 18 heures directement après irradiation + inj. MEA avant* ..	30	12,7	6 (30 %)
29	Gr. IX. *Inj. intrapérit. moëlle osseuse jeune rat maintenu à* —40° C. *durant 18 heures directement après irradiation + inj. MEA avant* ..	17,2	13,4	4 (13,8 %)
40	Gr. X. *Inj. intrapérit. moëlle osseuse jeune cobaye directement après irradiation + inj. MEA avant* ..	35	15,6	4 (10 %)
20	Gr. XI. *Protection région flanc droit et gauche* (8 cm²) *+ inj. intrapérit. moëlle osseuse jeune rat, directement après irradiation*	30	26,6	3 (15 %)
20	Gr. XII. *Protection flanc droit et gauche* (8 cm²)	35	33,6	0 (*après* 9 j.)
80	*Inj. MEA avant*	23,8	13,2	5 (6,25 %)
40	*Contrôles*	100	33,5	0 (*après* 8 j.)

intéressante. La conservation de la moëlle à 0° C diminue également l'efficacité de la moëlle injectée.

Les très basses températures sont néfastes à la conservation d'une moëlle encore active, les résultats obtenus ne diffèrent guère de ceux obtenus chez des rats uniquement injectés de MEA.

Nous croyons être en droit de pouvoir dire à l'heure actuelle que ces résultats ne sont pas nécessairement dus à la pullulation sur place des cellules injectées. En effet, nous n'avons pu trouver trace dans le péritoine, de la moëlle injectée, 2, 3 et 6 jours après cette injection. Ces observations ont portés chaque fois sur 3 animaux.

Les cellules injectées ont-elles été absorbées comme telles ou sont-elles détruites sur place? En d'autres mots sont-ce les cellules injectées ou un facteur qui est responsable de la survie de certains animaux? Nous ne sommes guère encore en mesure de répondre à ce dilemme. Remarquons, toutefois, que les survies obtenues à la suite d'injection de moëlle de cobaye ne sont pas démonstratives. Ces derniers résultats ne plaident donc pas pour l'existence d'un facteur. D'autres travaux sont cependant nécessaires avant de pouvoir conclure.

Pour terminer, nous constatons que la protection pendant l'irradiation des anses intestinales par deux écrans de plomb de 4 cm^2 placés au niveau de chacun des flancs chez des animaux injectés de moëlle osseuse après l'irradiation, permet comme on était en droit de s'y attendre à un certain pourcentage d'animaux de survivre. La différence de survie entre ces rats et ceux qui ont seulement eu les anses intestinales des deux flancs protégées est significative. La survie des animaux dont l'intestin a été protégé de cette façon est néanmoins beaucoup moins intéressante que celle des animaux chez qui l'on a remplacé cette protection par une injection de MEA. Peut-être ne protégeons-nous pas suffisamment d'anses intestinales? La perte maximum de poids semble plaider en faveur d'une telle hypothèse. Nous avons essayé de répéter une expérience semblable en protégeant chez 20 animaux toute la masse des anses intestinales, extériorisée dans une boîte de plomb, mais sans succès. En effet, à 850r, ces animaux ne résistent pas au choc opératoire et meurent tous endéans les 9 jours.

Les courbes de poids des animaux injectés de MEA avant l'irradiation et après celle-ci de moëlle osseuse sont, chaque fois que l'on injecte de la moëlle de jeune rat dans un délai de 6 heures et quelque soit la quantité injectée, semblables à celles d'animaux chez qui l'on aurait protégé de la moëlle osseuse mécaniquement après les avoir injectés de MEA. Remarquons que la courbe de poids des animaux qui ont reçu, outre, l'injection de MEA une injection de moëlle de cobaye se comporte d'une façon assez semblable à celles des rats uniquement injectés de MEA. L'allure de la courbe de poids des animaux injectés de moëlle de jeune rat conservée à $-40°$ C n'est guère meilleure. Les courbes de poids des animaux injectés de moëlle de rat adulte ou de moëlle de jeune rat conservée à $0°$ C sont intermédiaires entre ces dernières et celles de ceux injectés endéans les 6 heures après l'irradiation. La courbe de poids des animaux à intestin protégé est la plus mauvaise. Elle se caractérise surtout par le fait que la première chute pondérale est très importante, elle atteint 26,6% et elle reste basse jusqu'au 16ème jour, comme si le volume d'intestin protégé était nettement insuffisant, hypothèse que nous avons déjà formulée plus haut.

RÉSUMÉ

Nous avons notamment montré dans la deuxième partie de ce travail que l'injection de MEA avant l'irradiation diminue les phénomènes gastro-intestinaux et la perte de poids concommitante à ceux-ci, comme si on

protège l'intestin de ces animaux mécaniquement. Ces animaux présentent d'ailleurs, tout comme ceux chez qui on n'aurait protégé que l'intestin mécaniquement, une deuxième chute pondérale et une survie finale peu importante ; à 850r il n'y en a que 6,25% qui survivent 30 jours. Pour augmenter ce taux de survie et le faire passer à 75% par exemple et supprimer la deuxième chute pondérale, il faut protéger concomitamment un volume suffisant de moëlle osseuse. Nous montrons par ailleurs que l'injection de MEA avant une irradiation de 850r permet d'obtenir un taux de survie atteignant de 45–60% après 30 jours chez des animaux injectés d'une suspension de moëlle osseuse homologue endéans les 6 heures après l'irradiation; ces animaux ne présentent pas de deuxième chute pondérale. L'injection de moëlle osseuse seule ne permet pas aux animaux de survivre au delà de 8 jours. Les résultats obtenus en injectant de la moëlle de cobaye à des rats préalablement injectés de MEA, sont peu concluants.

Nous tenons à remercier notre laborantine Mademoiselle A. Guillaume de son assistance technique dévouée.

La mercaptoéthylamine utilisée au cours de ce travail provient des Laboratoires Labaz à Bruxelles.

REFERENCES

[1] Jacobson, L. O., Marks, E. K., Robson, M. J., Gaston, E. et Zirkle, R. E. *J. Lab. clin. Med.* 1949, **34** 1538.

[2] Mandart, M., Lambert, G., Maisin, H. et Maisin, J. *C.R. Soc. Biol. Paris*, 1952, **146** 1647.

[3] Maisin, J., Mandart, M. et Lambert, G. Communication faite au premier Congrès des médecins électroradiologistes de culture latine (Bruxelles, juillet 1951). *J. belge Radiol.* 1952, **35** 337.

[4] Gershon-Cohen, J., Hermel, M. B. et Griffith, J. Q., Jr. *Science*, 1951, **114** 157.

[5] Maisin, J., Dunjic, A., Van Lancker, J., Lambert, G. et Passau, L. *C.R. Soc. Biol. Paris*, 1953, **147** 1520.

[6] Mandart, M., Lambert, G. et Maisin, J. *ibid*, 1952, **146** 1305.

[7] Maisin, J., Van Lancker, J., Dunjic, A., Lambert, G. et Passau, L. *ibid*, 1952, **147** 1517.

[8] Mandart, M., Lambert, G. et Maisin, J. *ibid*, 1952, **146** 1645.

[9] Mandart, M., Lambert, G. et Maisin, J. *ibid*, 1952, **146** 1392.

[10] Maisin, J., Dunjic, A. et Maisin, H. *ibid*, 1954, **148** 611.

[11] Maisin, J., Maisin, H. et Dunjic, A. *ibid*, 1954, **148** 743.

[12] Bacq, Z. M., Herve, A., Lecomte, J., Fischer, P. et Blavier, J. *Arch. int. Physiol.* 1951, **59** 442.

[13] Bacq, Z. M. et Herve, A. *Bull. Acad. Méd. roy. Belg.* 1952, **17** 13.

[14] Lambert, G., Maisin, J. et Mandart, M., *C.R. Soc. Biol. Paris*, 1952, **146** 1434.

[15] Maisin, H., Wolfe, R., Tobias, C. A. et Lawrence, J. H. Observation non publiée, 1953.

[16] Maisin, J., Maisin, H. et Dunjic, A. Communication faite à la Soc. de Biologie du 29 mai 1954. *C.R. Soc. Biol. Paris*, 1954, **148** 1293.

[17] Maisin, J., Maisin, H. et Dunjic, A. Observation non publiée, 1954.

[18] Maisin, H. et Fievez, C. *Symposium de Radiologie, Liège, 1954.* Butterworths, Londres, 1955.

DISCUSSION

C. Burg : On a l'impression que le problème des substances protectrices présenterait un aspect différent s'il était envisagé, non pas vis-à-vis d'une dose unique,

léthale de rayons X, mais par rapport à une irradiation en quelque sorte chronique ; mais finalement léthale.

Sans nier l'intérêt du premier aspect de la question, on peut se demander si pour l'étude du mécanisme d'action des agents de protection, il ne serait pas plus simple, malgré les apparences, de s'adresser au second cas. En effet, l'effet brutal des radiations déclanche une intervention de toute la série des glandes endocrines et met probablement en jeu une série de processus surajoutés, qui peuvent compliquer singulièrement la question.

B. Jolles : I should like to ask Maisin whether he noted if any of the deaths of the animals in his experiments with regional shielding fell in the '3-5 days' category of deaths described by Rajewski. I ask this question because in a series of experiments with sieve irradiation when studying survival curves of animals irradiated with open and sieve fields (double the open field dose through a 50 per cent sieve) it was noted that while the number of survivors in the 'sieve' group was greater than in the 'open' field group, death when it did occur in the sieve animals, took place at an earlier date than in the open field group.

Betz : Les expériences que Maisin vient de nous exposer sont intéressantes en ce sens qu'elles montrent la multiplicité des facteurs réglant la régénération hématopoiétique du rat irradié. J'aurai l'occasion de présenter ici même des résultats qui plaident dans le même sens. S'il est difficile de dire comment agit la protection de l'intestin, je voudrais ajouter une hypothèse à celles formulées par Maisin. Nous avons montré que l'hypercorticisme de l'animal irradié exerce une action inhibitrice sur la régénération hématopoiétique. On peut penser qu'en protégeant l'intestin, on diminue considérablement l'hyperactivité du cortex surrénalien. On sait en effet que la réaction surrénalienne est particulièrement intense lorsque la masse intestinale est comprise dans le champs de l'irradiation.

LABORATORY STUDIES AND CLINICAL TRIALS OF SOME CHEMICAL RADIO-SENSITIZERS

J. S. Mitchell

Department of Radiotherapeutics, University of Cambridge

This paper deals mainly with work completed and in progress during the past year and is intended to supplement the review of the subject which I gave in 1953 at the 7th International Congress of Radiology (Mitchell[1]).

The aim of this investigation is to try to improve the results of radiotherapy of some types of cancer by the ancillary use of chemical agents designed to act as radio-sensitizers. It is emphasized that in general these chemicals when acting alone are not chemotherapeutic agents. In addition to its practical importance, the possibility of chemical radio-sensitization appears to be of theoretical interest.

The first compound studied, which is still perhaps the most interesting, is tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate ('Synkavit' ; Compound I). Clinical trials of its use have been in progress since November, 1946, and I have employed it in the treatment of about 1070 patients mainly in conjunction with radiotherapy. I wish to emphasize the more recent clinical studies which are in progress and include the design of a clinical trial for evaluation of a radio-sensitizer. In August, 1953, a special clinical trial was started to examine the combined use of intravenous Compound I (Synkavit), oxygen administration before and during irradiation and X-ray therapy, in the treatment of some patients with advanced cancer.

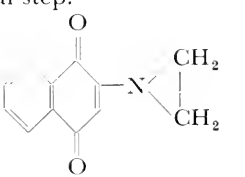
The results of various aspects of this work have been published by Mitchell and Simon-Reuss[2] in 1947, Mitchell[3-8] in the years 1948–52, Mitchell and Simon-Reuss[9, 10] in 1952, Hughes and Simon-Reuss[11] in 1953. In addition, a number of papers dealing mainly with mitotic inhibition have been published on the following groups of compounds and related aspects of the problem : Certain quinones by Friedmann, Marrian and Simon-Reuss[12] (1948), sulphydryl addition compounds of some quinones and related compounds by Friedmann, Marrian and Simon-Reuss[13] (1948), maleimide and related substances by the same authors[14] in 1949, the reactions of substituted maleimides with thiols (Marrian[15] in 1949), the condensation of N-substituted maleimides with thiourea (Marrian[16] in 1949), the action of 1 : 4-naphthohydroquinone diphosphate (Friedmann and Bailey[17]), unsaturated imides with special reference to their reaction with sulphydryl groups (Friedmann, Marrian and Simon-Reuss[18], 1952), halogen derivatives of the 1 : 4-naphthoquinone group and maleic acid series (Friedmann, Marrian and Simon-Reuss[19], 1952), a spectrometric investigation of the interaction of glutathione with maleimide and N-ethylmaleimide (Friedmann[20], 1952), and the action of X-rays on the glutathione 1 : 4 naphthoquinone reaction (Friedmann[21], 1954). As a different method of approach, studies have been reported on the antagonization of the antimitotic action of

tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Compound I) by nucleotides, nucleosides, purines and pyrimidines and some other compounds by Mitchell[6, 7] in 1950 and 1951. The report by Mitchell[3] has been criticized by Gellhorn and Gagliano[22] (1950). Berkman[23] (1951, 1953) published clinical results similar to those of Mitchell. Clinical and laboratory studies have been published by Jolles[24] (1952). Experiments on the Ehrlich mouse carcinoma have been described by Dittrich and Schmermund[25] (1953). An account of six years' clinical studies and attempts at quantitative clinical assessment of Compound I as a radio-sensitizer in the radiotherapy of malignant tumours has been published (Mitchell[1], 1953). Recently, oxidation-reduction potential and pH measurements have been made *in vivo* using the Walker rat carcinoma 256 and the Jensen sarcoma to study the effects of oxygen, Synkavit and X-rays alone and in combination (Cater and Phillips[26]).

New Compounds

The attempt to devise compounds which incorporate radioactive atoms and may concentrate in malignant tumours has led to the development of tetra-sodium 6-iodo-2-methyl-1 : 4-naphthohydroquinone diphosphate ('6-iodo Synkavit'). The synthesis of this compound was carried out by Dr. K. J. M. Andrews, of Roche Products, Welwyn Garden City, by the kind arrangement of Dr. A. L. Morrison. The tissue-culture studies made on chick fibroplasts by Mrs. Simon-Reuss show that as expected the introduction of the iodine atom in the 6-position slightly reduces the activity as measured in terms of the concentration producing 50 per cent mitotic inhibition by a factor of about 1·5 but otherwise produces cytological effects very similar to those of Compound I.

The idea which I had of incorporating a 'radio-mimetic' group and a radio-sensitizer in the same molecule as a related method of improvement upon Synkavit, has led to the development of 2-ethylenimino-1 : 4-naphthoquinone. After reading the paper by Domagk, Petersen and Gauss[27] (1954), this seemed a natural step.



2-ethylenimino-1 : 4-naphthoquinone

The compound 2-ethylenimino-1 : 4-naphthoquinone and the corresponding known open-chain compound 2-β-hydroxyethylamine-1 : 4-naphthoquinone (Fieser, L. F. *et al*[28], 1948), have been synthesized in this Department by Mr. D. R. Maxwell, with the advice of Dr. D. H. Marrian. Animal experiments in progress using the Walker rat carcinoma suggest that 2-ethylenimino-1 : 4-naphthoquinone has some action in producing tumour retrogression by itself, in addition to radio-sensitization when used in combination with therapeutic doses of X-rays. The tissue-culture studies by Mrs. Simon-Reuss using chick fibroblasts show that 2-ethylenimino-1 : 4-naphthoquinone produces 50 per cent mitotic inhibition after 24 hours in concentration approximately 4×10^{-7}M with a striking accumulation of

cells in prophase and metaphase especially in lower concentrations. Similar changes are found with the Ehrlich ascites tumour.

Tissue-culture studies

With regard to the general technique with tissue-culture experiments, experimental developments during the past few years to study mitotic inhibition, cytological effects generally and radio-sensitization by chemical agents have proved satisfactory. Compound I and Compound XXVIII are the usual reference substances. Probit analysis has been used. The study of the combination of the action of X-radiation and Compound I by the summation method has demonstrated potentiation of both mitotic inhibition and chromosome fragmentation (similar results have been obtained in some experiments on the roots of Allium Cepa by Mr. K. C. Bora; it has also been found that in this material the compound 2-ethylenimino-1 : 4-naphthoquinone produces gross inhibition of the growth of roots in concentration 10^{-4}M).

The problem of the thermal instability of Compound I which appeared to account for some erratic results in some animal experiments and clinical trials, appears to have been solved by the finding that this thermal instability is associated with the presence of an easily removable impurity in the aqueous solutions in the ampoules in the absence of oxygen (Dr. A. L. Morrison). With the purified preparations of Compound I, it now seems possible to obtain consistent results without the necessity to store the ampoules at 3° C.

An interesting experiment by Mrs. Simon-Reuss is reaching completion and will be reported in detail elsewhere. The influence of Synkavit and its effects as a radio-sensitizer on chick fibroblasts in culture have been followed after subcultivation for up to 17 passages. A pure strain of chick fibroblasts was divided into batches of cultures; one batch was treated with Synkavit for 24 hours in concentrations 2, 3, 4×10^{-6}M respectively in different experiments. The other cultures were kept for controls for testing the effects of Synkavit and of irradiation. The whole strain was subcultured every 48 hours, washed in Tyrode solution and transferred to fresh medium consisting of plasma and embryo extract only. At various intervals six cultures were taken from each group and the group formerly treated with Synkavit was irradiated at 18 hours and a control group was similarly irradiated. Further, one of the treated groups and one of the untreated groups were kept as controls. These cultures were fixed and stained and counted after 24 hours. The experiments lasted for 16, 16 and 17 passages, respectively for 5–5½ weeks. The cultures originally treated with Synkavit alone showed no abnormality and no mitotic inhibition and were in no way different in appearance from the controls. The untreated cultures irradiated with 150r showed 22–28 per cent mitotic inhibition throughout the whole experiment. Those treated originally with 2×10^{-6}M Synkavit and irradiated with 150r showed mitotic inhibition starting off with 75 per cent for three passages, then falling to 60 per cent at the sixth passage and 26 per cent at the tenth passage. The cultures originally treated with 4×10^{-6}M Synkavit showed no inhibition and no cytological abnormality. 300r produced 40 per cent inhibition in the controls, but 300r administered to the subcultures of the strain originally treated with Synkavit showed 94 per cent mitotic inhibition persisting for several subcultures with 80 per cent at the fourteenth passage

and 60 per cent inhibition at the sixteenth passage. It appears from these experiments that initial treatment of the cultures leads to a persistence of radio-sensitivity in the subcultures. To assess the part played by retained Synkavit in the cultures, Mrs. Simon-Reuss has carried out independent experiments in which it has been found that radio-sensitization persists down to a concentration between 10^{-9} and 10^{-10} M Synkavit. Some experiments are in progress with ^{14}C labelled Synkavit.

Possible mechanism of radio-sensitization

Compound I appears to be selectively concentrated by the tumour cells with a high differential absorption ratio. The phosphate groups are probably essential for its passage through the cell membranes. Inside the tumour cell it appears to be concentrated in the perinuclear region perhaps in the mitochondria. The parent 2-methyl-1 : 4-naphthoquinone is likely to be formed *in situ* and react specifically with sulphydryl compounds. In this way, selective radio-sensitization of the tumour cells by Compound I is envisaged as the converse of —SH protection against ionizing radiations.

Then increase in the oxygen tension in the tumour cells is likely to be associated with optimum use of the available oxygen for increasing the effects of the radiation. It is of interest that in the measurements by Drs. Cater and Phillips of oxidation-reduction potentials *in vivo* in the Walker rat carcinoma it was found when the animal breathed oxygen the potential taken up by the platinum electrode relative to the silver-silver chloride electrode increased by 10–50 mV, while intravenous injection of Synkavit (5–10 mg per 200 g rat) caused an immediate fall of 30 to 200 mV followed by gradual recovery.

The observation that the mitotic inhibition produced in chick fibroblast cultures by Compound I is abolished by an equimolecular concentration of guanosine, but not by isoguanine riboside, guanylic acid, guanine or any other related compound suggests a chemical specificity in the action of Compound I. It is not impossible that like some 2-OH-3-alkyl-naphthoquinones (Ball, Anfinsen and Cooper[29], 1947, *see* Potter and Reif[30], 1952), it prevents the reduction of cytochrome C and interferes with some process of phosphorylation. A problem is raised by the observation that tetrasodium 2 : 3-dimethyl-1 : 4-naphthohydroquinone diphosphate, Compound XXVIII (Mitchell and Simon-Reuss[9, 10], 1952, and Mitchell[1], 1953) is a good radio-sensitizer for both chick fibroblast cultures and the Walker rat carcinoma and does not react *in vitro* with sulphydryl compounds.

Animal experiments

The remarkably low acute and chronic toxicity of Compound I has been confirmed in experiments on rats, mice and rabbits. In rats, the repeated intramuscular administration of the compound in large doses increased the mortality following exposure of the whole body to X-radiation. Experiments by Jolles[24] (1952) in the rabbit and guinea-pig suggests species differences. Further experiments are in progress. It has recently been found that using similar methods 2-ethylenimino-1 : 4-naphthoquinone shows no acute toxic effects after intravenous administration in doses in the region of 2–5 mg per kilo in rats, mice and rabbits.

The earlier therapeutic experiments with Compound I were sufficiently suggestive to provide a basis for clinical trials, but were far from satisfactory because of the rather small number of animals and the high rate of spontaneous retrogression of the tumours. This difficulty appears to have been overcome by maintaining the Walker rat carcinoma as ascites tumour and in recent experiments almost invariably using solid tumours at the second passage from the ascites tumour. I wish to thank Professor A. Haddow for his help in supplying animals and tumours, with which to start these experiments.

Two large-scale therapeutic experiments with tetra-sodium 2-methyl 1 : 4-naphthohydroquinone diphosphate (Compound I) and tetra-sodium 2 : 3-dimethyl-1 : 4-naphthohydroquinone diphosphate (Compound XXVIII), on the production of permanent retrogression of the Walker rat

Figure 1. Arrangements for roentgen irradiation of experimental tumour in rat. The rat is wrapped in a flexible lead plate with the tumour projecting through an elliptical aperture.

carcinoma 256 have now been completed. The details of the experiment with Compound I are summarized in *Table I.* The results for Compound XXVIII (Mitchell[1], 1953) are compared with those of Compound I in *Table II.*

Experimental Methods—The main features of this type of animal experiment with the Walker rat carcinoma 256 in the rat, are given in *Tables I* and *II.* The following experimental details may be added.

For the irradiation of the tumours in groups C and D the rat is wrapped up in a lead plate of thickness 1·3 mm with the tumour projecting through an elliptical aperture in the plate of dimensions 4 × 3·3 cm. A convenient size for the lead plate is 21 cm × 17·5 cm transversely ; the elliptical aperture reaches to about 1 cm distance from the middle of the longer edge. The experimental arrangement with the rat held in position in this way for irradiation is shown in *Figure 1.* The selection of the tumour size in relation

Table I

Radio-sensitization by tetra-sodium 2-methyl-1 : 4-naphtholhydroquinone diphosphate (Compound I), of the Walker carcinoma 256 in the rat. A single intravenous dose of compound was administered at 30 minutes before X-ray treatment of the tumour in the individual rat. The effects were assessed in terms of permanent retrogression of the primary tumour, note being taken also of the times of survival of the animals with unhealed tumours, and of metastases. Measured tumours of maximum dimensions not less than 2·0 cm and not more than 3·2 cm growing after transplantation in rats of selected weight and sex were allocated in batches of 6 to 10 in the following groups :

A. Control.

B. Compound I only. By intravenous injection, one single dose was administered at 30 minutes before the time corresponding to irradiation.

C. X-ray treatment only. Central tumour dose 1100 r with field 5·0 × 4·0 cm ellipse in thick lead shield. Dose rate at centre of tumour 158 r per minute.

D. Compound I with X-ray treatment as in C. The intravenous injection was given at 30 minutes before the beginning of the irradiation.

Experiment number	*Sex*	*Dose of Compound I* mg	*Date of irradiation*	*Ratio of number of animals with permanent retrogression of primary tumour to total number of animals in each group. Also mean initial weight of animals including tumours and survival times in days from implantation for tumours not cured.** Group A	Group B	Group C	Group D	*Comments*
99	F	15	9.XI.53	1/6 213 g ; 4 × 16, 14 d	2/6 217 g ; 4 · 22 d	0/6 220 g ; 3 · 32, 3 · 29 d	2/6 211 g ; 28, 31, 2 × 83 d	
100	M	10	17.XI.53	1/10 311 g ; 9 · 10 d	2/10 305 g ; 8 · 10 d	7/10 275 g ; 2 · 34, 18 d	10/10 304 g —	
101	M	10	19.XI.53	0/10 279 g ; 10 · 9 d	0/10 286 g ; 10 · 9 d	5/10 317 g ; 3 · 20, 26, 32 d	8/10 300 g ; 7, 16 d	
107	F	10	12.XII.53	0/8 152 g ; 8 · 7 d	0/8 153 g ; 8 · 7 d	2/8 160 g ; 10, 3 · 11, 18, 31 d	7a/8 141 g ; 11 d	*a*. One of these died with axillary metastases
108	F	10	18.XII.53	0/9 117 g ; 9 · 12 d	0/9 115 g ; 9 · 12 d	1/9 133 g ; 3 · 25, 2 · 13 d	6/8*b* 139 g ; 2 · 13 d	*b*. Excluding one which died during irradiation
109	M	10	23.XII.53	0/10 130 g ; 2 · 8, 7 · 16, 18 d	1/10 145 g ; 8, 8 · 16 d	5/10 145 g ; 1 · 16, 28 d	5/10 154 g ; 3 · 16, 2 × 28 d	
110	M	10	10.II.54	0/10 204 g ; 10 · 11 d	0/10 194 g ; 10 · 11 d	1/10 196 g ; 12, 11, 31, 2 · 39, 43 d	8/10 170 g ; 21, 39 d	
112	M	10	1.III.54	1/8 181 g ; 7 · 16 d	1/8 158 g ; 7 · 16 d	3/8 161 g ; 15, 2 · 17, 2 · 18 d	2/8 170 g ; 2 · 15, 17, 2 · 18, 37 d	
113	M	10	18.III.54	0/7 151 g ; 7 · 22 d	0/8 165 g ; 8 · 22 d	2/7 169 g ; 2 · 22, 3 · 36 d	4/7 168 g ; 1 · 22, 2 · 52 d	
Total permanent retrogression of primary tumour				3/78	6/79	32/78 (41·0%)	52/77 (67·5%)	
Mean survival time of tumours not cured				13·0 days	13·3 days	27·8 days	29·2 days *	* Animals were killed when maximum tumour dimension reached 5·0 cm or looking ill before that

Conclusion : The difference between the ratios for permanent retrogression in C and D is regarded as significant, χ^2 [1] c = 9·921 and P = 0·0012. With Abbott's correction for spontaneous retrogression in A, χ^2 [1] c = 10·25 and P = 0·0010.

to the dimensions of the aperture is of great importance in order to avoid the 'edge miss'. The dose-rate was measured in a wax phantom by Miss R. Saunders, who has kindly supplied the following information. The phantom made of wax of density 0·90 g per c.c. is shaped to represent a tumour 4 × 3 cm and 2·6 cm high on a base of dimensions representing a 200 g rat. A hole was made to accommodate a 250 r Victoreen chamber horizontally along a 3 cm axis the external diameter of the chamber being 0·9 cm and the centre of the chamber 1 cm from the upper surface of the phantom. The wax phantom was wrapped in a lead sheet through which the 'tumour' projected. The end of the chamber was supported by a bolus bag. The Maximar X-ray machine was run at 220 kV and an *added* filter of 1 mm aluminium was used (H.V.L. = 0·4 mm copper ; effective wavelength 0·25 A). The chamber was exposed for 1 minute. The end of the 5 cm circular applicator just touched the top of the phantom. The F.S.D. of the applicator was 41·5 cm. The Victoreen readings were corrected for wavelength, temperature and pressure. The dose-rate at the centre of the tumour was in the region of 160 r per minute, but the exact value as measured was used in each experiment.

While permanent retrogression of the tumour is the main criterion studied, measurements of the tumour dimensions and the weight of the rats have been recorded three times weekly before and indefinitely after irradiation in all these experiments. Many histological examinations have been made to confirm the presence of tumour and the effects of radiation and chemicals on the tumour and on normal tissues. It is to be noted that throughout these experiments the animals have all been treated as individuals. I wish to express my thanks to Mr. E. A. King for his skilled help in these experiments.

The results of the experiments with the Walker rat carcinoma 256, in *Tables I* and *II* show that Compound I, when administered by the intravenous route at 30 minutes before a single therapeutic dose of X-radiation, produces definite radio-sensitization as estimated by a significant increase in the proportion of primary tumours showing permanent retrogression. The administration of Compound I by intramuscular injection (*Table II*) shows that the intramuscular route is completely ineffective except possibly when given in very large doses over very prolonged periods. In doses comparable with those used clinically, intramuscular administration has no effect as a radio-sensitizer and no significant effect on the tumour. The ineffectiveness of the intramuscular route serves as a control for the experiments with intravenous administration. The experiments with intramuscular injection of Compound I were carried out before the technique of transplantation of the tumour was fully developed. It can be seen from the experiments in the intravenous series that for radio-sensitization with Compound I the best results are obtained when the intravenous injection is made at about 30 minutes before the beginning of the X-ray exposure.

The results of the similar experiment with Compound XXVIII, reported elsewhere (Mitchell[1]), are given in more detail in *Table II*. Again, the ineffectiveness of the intramuscular route serves as a control. The experiments with intravenous administration include a number with unsuitable timing. With Compound XXVIII by intravenous injection it appears

Table II

Summary of experiments with tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Compound I) and tetra-sodium 2 : 3-dimethyl-1 : 4-naphthohydroquinone diphosphate (Compound XXVIII) on the production of permanent retrogression of the Walker rat carcinoma 256. Studies of radio-sensitization and comparison of effects of intravenous and intramuscular routes of administration.

Measured tumours of maximum dimensions not less than 2·0cm and not more than 3·2cm (or not more than 4.0cm in the case of intramuscular Compound I) growing after transplantation in rats of selected weight and sex were allocated to batches of 5 to 10 in the following groups :

A. Control.

B. Compound only, either by intravenous injection usually in one single dose at 0 to 30 minutes before the time corresponding to irradiation or by repeated daily intramuscular injections.

C. X-ray treatment only, with central tumour dose either 1100, 1250, 1350, 1500 or 1600r with field 5·0 × 4·0cm ellipse in thick lead shields.

D. Compound as in B with X-ray treatment as in C.

The experiments in each class are pooled and include comparable numbers of male and female animals.

Compound number	*Method of administration*	*Dose of compound and time factors of administration*	*Central tumour dose of X-radiation*	*Ratio of number of animals with permanent retrogression of primary tumour to total number of animals in each group*				*Date of completion of experiment*
				A	*B*	*C*	*D*	
I	Intravenous	10–15 mg at 30 minutes before beginning of irradiation	1,100r	3/78	6/79	32/78	52/77	7.VI.54
	,,	15 mg immediately before irradiation	1,100r	1/30	0/30	3/29	6/30	4.II.54
	,,	5 mg at 30 minutes before irradiation	1,100r	1/20	2/20	10/20	7/20	8.XII.53
	Intramuscular	10 mg daily for 2 to 8 days before irradiation, 15 mg on day of irradiation at 30 minutes before beginning, and subsequently 10 mg daily for varying intervals from 2 days after irradiation up to 28 days after retrogression of the tumour in the earlier experiments.	1,250r	3/17	2/17	1/22	8/24	14.VI.54
			1,350r	3/23	9/22	6/23	16/23	17.IX.54
			1,500r	7/70	16/65	35/67	34/70	14.II.52
			1,600r	1/18	6/17	10/19	9/18	14.II.52
XXVIII	Intravenous	Single dose of 10 to 25 mg at 30 minutes before irradiation	1,100r	0/53	1/54	19/50	29/54	16.I.53
		Single dose of 25 mg immediately before	1,100r	0/40	1/40	14/37	23/39	16.I.53
		Single dose of 10 to 25 mg on the day of irradiation with 2 to 3 injections each of 10 mg on the preceding days.	1,100r	0/40	2/38	20/38	24/37	13.V.53
		Pooled intravenous doses.	1,100r	0/133	4/132	53/125	76/130	13.V.53
	Intramuscular	3 to 7 daily injections each of 10 mg before day corresponding to irradiation, then 10 mg on that day and usually on the two following days	1,100r	0/65	18/60	39/62	40/58	13.V.53

that radio-sensitization is obtained for intravenous injection either immediately before or at 30 minutes before starting the X-ray treatment.

The difference between groups C and D for intravenous injection is regarded as significant. For the pooled intravenous doses $\chi^2_{[1]C} = 5{\cdot}953$, $P = 0{\cdot}014$ and for the single intravenous doses only, $\chi^2_{[1]C} = 5{\cdot}133$ and $P = 0{\cdot}023$. These experiments suggest that Compound XXVIII when administered by intravenous injection in doses approximately twice those of Compound I is an effective radio-sensitizer for the Walker rat carcinoma 256, though the findings suggest that Compound XXVIII is slightly less effective as a radio-sensitizer than Compound I. However, there is no very convincing difference in effectiveness between the two compounds in these animal experiments.

Distribution studies—Distribution studies of Compound I in the rat with the Walker carcinoma 256 have demonstrated increased fluorescence of a derivative of the compound with Wood's light (wavelengths 3,650, 3,655 and 3,663 A) in the actively growing parts of the tumour and some other tissues, including sternal marrow, lymphoid tissue, testis, ovary and kidney after intramuscular injection. After intravenous injection with small doses there is much more selective concentration of the compound in the tumour. With larger intravenous doses there is spill-over into the other organs mentioned. The compound responsible for the yellow fluorescence appears to be 2-methyl-1 : 4-naphthoquinone-2 : 3-oxide, as confirmed by the fluorescence spectrum. These distribution studies were summarized in Table II of MITCHELL[1] (1953). Other compounds which accumulate in the actively growing parts of the Walker rat carcinoma 256 are the tetra-sodium salts of 2 : 3-dimethyl-1 : 4-naphthohydroquinone diphosphate (Compound XXVIII), and 2-methyl-3-bromo-1 : 4-naphthohydroquinone diphosphate (Compound IX). In the case of Compound IX, there is a considerably lower differential absorption ratio in the tumour, as shown by fluorescence, than in the case of Compound I, and this has been confirmed by Mr. D. R. Maxwell by radioactive tracer studies with Compound IX labelled with ^{82}Br. Distribution studies using Compound I labelled with ^{14}C in the methyl group are in progress.

Clinical trials of tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate as a radio-sensitizer

Attempts to evaluate tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Synkavit, Compound I) as a radio-sensitizer in the radiotherapy of malignant tumours have been in progress since November 1946. A summary of the results as assessed on 31 May, 1953, has been published (Mitchell[1]). The investigation is continuing. It is emphasized that in general the compound alone has no therapeutic effect on malignant tumours.

The preliminary general survey of the use of Compound I in the treatment of patients with various types of malignant tumours other than carcinoma of the bronchus with follow-up for at least 5 years, suggests that the proportion of cases showing unexpectedly good clinical response is greater following radiotherapy combined with the compound administered by intravenous injection than following radiotherapy combined with the compound

administered by intramuscular injection. Of particular interest are the results obtained in carcinoma of the mouth and carcinoma of the ovary. Of interest is the frequent clinical observation of focal pain and sensations in the region of the tumour after intravenous injection of large doses of Compound I.

The preliminary clinical studies of the influence of the ancillary use of the compound on the survival times of inoperable cases of carcinoma of the bronchus treated by X-ray therapy have been discussed in detail. The evidence obtained was sufficiently suggestive to justify further work, but the methods used were clearly inadequate.

It is suggested that the design of a clinical trial for quantitative evaluation of any proposed method of treatment of cancer is one of the most important problems at the present time. The most important single feature of the design is random allocation of patients to two or more alternative forms of treatment. At the same time it is absolutely essential to provide for each individual patient that form of treatment which is the best according to present knowledge at the beginning of the investigation. Accordingly, it is often necessary to plan to depart from what might be termed the theoretically ideal form of experiment with deliberate sacrifice of some information. In the present example, cases were allocated at random with the aid of a table of random numbers to one or two alternative forms of treatment, X-ray therapy combined with the compound administered by intravenous injection and X-ray therapy combined with the compound administered by intramuscular injection, the latter being regarded as the control group. This type of design of a clinical therapeutic trial must be justified *a posteriori*. This has been done, and includes consideration of all the various factors which may be relevant. To date, this trial includes 173 patients but the follow-up must be continued. Already for the first 91 cases the two groups are substantially identical in composition and there is evidence of a small but definite improvement in survival of the male patients with reasonably certain diagnosis (excluding those treated surgically) in the group treated with X-ray therapy combined with intravenous compound in relation to those in the group treated with X-ray therapy combined with intramuscular compound. Statistical details have been published (Mitchell[1], 1953, especially Table II). In general terms, in comparable groups of cases of inoperable carcinoma of the bronchus, the mean survival time after the first X-ray treatment was only about 4 months with X-ray therapy only, about 6 months with X-ray therapy combined with intramuscular compound and about 11 months with X-ray therapy combined with intravenous compound. The improvement in this last group is certainly in accordance with clinical impressions and indicates a small prolongation of useful life. The mean interval from the first symptom to the first X-ray treatment is about 7 (6–8) months in all the groups. One unsolved problem to which much attention is being devoted is to decide when to stop a clinical trial such as the present one.

It is concluded that the results obtained so far indicate that intravenous administration of Compound I has a small but useful effect as a clinical radio-sensitizer. Even though large effects have not been produced, the results provide evidence that it is possible to improve the response of some

human malignant tumours to radiotherapy by means of chemical radio-sensitizers.

Combination of Compound I, oxygen and X-ray therapy—Since August, 1953, a clinical trial has been in progress of the combined use of intravenous Compound I (Synkavit), oxygen administration before and during irradiation and X-ray therapy in the attempted treatment of patients with advanced cancer. The compound has been given in the usual manner with maximum unit doses in the region of 100 to 150 mg at 30 minutes before starting the X-ray treatments at each visit. The oxygen is administered at atmospheric pressure by means of either a B.L.B. mask or an anaesthetic mask, with a flow-rate meter at about 6 litres per minute, starting usually 20 minutes before and continued throughout irradiation. Every endeavour has been made to employ the normal methods of X-ray therapy, often with large fields, and to avoid any modification of the techniques as a result of the combined treatment. So far 34 cases have been treated, mainly advanced cases of carcinoma of the stomach, colon, and ovary and inoperable cases of carcinoma of the bronchus after thoracotomy. It is of course too early to assess the results. With the combined treatment, both the skin reactions and the general reactions appear to be no more severe than would be expected with the X-ray therapy only. I wish to thank Dr. S. D. Sturton for his help in the initial part of this investigation. These trials are being continued, together with studies of the combination of oxygen and X-ray therapy. A randomized trial has been started using four groups, X-ray therapy combined with intramuscular Synkavit, oxygen combined with X-ray, intravenous Synkavit combined with X-ray and intravenous Synkavit, oxygen and X-ray. Experiments with animal tumours in these fields are being carried out by Dr. D. B. Cater and Dr. A. F. Phillips.

SUMMARY

The aim of this investigation is to try to improve the results of radiotherapy of some types of cancer by the ancillary use of chemical agents designed to act as radio-sensitizers. This paper deals mainly with recent results but also refers to the previous studies carried out since 1946. The first compound studied, tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Synkavit ; Compound I), is still perhaps the most interesting. Clinical trials of its use have been in progress since November, 1946, and about 1,070 patients have been treated by the combination of radiotherapy with this compound.

In the laboratory studies we have used :—

(*1*) tissue cultures mainly of chick fibroblasts, for the primary sorting test and for studies of the relation between chemical structure and biological action.

(*2*) animal experiments with rats mainly, but also using mice and rabbits, for :—

(*a*) tests of chemical toxicity and radiotoxicity in the case of compounds incorporating radio-isotopes.

(*b*) studies of radio-sensitization of normal tissues with total body radiation.

(*c*) studies of the distribution of the chemicals in the tumour and normal tissues, using mainly both fluorescence methods and radioactive labelling.

(*d*) radiotherapeutic experiments to study radio-sensitization in transplanted tumours, mainly the Walker rat carcinoma 256.

New compounds examined recently include tetra-sodium 6-iodo-2-methyl-1 : 4-naphthohydroquinone diphosphate and 2-ethylenimino-1 : 4-naphthoquinone, the latter compound appearing to be of particular interest.

The problem of the thermal instability of Compound I appears to have been solved by the finding that this thermal instability is associated with the presence of an easily removable impurity so that stable aqueous solutions of Compound I can now be prepared.

An interesting experiment by Mrs. Simon-Reuss is summarized ; it appears that initial treatment of chick fibroblast cultures with Compound I leads to a persistence of radio-sensitivity in subcultures even after 16 passages.

The possible mechanisms of radio-sensitization are discussed ; the selective sensitization of the tumour cells is envisaged as the converse of —SH protection.

Two large-scale therapeutic radio-sensitization experiments with Compound I and Compound XXVIII have now been completed and are reported in detail. With both compounds, significant radio-sensitization is observed when the compound is administered by the intravenous route but not when the compound is given by the intramuscular route.

The clinical trials of tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Compound I) as a radio-sensitizer are summarized. The preliminary general survey in the treatment of patients with various types of malignant tumours other than carcinoma of the bronchus, with follow-up for at least 5 years, suggests that the proportion of cases showing unexpectedly good clinical response is greater following radiotherapy combined with the compound administered by intravenous injection than following radiotherapy combined with the compound administered by intramuscular injection. Of particular interest are the results obtained in carcinoma of the mouth and carcinoma of the ovary. The preliminary clinical studies of the influence of the ancillary use of the compound on the survival times of inoperable cases of carcinoma of the bronchus treated by X-ray therapy were very suggestive, but the methods of investigation used were inadequate.

It is suggested that the design of a clinical trial for quantitative evaluation of any proposed treatment of cancer is one of the most important problems at the present time. The most important single feature of the design is random allocation of patients to two or more alternative forms of treatment. At the same time it is absolutely essential to provide for the individual patient that form of treatment which is the best according to present knowledge at the beginning of the investigation. A clinical trial of Compound I as a radio-sensitizer in the treatment of inoperable cases of carcinoma of the bronchus is discussed ; so far it includes 173 patients. In general terms, in comparable groups of cases of inoperable carcinoma of the bronchus, the mean survival time after the first X-ray treatment was about 4 months with

X-ray therapy only, about 6 months with X-ray therapy combined with intramuscular compound and about 11 months with X-ray therapy combined with intravenous compound. It is concluded that the results obtained so far show that intravenous administration of Compound I has a small but useful effect as a radio-sensitizer.

Since August, 1953, clinical trials have been in progress of the combined use of Compound I, oxygen administration before and during irradiation, and X-ray therapy in the treatment of patients with advanced cancer. After preliminary studies a randomized trial has been started.

ADDENDUM

ANIMAL EXPERIMENTS WITH THE WALKER CARCINOMA 256 IN THE RAT

(1) Distribution Studies

With reference to the fluorescence method for study of the distribution of Compound I in the rat with the Walker carcinoma 256, further measurements of the fluorescence spectrum with Wood's light (wavelengths 3,650, 3,655 and 3,663 A) and of its behaviour with pH confirm that the compound responsible for the fluorescence of the tissues is probably 2-methyl-1 : 4-naphthoquinone-2 : 3-oxide. Cut surfaces of tissues require about 6 hours at room temperature in the presence of air for full development of this fluorescent derivation of Compound I but the fluorescence develops within 15 to 30 minutes in the presence of alkali and hydrogen peroxide ; varying concentrations between N/100 and 3 N NaOH and 10 to 100 volume H_2O_2 have been used, the fluorescence developing most rapidly at the highest concentrations. The fluorescence does not appear when the tissues are kept in air at -20° C and -4° C, or are kept in nitrogen at room temperature for 15 days, though the fluorescence starts to develop within 10 minutes after the admission of air.

In continuation of these investigations, the method of fluorescence microscopy has been used to obtain information about the localisation of Compound I or the compounds derived from it within the tumour cells. In *Figure 2* the appearances with fluorescence microscopy and the same area of the tumour stained with toluidine blue are compared for a region in the growing edge of the Walker carcinoma after treatment of the rat with large intramuscular doses of Compound I. The essential features of the technique are summarized in the legend to *Figure 2*. It must be mentioned that the development of the fluorescence was accelerated by running N/100 NaOH followed by 30 volume H_2O_2 and then further N/100 NaOH under the coverslip. The upper left-hand part of the plates shows an area of actively growing tumour ; below the margin of this is an area of mainly necrotic and partly relatively inactive tumour. Comparison of the appearances with fluorescence microscopy and toluidine blue staining of the actively growing parts of the tumour show that in the proliferating cells not in mitosis the fluorescent derivative of Compound I is concentrated mainly in the perinuclear region of the cytoplasm of the tumour cells, with a paler peripheral region and some fluorescent granules in the cytoplasm.

2. Therapeutic Experiments

In the therapeutic experiments to test radio-sensitization by tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (Compound I) of the

Table III.—Clinical Therapeutic Trial of Compound I as a Radio-sensitizer
Summary of Results to 30th June 1954

Treatment of inoperable cases of carcinoma of the bronchus with randomized allocation to alternative treatments, X-ray therapy combined with intravenous Compound I (X + I-VS) and X-ray therapy combined with intramuscular Compound I (X + I-MS) which is the control group. Results assessed 30th June 1954. Cases No. 1–151 inclusive in which the first X-ray treatment was given between 1st April 1951 and 31st December 1953.

Treatable less advanced male cases

Selected from these cases are those with no evidence of extra-thoracic spread, metastases in ribs or the syndrome of superior mediastinal obstruction at the first X-ray treatment and those in which the minimum tumour dose was not less than 1,200r. For the present purpose cases treated surgically at any stage, cases regarded as operable but not treated surgically on account of poor general condition and female patients are excluded. Survival is estimated in months to the nearest month from the first X-ray treatment and also from the first symptom.

	Number of cases surviving 8 months or more after first X-ray treatment / Total number of cases in each group	
Group	*X + I-VS*	*X + I-MS*
(a) Histology positive for carcinoma of the bronchus (one p.m. and sputum, all others bronchial biopsy) ..	7/11	3/15
(b) Conclusive evidence of malignant cells in sputum and/or bronchial aspirate, or probable malignant cells in sputum and/or bronchial aspirate, together with subsequent development of definite metastases	5/7*	0/6
(c) Reasonably certain diagnosis with typical broncho-scopic and radiological appearances and clinical course, including subsequent development of definite metastases	3/3	0/4
	15/21	3/25

Pooled groups (a), (b) and (c)

* In this group is included one case surviving 4 months from the first X-ray treatment and 10 months from the first symptom who was given intramuscular compound because of incorrect suspicion of cerebral metastases ; for calculation of mean survival times, this case is transferred to the group X + I-MS.

Mean survival times (to date) from first X-ray treatment : 10·2 months for X + I-VS, and 4·8 months for X + I-MS. The difference is significant : for 44 d.f., t = 4·448, and P<0·001.

from first symptom : 15·8 months for X + I-VS, and 11·2 months for X + I-MS. The difference is significant : for 44 d.f., t = 2·520 and P = 0·0163.

It is to be noted that at the date of assessment, 4 patients were still living in the series X + I-VS and one patient was living in the series X + I-MS.

Walker carcinoma 256, in the rat, a number of experiments have been carried out which are not included in this paper and which will be reported elsewhere, together with further experiments in progress.

The increased proportion of tumours showing permanent retrogression in Group D of *Table I* (page 175), that is with intravenous Compound I and

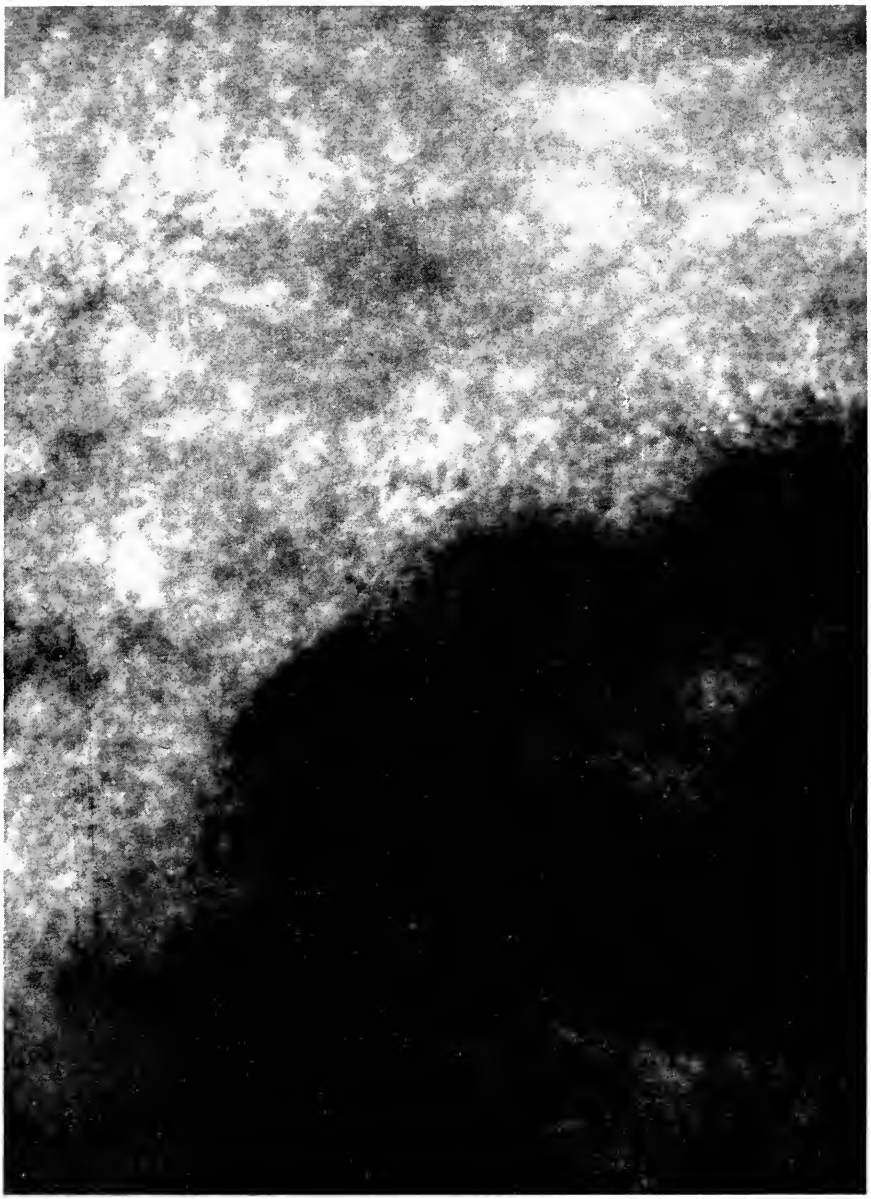


Figure 2. Comparison of fluorescence microscopy and toluidine blue staining of an area in the growing edge of the Walker rat carcinoma after treatment with large intramuscular doses of Synkavit.
Fluorescence microscopy with Wood's light.
Frozen section. Fluorescence developed with N/100 NaOH *and* 30 volume H_2O_2.

Original magnification × 570. *Zeiss* 1 mm *water immersion objective.*
Rat received 23 daily doses each of 10 mg *of Synkavit, with* 100 mg *at 20 minutes before death.*
The compound responsible for the yellow fluorescence appears to be 2-methyl-1 : 4-naphthoquinone-2 : 3-oxide.

central tumour dose 1,100 r, would be given by a central tumour dose of X-radiation alone of at least 1,400 r, and probably about 1,500 r.

In the early stages of this work no effect appeared to be obtained with intravenous Compound I given immediately after the end of the X-radiation of the tumour, but this experiment will be repeated.

Table IV.—Clinical Therapeutic Trial of Compound I as a Radio-sensitizer (Contd.)

Group	Number of cases surviving 8 months or more after first X-ray treatment/ Total number of cases in each group *X+I-VS*	*X+I-MS*
(d) Verified as in (a) and (b) but minimum tumour dose less than 1,200 r	0/4 (None survived more than two months)	0/5 (None survived more than two months)
(e) Male patients with extra-thoracic spread and/or metastases in ribs at first X-ray treatment, or with syndrome of superior mediastinal obstruction	5/26	3/16
(e1) Excluding cases with minimum tumour dose less than 1,200 r	4/21	2/11
(f) Male patients treated surgically at some stage	2/7	7/7
(g) Male patients with diagnosis of carcinoma of the bronchus *not* verified :		
(g1) diagnosis of carcinoma of bronchus *unproved*	0/5	3/5
(g2) diagnosis of carcinoma of bronchus *unlikely* or *disproved*	1/5	6/6
(h) Female patients, all groups except bronchial adenoma	2/9	2/4
(i) Bronchial adenoma	0/2	0/1
(j) No X-ray therapy : compound only		0/1
(k) Operable but not treated surgically on account of poor general condition		2/2

For the unselected inoperable male patients treated with effective doses of X-radiation in the pooled groups (a), (b), (c) and (e1), the proportions surviving 8 months or more after the first X-ray treatment in the series X+I-VS and X+I-MS are respectively 19/42 and 5/36 ; $\chi^2_{c[1]}=8\cdot64$, so that $P=0\cdot003$.

Survival from the first symptom is a rather less sensitive and less objective test. The proportions surviving 11 months or more after the first symptom are respectively (after transfer of the case marked * to the series X+I-MS) for the series X+I-VS, 26/41 and for the series X+I-MS, 14/37 ; $\chi^2_{c[1]}=4\cdot12$, so that $P=0\cdot0043$ and this difference between the two series can be regarded as significant, even after the inclusion of the unfavourable cases in group (e1).

With reference to *Table I*, the animals were killed when the maximum dimension of the tumour reached 5·0 cm or when the animal was looking ill and cachectic before that size of the tumour was reached. Under these conditions, there were very few animals with metastases detectable macroscopically.

CLINICAL TRIALS OF TETRA-SODIUM-2-METHYL-1:4-NAPHTHOHYDROQUINONE DIPHOSPHATE, COMPOUND I, AS A RADIO-SENSITIZER

The results in this clinical trial as assessed on 30th June 1954 are summarized in *Tables III* and *IV*. These tables are a revision of *Table II* of my earlier report[1] ; the basis for the classification and assessment of the results is sub-

stantially the same in *Tables III* and *IV* as in that paper. It is to be noted in groups (a), (b) and (c) of *Table III* that at the date of assessment 4 patients are still living in the series X+I-VS and one patient is living in the series X+I-MS, and it is almost certain that the final mean survival time in the group X+I-VS will be substantially greater than the value given of 10·2 months from the first X-ray treatment. As this clinical trial with randomized allocation proceeds and the number of cases included increases, the difference between the results in the series X+I-VS and X+I-MS increases in significance.

In addition to the statistical estimation of survival times from the first X-ray treatment and from the first symptom, I think it is of the greatest importance to assess the detailed progress of the course of the disease in the individual patients and, in particular, to try to obtain an estimate of the

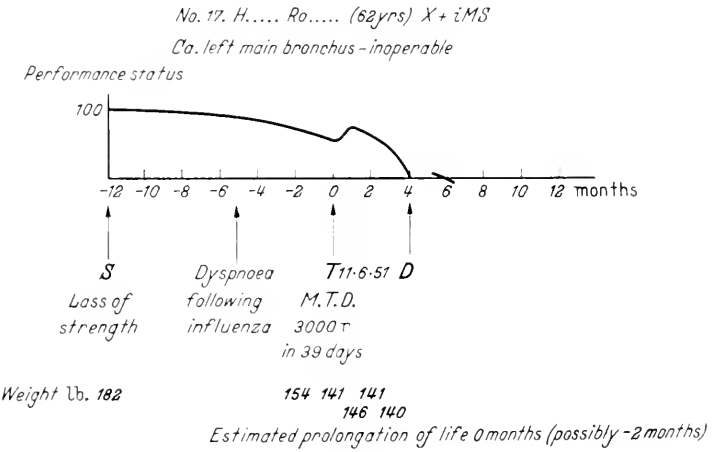


Figure 3.

effect of the different types of treatment. I have seen nearly all the patients referred to in *Tables III* and *IV* personally but it is essential to try to present the results for the individual patients objectively. A useful method of approach is by the use of the Performance Status defined by KARNOFSKY *et al*[31] (Table 3). Selected examples for two patients who appeared to be reasonably typical are given in *Figures 3* and *4*. The former is for a case treated with X-ray therapy combined with intramuscular Compound I and the latter for a case treated with X-ray therapy combined with intravenous Compound I. By extrapolation of the curve of deteriorating Performance Status before treatment, an attempt is made to estimate the duration of life in the absence of the treatment given and from this to estimate the prolongation of life, and also prolongation of useful life, as a result of the form of treatment administered. In *Figure 3* in the case treated with X-ray therapy combined with intramuscular compound, it appears unlikely that the treatment resulted in any prolongation of life but that there was possibly a slight palliation of symptoms, although even this conclusion is by no means

certain. With regard to the case shown in *Figure 4* and treated by X-ray therapy combined with intravenous compound, the graphical presentation indicates a substantial prolongation of life and also of useful life as a result of the treatment ; this conclusion supports the clinical findings in the case of this patient throughout the course of his illness.

Other compounds

With regard to the investigations of 2-ethylenimino-1 : 4-naphthoquinone both in animal experiments and clinical trials (see page 171), the low solubility of this compound presents considerable difficulty and it appears to be desirable to attempt the difficult problem of preparing the corresponding phosphorylated compound, 2-ethylenimino-1 : 4-naphthohydroquinone diphosphate. It is of interest that a focal reaction in the region of the tumour was observed several times in a patient with advanced carcinoma of

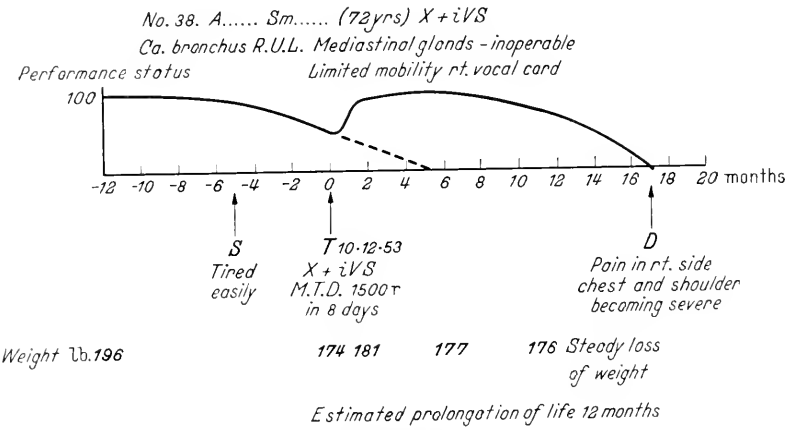


Figure 4.

the floor of the mouth after intravenous injection of about 10 mg of 2-ethylenimino-1 : 4-naphthoquinone in 170 ml of normal saline. It is suggested that further study of this compound and related compounds is desirable.

REFERENCES

1 Mitchell, J. S. *Brit. J. Cancer*, 1953, **7** 313 ; *Acta radiol. Stockh.* Supp. **116** 341.
2 Mitchell, J. S. and Simon-Reuss I. *Nature, Lond.* 1947, **160** 98.
3 Mitchell, J. S. *Brit. J. Cancer*, 1948, **2** 351.
4 Mitchell, J. S. *Experientia*, 1949, **5** 293.
5 Mitchell, J. S. *Ann. Rep. Brit. Emp. Cancer Campaign*, 1949, **27** 214.
6 Mitchell, J. S. *ibid*, 1950, **28** 213.
7 Mitchell, J. S. *ibid*, 1951, **29** 192.
8 Mitchell, J. S. *ibid*, 1952, **30** 238.
9 Mitchell, J. S. and Simon-Reuss, I. *Brit. J. Cancer*, 1952, **6** 305.

[10] Mitchell, J. S. and Simon-Reuss, I. *Brit. J. Cancer*, 1952, **6** 317.
[11] Hughes, A. and Simon-Reuss, I. *ibid*, 1953, **7** 142.
[12] Friedmann, E., Marrian, D. H. and Simon-Reuss, I. *Brit. J. Pharmacol.* 1948, **3** 263.
[13] Friedmann, E., Marrian, D. H. and Simon-Reuss, J. *ibid*, 1948, **3** 335.
[14] Friedmann, E., Marrian, D. H. and Simon-Reuss, J. *ibid*, 1949, **4** 105.
[15] Marrian, D. H. *J. Chem. Soc.* 1949, 1515.
[16] Marrian, D. H. *ibid*, 1949, 1797.
[17] Friedmann, E. and Bailey, N. T. J. *Biochim. Biophys. Acta*, 1950, **6** 274.
[18] Friedmann, E., Marrian, D. H. and Simon-Reuss, I. *ibid*, 1952, **9** 61.
[19] Friedmann, E., Marrian, D. H. and Simon-Reuss, I. *ibid*, 1952, **8** 504.
[20] Friedmann, E. *ibid*, 1952, **9** 65.
[21] Friedmann, E. *Brit. J. Radiol.* 1954, **27** 137.
[22] Gellhorn, A. and Gagliano, T. *Brit. J. Cancer*, 1950, **4** 103.
[23] Berkman, A. *Türkiye Tip Encümeni Arsive (Istanbul)*, 1951, **2** 1.
Berkman, A. *Seventh Int. Congress Radiology Abstract T100*, 1953.
[24] Jolles, B. *Ann. Rep. Brit. Empire Cancer Campaign*, 1952, **30** 324.
[25] Dittrich, W. and Schimermund, H.-J., *Strahlentherapie*, 1953, **90** 88.
[26] Cater, D. B. and Phillips, A. F. *Nature, Lond.* 1954, **174** 121.
[27] Domagk, G., Peterson, S. and Gauss, W. *Zs. Fd. Krebsforsch.* 1954, **59** 617.
[28] Fieser, L. F., *et al. J. Amer. Chem. Soc.* 1948, **70** 3215.
[29] Ball, E. G., Anfinsen, C. B. and Cooper, O. *J. biol. Chem.* 1947, **168** 257.
[30] Potter, V. R. and Reif, A. E. *ibid*, 1952, **194** 287.
Fisher, R. A. *Statistical Methods for Research Workers*, Oliver and Boyd, Ltd., Edinburgh, 1925–46.
Fisher, R. A. *The Design of Experiments*, Oliver and Boyd, Ltd., Edinburgh, 1935–46.
Medical Research Council. *Brit. Med. J.* 1948, **2** 769.
Medical Research Council. *ibid*, 1950, **2** 1073.
Hill, A., Bradford, *Principles of Medical Statistics*, Pub. by Lancet, Ltd., London, 1952, Fifth Edn., pp. 5, 233.
[31] Karnofsky, D. A., Burchenal, J. H., Armistead, G. C., Jnr., Southam, C. M., Bernstein, J. L., Craver, L. F. and Rhoads, C. P. *Arch. Int. Med.* 1951, **87** 477.

DISCUSSION

S. Neukomm : For four years, our research team has devoted part of its activity to the study of the metabolism of Synkavit, injected in rats, and its mode of action *in vitro* on fibroblast from the heart of mice. We used Synkavit labelled with radioactive phosphorus*. This substance undergoes a more or less rapid hydrolysis in the organism, according to the nature of the organs in which it is fixed. This hydrolysis brings about the liberation of the phosphoric groups (PO_4), which then follow the general metabolism of the PO_4 ions. From then on, the destiny of the basic molecule (2-methyl-1, 4-naphto-hydroquinone) can no longer be followed. Still, it is most probable that this molecule remains locally fixed in the cells in an oxidized form, as Mitchell's report seems to show.

With reference to the distribution of Synkavit in the various organs of rats, our results confirm and extend Mitchell's statements. Synkavit is mainly fixed and accumulates (*I*) in the organs which have intense cellular proliferation centres and

* Neukomm, S., Péguiron, L., Lerch, P., and Richard, M. *Arch. internat. Pharmacodyn Thérapie*, XCIII, 1953, 373.
Péguiron, L. and Neukomm, S. *Acta Anat.* 1954, **21** 46.

(*2*) in storage, detoxication or elimination organs. There is a close correlation between the capacity of the cells to absorb Synkavit, and the rate of renewal of deoxyribonucleic acids. Further, the retention of the product is greater when the proliferation is more intense.

It has been clearly shown that Synkavit does not provoke any important morphological lesions in mitosis. It is a weak antimitotic. By histophotometric measurements of fibroblast nuclei cultivated *in vitro* and stained by the Feulgen reagent, we have established that Synkavit decreases the proportion of cells containing high concentration of deoxyribonucleic acid (pre-prophasic nuclei).

With the concentrations we have used the proliferation of the cultures is not hindered. The observed effect is thus not bound to a decrease in the number of mitosis, but rather to change of the time of synthesis of the deoxyribonucleic acid, leading to a delay of prophase. Our research shows that the quantity of Synkavit necessary to obtain this effect is extremely low. This corroborates and reinforces the idea that the basic molecule of Synkavit remains fixed in the cell and intervenes in the metabolism of the deoxyribonucleic acid.

The damage caused to the fibroblast cultures immersed in Synkavit solutions containing proteins is only half that found in solutions (Tyrode) that do not contain any proteins. On the other hand, exposure of cultures for 50 minutes to Synkavit in Tyrode solution shows damages equal to that caused by Synkavit directly introduced in the culture medium ; in other words, with equal concentrations, the effect of Synkavit develops in 50 minutes in Tyrode and in 48 hours in plasma. Other experiments (cultures treated with Synkavit compared with non-treated cultures, influence of the number, the duration and the volume of the wash-liquid after exposure of the cultures to Synkavit) show that Synkavit is for the greatest part adsorbed on the proteins or on the cellular membranes.*

These data help to explain the important difference in the therapeutic effect obtained by administering Synkavit by the intramuscular or intravenous route. The combination ' Synkavit-proteins ' cannot be effected instantaneously and depends on the local concentration of Synkavit. When the latter is introduced into the muscular tissues, it remains there longer and at a higher concentration than when it is introduced in the blood-stream and therefore cannot reach distant organs in sufficient concentration.

B. Jolles : Having used Synkavit in over two hundred patients with advanced malignant disease undergoing radiotherapy, and noted the beneficial effects of the administration of this compound as a coadjuvant of radiotherapy, I should like to draw attention to the formula of Karnofsky mentioned by Mitchell in view of the fact that often, particularly in the case of patients with carcinoma of the lung, the results assessed on the basis of survival times do not give the true picture of the efficacy of the treatment. This is often better assessed instead on the basis of criteria concerning comfort and relief of symptoms which accrue from a particular method of treatment.

J. O. Laws and B. Jolles : Amongst the attempts to improve the results of the radiotherapy of some types of cancer, especially those in which results are unsatisfactory, by the ancillary use of chemical agents designed to act as radio-sensitizers, the work of Mitchell† (1948) has been most consistently and thoroughly pursued. After many clinical trials Mitchell‡ (1952) has shown that the administration of tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (synthetic vitamin K derivative, Synkavit, Roche) to patients with advanced malignant disease improves

* Richard, M., Péguiron, L. and Neukomm, S. *Arch. internat. Pharmacodyn. Thérapie*, in press.

† Mitchell, J. S. *Brit. J. Cancer*, 1948, **2** 351-358.

‡ Mitchell, J. S. *Brit. Empire Cancer Camp. 30th Ann. Rep. London*, 1952, p. 239.

significantly the survival results of treatment. Laboratory tests with tissue cultures and animal experiments with a large series of chemical compounds (Mitchell and Simon-Reuss*, 1952), have shown the superiority of this temporarily solubilized quinol.

The work here described is concerned with the animal experiments only as the clinical material available would not allow as yet any data regarding the effectiveness of Synkavit as a coadjuvant of radiotherapy to be presented.

Thirty-five rabbits and 37 rats were given subcutaneous injections of Synkavit, the amounts given to rabbits ranging from 10–50 mg daily, but constantly 10 mg in the case of rats. The drug was given in some cases for a few days before the delivery of the X-ray dose and continued for a time varying from 10–17 days. The dose of X-rays given in the case of rabbits ranged from 600–1,500 r. There were three groups of rats which received respectively 480 r, 585 r and 650 r; the first two groups contained 25 rats and the last 26.

All the rabbits and all the rats were exposed to a beam of radiation covering the whole body. The physical factors were 180 kV, 10 mA, 50 cm F.S.D. with a H.V.L. varying according to the filtration, which was either the inherent tube shield filtration alone or 0·5 mm Cu (H.V.L. 0·85 mm Cu). The irradiation was carried out with the tube head and the applicator inverted, with the box containing the animals standing on top of the applicator end.

RESULTS

Rabbits

The difference in survival rates between the Synkavit plus X-rays series of rabbits with the series of rabbits which received X-rays alone was not significant when the assessment was made on the whole series of 35 rabbits which received Synkavit injections.

In the Synkavit group the majority maintained their weight fairly well throughout the observation period or gained ground slightly. This was particularly so in the case of the rabbits which received Synkavit injections for a few days prior to exposure to X-rays. In the series of rabbits exposed to X-rays without Synkavit there was a consistent drop in the weight curve, with the exception of one surviving rabbit which gained some 10 per cent in weight over a period of 30 days. The Synkavit rabbits were on the whole more lively and taking food more liberally than the animals without Synkavit.

Rats

The 76 rats used in these experiments were dealt with in three separate series.

Group I—13 rats received 10 mg of Synkavit injections daily (7 rats for four days and 6 rats for five days) prior to exposure to a dose of 580 r and subsequently daily until their death, and a group of 13 rats received a similar dose of X-rays to the whole body without any injections. In the Synkavit group there was 1 survivor and in the non-Synkavit series there were no survivors at 30 days.

Group II—Of 25 rats in this group 24 were exposed to a dose of 650 r to the whole body. Of these, 11 were given injections of 10 mg of Synkavit daily, and 13 were given daily injections of 1 c.c. of sterile normal solution of saline. With the exception of the control rat which survived the 30 days' period there were no 30-day survivals in the whole series.

Group III—A group of 25 rats were exposed to a dose of 480 r to the whole body. Of these 9 received seven and 4 received five injections, 10 mg daily, of Synkavit prior to exposure to X-rays and eight to eleven daily injections after exposure. 8 rats received six pre-X-ray treatment and 10 post-treatment injections of 1 c.c. of

* Mitchell, J. S. and Simon-Reuss, I. *Brit. J. Cancer*, 1952, **6** 305–317.

sterile normal solution of saline and 4 received no injections whatsoever. In the group of rats which received saline injections there were no deaths at 31 days after irradiation. In the group of rats which received Synkavit injections there was only 1 survival at 31 days, the remainder dying within 12–16 days. The 4 control (X-rays only) rats survived 31 days.

MICROSCOPICAL

The spleen and adrenals of both rats and rabbits were examined in these experiments. The adrenal has been comparatively little studied, and yet is considered in the recent literature to play some part in the syndrome of irradiation sickness (PORTER*, 1952), and in the repair of tissue damage following irradiation (CRAVER†, 1948). The importance of adrenal hormones in maintaining many vital functions also suggest a potential connection between this organ and the widespread changes present after irradiation.

Spleen

The changes in the spleen followed the same pattern in both species and were of the type previously described in the mouse by JACOBSON *et al*‡ (1950). The lymphoid follicles disappeared rapidly in the first three weeks. In many of the animals dying in the earlier period there were further changes of an atrophic nature, in some cases little except blood vessels and fibrous tissue being seen. Iron containing pigment was often prominent in those specimens. There was in general no difference between animals given Synkavit and the controls dying after the same period.

Rabbits

In general the findings in these species are similar to those of ENGELSTAD and TORGERSEN§. The changes in the cytoplasm consist of loss of lipoid at an early stage and in some cases a marked basophilia. This latter tends to disappear but lipoid is slow to reappear in comparison with what is usually seen in secondary adrenal involvement, often being small in amount at the end of 31 days. The nuclear changes affect particularly the zona fasciculata extending up to the medulla (the zona reticularis is hardly distinguishable in the rabbit). These come on after 3–5 days and appear to persist for weeks. The nuclei may undergo any of the commonly seen forms of degeneration, pyknosis, fragmentation or loss of basophilia followed by dissolution. Apart from occasional initial hyperaemia there is no inflammatory reaction and one has the impression that some of the degenerated cells remain *in situ* almost indefinitely. How much function may remain in spite of the nuclear changes is an interesting point for further study.

In contrast to the constant degenerative changes in the inner zones of the cortex, the zona glomerulosa in the rabbit shows comparatively slight damage. Both cytoplasm and nuclei appear relatively normal. No mitosis was seen in the rabbit material, but in the presence of some of the relatively normal cells its occurrence cannot be excluded on the present evidence.

Rats

In the irradiated animals it was found that, unlike what had been seen in the rabbit, all zones seemed equally affected, showing cytoplasmic and nuclear changes. The cytoplasmic damage was similar to that seen in the rabbit. The nuclear alterations

* PORTER, E. C. *Radiol.* 1952, **58** 246–257.

† CRAVER, B. N. *Amer. J. Roentgenol.* 1948, **59** 404–407.

‡ JACOBSON, L. O., SIMMONS, E. L., MARKS, E. K., ROBSON, M. J., BETHARD, W. F. and GASTON, E. O. *J. Lab. Clin. Med.* 1950, **35** 746–770.

§ ENGELSTADT, R. B. and TORGERSEN, O. *Acta Radiol.* 1937, **18** 671–687.

varied according to the normal patterns seen in the various éones. In the reticularis, pyknosis was the rule ; in the fasciculata, either pyknosis or a loss of basophilia and 'fading' ; in the reticularis, a breaking up of the nucleus, the stroma disappearing and leaving the chromatin particles scattered.

In animals dying in the first few days after irradiation, hyperaemia in the zona reticularis was common. As in the rabbit no evidence of an inflammatory reaction was seen at any time. Mitosis was in general absent but a few mitotic figures were seen in two rats dying on the 29th and 31st days. They were all in the outer part of the zona fasciculata.

In neither species was there any unequivocal evidence of the effect of Synkavit. In the rat, however, the general impression was one of greater damage in those receiving the compound than in the controls.

SUMMARY

(1) In rabbits the administration of Synkavit prior and after irradiation has shown slight increase in tolerance of irradiation, and although the difference in the mortality rate of rabbits and rats is not significant, the weight curve and the well-being of the animals suggested an increased tolerance of radiation in this species. In rats an opposite effect was found (MITCHELL*, 1951 ; JOLLES†, 1952). The histological changes in the adrenals stained only with haematoxylin and eosin can clearly give no more than a hint of the changes which take place in the irradiated animals. Nevertheless, enough has been found to suggest that a more detailed study of adrenal pathology and function might be fruitful in elucidating the mechanism of radiation effects.

(2) The histological findings of the effects of Synkavit were disappointing, but as the main demonstrable effect of this compound is mitotic inhibition (MITCHELL‡, 1949), it is perhaps not surprising that the histological appearance of tissue already showing nuclear damage and mitotic arrest from the effect of the X-rays, should be little altered by the chemical. Nevertheless the suggestion of greater damage in the adrenals of rats receiving Synkavit makes it possible that this may at least have contributed to the deleterious effect of this compound in this species.

Although it is hazardous to generalize from the described experiments the fact that patients suffering from malignant tumours who receive daily injections of Synkavit while undergoing radiotherapy stand treatment better, induces one to venture an opinion that the administration of this compound in humans produces a response similar to that found in rabbits rather than that in rats. In this context however it has to be borne in mind that a selective concentration of the drug in tumours has been shown by Mitchell by means of ultra-violet microphotography and that the use of Synkavit in normal individuals probably produces effects different from those to be expected in tumour-bearing patients receiving treatment to a part of the body only.

* MITCHELL, J. S. *Brit. Empire Cancer Camp. 29th Ann. Rep. London*, 1951, p. 192.

† JOLLES, B. *Brit. Empire Cancer Camp. 30th Ann. Rep. London*, 1952, p. 325.

‡ MITCHELL, J. S. *Brit. Empire Cancer Camp. 27th Ann. Rep. London*, 1949, p. 214.

AUGMENTATION DE LA SYNTHÈSE DE L'HÉMOGLOBINE *IN VITRO* PAR LES RÉTICULOCYTES APRÈS IRRADIATION

A. Nizet et A. Herve

Laboratoires de Clinique Médicale B (Prof. L. Brull), de Pathologie Générale (Prof. Z. M. Bacq) et de Radiothérapie (Prof. P. Desaive) de l'Université de Liège

Les expériences que nous allons vous décrire ont pour but de mettre en évidence l'influence de l'irradiation, soit de l'organisme tout entier, soit du sang ou du plasma *in vitro*, sur la synthèse de l'hémoglobine. Cette synthèse est mesurée à partir de l'incorporation du carbone radioactif de la glycine-2-^{14}C et de la phénylalanine-2-^{14}C à l'hémine et à la globine par les hématies jeunes *in vitro*.

L'un de nous (A. N.) a démontré, avec Robscheit-Robbins, que les hématies jeunes anucléées ou réticulocytes ont besoin d'une série d'acides aminés pour mûrir *in vitro*[18]. Par ailleurs, une série de travaux, basés sur la méthode des indicateurs isotopiques, ont démontré l'utilisation par ces cellules de la glycine pour la synthèse de l'hémine (London, Shemin, Neuberger et coll.[7, 10]), de la glycine, de la leucine, de la lysine et de l'histidine pour la synthèse des protéines réticulocytaires (Borsook et coll.[3]), et de la glycine et de la phénylalanine pour la synthèse de la globine (Nizet et Lambert[15-17]).

La mesure comparative de l'incorporation d'anabolites radioactifs *in vitro* permet une étude précise de la vitesse de synthèse de l'hémoglobine dans différentes conditions.

TECHNIQUES UTILISÉES

(*a*) *Préparation des chiens*

Les animaux, adultes des deux sexes, sont soumis à deux saignées par semaine (environ 20 cm^3 par kg de poids), par ponction de la veine jugulaire externe, jusqu'à ce que le taux d'hémoglobine soit abaissé jusqu'à 7 à 10 grammes pour cent. A ce moment, le taux de réticulocytes dans le sang périphérique atteint 40 à 200 p. 1000. Les chiens reçoivent une alimentation complète avec un supplément de fer et de vitamines.

(*b*) *Incubation du sang avec les amino-acides marqués*

Les manipulations se font par voie aseptique. Le sang est additionné lors du prélèvement, et à raison de 1/10 de son volume, de la solution suivante : héparine en poudre (Liquémine Roche) 20 cg–glucose 2 g–chlorure sodique 90 cg–eau 100 cm^3. Les amino-acides marqués sont ajoutés au sang sous forme de solution dans le chlorure sodique à 9 p. 1000. Les échantillons de sang sont incubés pendant 5 à 10 heures à 37° C dans un bain thermostatique. Après incubation, le plasma est éliminé et les hématies sont lavées 4 fois au chlorure sodique 9 p. 1000, par centrifugations et décantations successives.

(c) Préparation de l'hémine et de la globine

Hémine—12 à 15 cm^3 de purée globulaire est additionnée d'un volume égal de solution à 1 p. 1000 de glycine ou de DL-phénylalanine dans le chlorure sodique 9 p. 1000 et la suspension globulaire versée dans 5 volumes d'acide acétique saturé de chlorure sodique et chauffé à 95° C (NENCKI et ZALESKI[11]). La température est ramenée à 95° C et maintenue à ce niveau pendant 5 minutes. Le liquide est filtré rapidement sur ouate et abandonné à la température du laboratoire. Après 24 heures, les cristaux d'hémine sont séparés par centrifugation, lavés successivement une fois à l'eau, une fois dans une solution aqueuse à 1 p. 1000 de phénylalanine ou de glycine, 2 fois à l'eau, 1 fois à l'alcool éthylique à 95° C, 1 fois à l'éther, et séchés sur chlorure calcique dans le vide.

(d) Mesures de radioactivité

Les mesures de radioactivité de l'hémine et de la globine se font en double, après combustion par voie sèche, sur sources épaisses de $BaCO_3$, au compteur de Geiger-Müller à courant d'hélium–isobutane.

On trouvera dans les publications antérieures de l'un de nous ([12, 16-18]) des détails complémentaires sur la préparation, l'hygiène et la diététique des chiens, ainsi que sur les techniques d'étude de la synthèse de l'hémoglobine *in vitro*.

(e) Conduite des expériences

Du sang hépariné et glucosé est prélevé aux chiens soumis à l'anémie hémorragique et partagé en plusieurs parties, de 50 cm^3 chacune lorsqu'il s'agit de préparer l'hémine, de 5 cm^3 dans le cas de la globine. La première partie, non irradiée, sert de référence ; la deuxième est irradiée *in vitro* (20.000 à 100.000 r). D'autre part, l'animal est irradié *in toto* à la dose de 500 r et du sang est prélevé $\frac{1}{2}$ h à 1 h après l'irradiation. Tous les échantillons de sang sont soumis à la centrifugation et le sang prélevé après irradiation (éventuellement dilué par rapport au témoin par suite de la soustraction sanguine précédente) est ramené au même volume globulaire par soustraction de plasma. Dans certaines expériences, le culot de globules rouges prélevés avant irradiation du chien et débarrassés du plasma est additionné de plasma de l'animal irradié. Dans d'autres expériences enfin, des globules intacts sont respectivement mis, dans les mêmes conditions, en présence de plasma intact et de plasma irradié *in vitro* à 20.000 r. Les hématies sont remises en suspension, saturées d'oxygène par agitation prudente, et les différentes fractions sont additionnées de glycine-2-^{14}C ou de DL-3-phénylalanine-2-^{14}C* aux mêmes concentrations dans tous les échantillons. La radioactivité est mesurée, après incubation, dans la globine et dans l'hémine.

(f) Données sur les conditions d'irradiation

A titre d'exemple, nous donnons ici un protocole :

Exp. No. 102—Irradiation du chien *in toto* (500 r) : 200 kV—18 mA—filtre de cuivre de 0,5 mm—distance focale 180 cm—champ 50 × 30 cm—durée 15 minutes.

* Glycine fournie par le Radiochemical Centre, Amersham, Bucks., Angleterre ; activité spécifique 46,7 microcuries par mg. Phénylalanine fournie par Tracerlab, Boston, Mass., U.S.A. ; activité spécifique 0,31 millicurie/millimôle.

Irradiation du sang : à 500 r : mêmes constantes.
à 100.000 r : 50 kV—2 mA—pas de filtre—distance focale 2 cm—champ 25 mm de diamètre—durée 15 minutes.

RÉSULTATS EXPÉRIMENTAUX

Les résultats sont transcrits dans les *Tableaux I–III.*

Tableau I.—Irradiation in vivo *et* in vitro *et Synthèse de l'hémine*

No. des expériences	61	87	102	108
No. des chiens	BN 49	N 56	B 58	N 61
Taux d'hématies (par mm³ *de sang)*	2.860.000	4.200.000	2,700.000	3.200.000
Taux de réticulocytes (par mm³ *de sang)*	147.000	55.000	111.000	162.000
Taux de glycine-2-^{14}C (d.p.m. *par* cm³ *de sang)*	222.000	222.000	74.000	92,500
Taux de glycine-2-^{14}C(*γ par* cm³ *de sang)*	2,36	2,36	0,79	0,98
Durée d'incubation du sang (heures)	7	5	6	7
Taux d'hémoglobine (g p. 100)	8,10	9,9	6,3	8,3
Activités (en désintégrations par minute *et par* cg *d'hémine)*				
Sang témoin	14.200	1.092	11.793	9.151
Sang irradié in vitro (500 r)	15.700	1.554	27.035	—
Sang irradié in vitro (20.000 r)	—	—	—	10.507
Sang irradié in vitro (100.000 r)	30.400	1.237	19.884	—
Sang du chien irradié in toto (500 r)	28.000	1.788	47.623	11.095
Hématies non irradiées (prélevées avant irradiation du chien) + plasma du chien irradié	—	—	19.180	13.088

Tableau II.—Irradiation et Synthèse de la Globine

No. de l'expérience	121	130	139
No. du chien	N62	R65	B66
Taux d'hémoglobine (g p. cent)	7,15	7,45	8,7
Taux d'hématies par mm³ *de sang*	3.000.000	3.100.000	3.500,000
Taux de réticulocytes par mm³ *de sang*	210.000	295.000	210.000
Taux de DL-*3-phénylalanine-2-*^{14}C, *en désintégrations par* minute *et par* cm³ *de sang*	277.500	294.500	590.000
Durée d'incubation du sang (heures)	5	5	7
Activités (en désintégrations par minute *et par* cg *de globine)*			
Sang témoin (avant irradiation)	851	999	703
Sang irradié in vitro (500 r)	1.244	—	—
Sang irradié in vitro (20.000 r)	1.019	1.234	722
Globules prélevés avant irradiation plus plasma prélevé après irradiation	929	1.097	—
Sang d'animal irradié in toto	1.302	2.116	908

Tableau III.—Irradiation du Plasma et Synthèse de l'hémine

No. de l'expérience	128	134
No. du chien	P63	P63
Taux d'hémoglobine (g p. cent)	5,15	5,75
Taux d'hématies par mm^3 *de sang*	3.000.000	3.500.000
Taux de réticulocytes (p. 1000 *hématies*)	51,5	87
Taux de glycine-2-^{14}C (*désintégrations par* minute *et par* cm^3 *de sang*)	49.400	17.000
Volume de purée globulaire (cm^3)	10,5	30
Volume de plasma (cm^3)	29,5	40
Durée d'incubation du sang (heures)	7	5
Activités (*en désintégrations par* minute *et par* cg *d'hémine*)		
Hématies non irradiées plus plasma non irradié	3.000	4.350
Hématies non irradiées plus plasma irradié (20.000r) ..	3.750	5.200

DISCUSSION DES RESULTATS

Les données expérimentales autorisent les conclusions suivantes :

(*1*) L'incorporation du carbone radioactif *in vitro* à l'hémine et à la globine des hématies jeunes de chien irradié est augmentée par rapport au sang prélevé avant l'irradiation ; elle peut être quadruplée. Cette donnée est en accord avec les résultats de RICHMOND et coll.[19].

(*2*) L'irradiation du sang *in vitro* entraîne, elle aussi, une augmentation de l'incorporation de ^{14}C à l'hémine et à la globine.

(*3*) Cette incorporation est également accélérée dans les globules rouges prélevés avant irradiation du chien et mis en présence de plasma prélevé 1/2 heure après irradiation de l'animal.

(*4*) L'incorporation du ^{14}C par les hématies jeunes intactes est plus importante en présence de plasma irradié qu'en présence de ce même plasma, non irradié.

REMARQUES

(*a*) Il est souvent difficile de conclure avec certitude à une synthèse protéique en se basant sur sur l'incorporation de métabolites radioactifs *in vitro*. On peut se demander s'il ne s'agit pas de phénomènes d'adsorption ou de liaisons chimiques anormales. A ce point de vue, l'hémoglobine nous donne de sérieuses garanties, grâce au contrôle que permet son groupement prosthétique dont la structure est bien connue. Une augumentation de la radioactivité de la globine peut être avec certitude attribuée à un accroissement de la synthèse si elle s'accompagne d'une augmentation concomitante de la radioactivité de l'hémine[13]. Au reste, le phénomène ne s'observe qu'en présence d'hématies jeunes et il est bloqué par des poisons métaboliques ou respiratoires[15]. D'autre part, l'activité spécifique des amino-acides utilisés est suffisamment élevée pour que leur introduction dans le sang ne modifie pas de façon anormale le taux de l'amino-acidémie.

(*b*) Il n'est pas *a priori* certain que les synthèses de l'hémine et de la globine marchent de pair. Dans les présentes expériences l'effet excitant

a été observé dans le cas de l'hémine et de la globine ; nous sommes donc autorisés à parler d'une synthèse de l'hémoglobine, intéressant à la fois la fraction protéique et son groupement prosthétique.

(*c*) Il n'y a pas lieu d'envisager l'influence possible d'une variation du volume globulaire consécutive au prélèvement de sang effectué avant l'irradiation du chien. Le volume globulaire de tous les échantillons est ramené à celui du témoin et tous les échantillons subissent des manipulations identiques. Il n'y a pas non plus de variation de taux des réticulocytes ; une crise réticulocytaire après saignée ne survient qu'après plusieurs heures. D'autre part, il ne s'agit pas non plus d'une libération de facteurs excitants sous l'influence des saignées. La mise en évidence d'une stimulation de la synthèse de l'hémoglobine dans le sang irradié *in vitro* et en présence de plasma irradié *in vitro* suffit à réfuter ces objections. Il est hautement significatif que la stimulation de la synthèse soit observée après irradiation de plasma seul, privé d'éléments figurés.

(*d*) La pureté radioactive de l'hémine et de la globine a été vérifiée par plusieurs cristallisations et précipitations successives[17].

(*e*) Il n'existe pas de relation simple de proportionnalité entre la dose de rayonnement ionisant et le degré de stimulation de la synthèse. Il est possible, et même vraisemblable, que des facteurs limitants, d'importance variable d'une expérience à l'autre, et non contrôlés, interviennent. Cette même absence de proportionnalité a été observée dans le cas d'autres facteurs stimulant la synthèse[6, 9].

(*f*) Nous pouvons confirmer notre observation antérieure[14] relative à l'absence de toute hémolyse dans le sang irradié à des doses atteignant 200.000 r, dans nos conditions expérimentales.

(*g*) Les résultats que nous venons d'exposer ont un caractère préliminaire : ils ne nous renseignent pas sur la chronologie des phénomènes observés. Ils ne nous apprennent pas à quel moment les propriétés stimulantes atteignent un maximum éventuel dans le sang de l'animal irradié, ni combien de temps elles persistent après l'irradiation. Ces points restent à éclaircir.

La stimulation de la synthèse de l'hémoglobine, observée en premier lieu par Richmond et ses collaborateurs, et rapportée par nous à un phénomène humoral, n'est pas le seul cas d'accélération d'un processus métabolique que l'on ait observé sous l'influence des radiations ionisantes. C'est ainsi que Back et Bloch-Frankenthal[2] ont constaté une augmentation temporaire de la respiration de noyaux d'érythrocytes d'oiseau soumis à une irradiation de 500.000 à 1.500.000 r. De même, Forssberg et Hevesy[5] ont montré que la fixation de phosphate marqué au ^{32}P par le foie de jeunes souris irradiées (2000 r) était considérablement augmentée par rapport aux témoins non irradiés. L'irradiation augmente la teneur en fer du foie, de la rate, des reins et du sérum du rat (10 à 1500 r)[4, 8].

En ce qui concerne nos expériences, on peut se demander s'il ne s'agit pas d'une libération ou d'une décharge sanguine brutale d'un facteur intervenant normalement dans l'anabolisme de l'hémoglobine (et peut-être dans d'autres anabolismes). Cette décharge pourrait être suivie de troubles par déficit secondaire. Si cette hypothèse se vérifiait, l'identification du facteur plasmatique pourrait présenter un intérêt pratique d'ordre thérapeutique.

CONCLUSIONS ET RÉSUMÉ

(*1*) La synthèse de l'hémoglobine *in vitro* par les réticulocytes de chien irradié est augmentée par rapport au sang prélevé avant l'irradiation de l'animal.

(*2*) La synthèse de l'hémoglobine est également augmentée dans les réticulocytes normaux mis en présence de plasma d'animal irradié.

(*3*) Cette même synthèse est accélérée dans le sang irradié *in vitro*.

(*4*) Le plasma irradié seul, *in vitro*, jouit également de propriétés stimulatrices de la synthèse.

(*5*) L'intervention d'un facteur humoral accélérant la synthèse de l'hémoglobine après irradiation est de la sorte démontrée.

REFERENCES

[1] Anson, M. L. et Mirsky, A. E. *J. Gen. Physiol.* 1930, **13** 469.
[2] Back, A. et Bloch-Frankenthal, L. *Proc. Soc. Exper. Biol. a. Med.* 1947, **66** 366.
[3] Borsook, H., Deasy, C. L., Haagen-Smit, A. S., Keighley, G. et Lowy, P. H. *J. Biol. Chem.* 1952, **196** 669.
[4] Chanutin, A. et Ludewig, G. *Amer. J. Physiol.* 1951, **166** 381.
[5] Forssberg, A. et Hevesy, G. *Arkiv för Kemi*, 1952, **5** 93.
[6] Lambert, S. *Arch. Intern. Physiol.* 1953, **61** 462.
[7] London, I. M., Shemin, D., West, R. et Rittenberg, D. *J. Biol. Chem.* 1948, **173** 797 ; 1949, **179** 463 ; 1950, **183** 749.
[8] Ludewig, S. et Chanutin, A. *Amer. J. Physiol.* 1951, **166** 384.
[9] Lybeck, H., Lambert, S., Nizet, A. et Barac, G. *Arch. Intern. Physiol.* 1954, **62** 304.
[10] Muir, H. M. et Neuberger, A. *Biochem. J.* 1949, **45** 163 ; 1950, **47** 97.
[11] Nencki, M. et Zaleski, J. *Z. Physiol. Chem.* 1900, **30** 384.
[12] Nizet, A. Recherches sur la Nutrition et la Régénération des Hématies, Liège, Vaillant-Carmanne, 1951.
[13] Nizet, A. *Rev. Méd. Liège*, 1953, **8** 506.
[14] Nizet, A., Bacq, Z. M. et Herve, A. *Arch. Intern. Physiol.* 1952, **60** 449.
[15] Nizet, A. et Lambert, S. *ibid*, 1953, **61** 120.
[16] Nizet, A. et Lambert, S. *Bull. Soc. Fr. Chim. Biol.* 1953, **35** 771.
[17] Nizet, A. et Lambert, S. *ibid*, 1954, **36** 307.
[18] Nizet, A. et Robscheit-Robbins, F. S. *Blood*, 1950. **5** 648.
[19] Richmond, J. E., Altman, K. I. et Salomon, K. *J. Biol. Chem.* 1951, **190** 817.

DISCUSSION

H. Suit : I would like to mention in connection with Nizet's report and the well-known depression of the marrow iron turnover rate following low dose (25 r) total body radiation in the rat, that we have examined the effect of 300–5,000 r *in vitro* on the ability of normoblasts of human bone marrow in culture to take up iron.

For the culture of the human bone marrow we used the technique of Lajtha of our laboratory. Irradiation was given at the beginning of the culture period (0 hr.). ^{59}Fe-globulin was added at the third hour of culture in the amount of 1 μc/ml culture medium. After 17–20 hours the culture was opened and autoradiographs prepared, which were developed and stained after a period of 4 weeks' exposure. This permitted a differential count on the erythroid series, a count of grains over individual cells, and the determination of the percentage of the cells of any group that showed a positive autoradiograph.

Figure 1 shows the result of such an experiment. Here a dose of 1,000 r was given in 3 mins. and the culture continued for 17 hours. The only change observed is that of a marked shift in the differential count of the normoblasts. There are 72 per cent early normoblasts (E.N.—pro, baso, and early polychromatic normoblasts) and 28 per cent late normoblasts (L.N.—late polychromatic and orthochromatic normoblasts) in the control culture while the irradiated specimen contained only 10 per cent E.N. but had 90 per cent L.N. The upper average grain count and the per cent positivity did not alter appreciably. These changes were not considered significant in any of the 12 marrows examined with doses varying from 300–5,000 r.

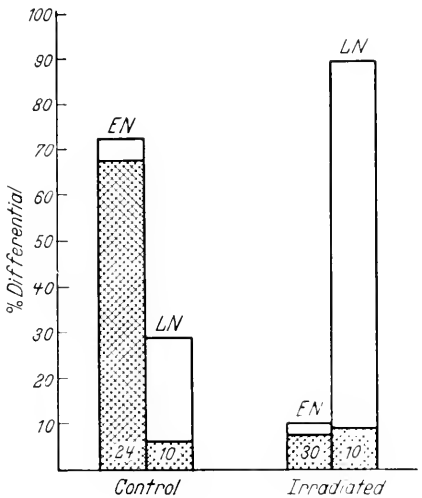


Figure 1

These data have suggested to us that those cells which had not received damage due to the *in vitro* radiation which was lethal by the time we observed them, had been able to take up iron at an essentially normal rate. We did not evaluate changes in reticulocytes properly and therefore cannot comment on them. However, we found nothing to indicate that iron uptake *in vitro* by human normoblasts was either accelerated or depressed by the rather large doses of radiation used. It seems, therefore, that the observed depression of marrow turnover rate *in vivo* following total body radiation may result from a mitotic inhibition or delayed maturation or some other factor than an interference with the actual uptake of iron.

PHOSPHORYLATING ACTIVITY OF MITOCHONDRIA AFTER TOTAL BODY IRRADIATION

D. W. VAN BEKKUM

Medical Biological Laboratory of the National Defence Research Council, Rijswijk, Netherlands

THE interference with mitosis and the death of living cells after exposure to ionizing radiation are commonly assumed to be the outcome of some primary damage to the cell nucleus. Most morphological and biochemical changes so far described after irradiation have been localized in the nucleus. Pycnosis and karyorrhexis, structural damage to chromosomes and inhibition of DNA synthesis are among the most widely recognized cellular effects of irradiation. There are however reasons to question the assumption that radiation damage is primarily to the nucleus (TROWELL[1] and MOLE[2]).

Therefore some years ago studies were initiated in this laboratory with the object of collecting information on the effect of ionizing radiation on metabolic processes in the cytoplasma. It was postulated that the interference with nuclear function, *e.g.* cell division and DNA synthesis, which follows exposure to irradiation, might well be the consequence of damage to biochemical reaction systems outside the nucleus. Since synthetic processes in the nuclei are generally supposed to be dependent on energy-generating reactions which occur in the cytoplasma, the oxidative phosphorylation of mitochondria seemed to present the most obvious subject for investigation.

In preliminary experiments with mitochondrial preparations from various tissues, it was found that spleen mitochondria showed a decreased phosphate uptake shortly after total body irradiation. Most of our subsequent work has been carried out with rat spleen tissue because of its radiosensitivity and its relative abundance per animal. A decrease of oxidative phosphorylation of isolated spleen mitochondria following total body exposure to X-rays has since been reported by several investigators. In 1952 POTTER and BETHEL[3] described a decrease of phosphate uptake by mitochondria, isolated from rat spleen after total body irradiation with 800 r. Similar observations were reported shortly afterwards by ourselves (VAN BEKKUM *et al*[4]). In these experiments also rather large doses of X-radiation, namely 800 r and 1,100 r, were administered to rats and spleen mitochondria were isolated 2, 4 and 24 hours afterwards. In addition to a diminished phosphate uptake, some decrease of oxygen consumption was observed in most experiments, although the latter effect has been generally of lower magnitude. Thus a decrease of P/O ratios has been consistently found with several substrates.

MAXWELL and ASHWELL[5] have published comparable results obtained with mitochondrial preparations from mouse spleen at 1–7 days after a lethal dose of total body X-irradiation. It should be pointed out that in general the study of biochemical reactions in highly radio-sensitive tissues

can only be expected to throw some light on the mechanism of *initial* radiation injury, if performed within a few hours after exposure. After a larger interval, especially when relatively large doses of radiation are being employed, the structural changes in the tissue are so radical that a change in tissue composition rather than in cellular function is most likely to be reflected in the results.

Therefore our experiments are usually performed at 4 hours or less after total body irradiation, when changes in organ weight are not yet apparent.

INFLUENCE OF DOSE

After the effect of large doses of total body irradiation had been established, we have attempted to assess the sensitivity of oxidative phosphorylation to this type of injury. Furthermore our studies have been extended to mitochondria isolated from rat thymus. The methods employed with spleen

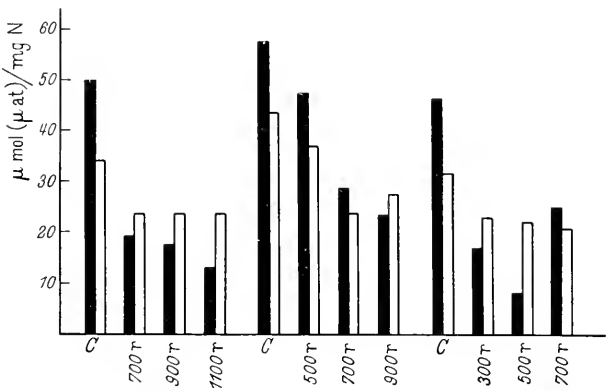


Figure 1. Oxidative phosphorylation of rat spleen mitochondria at 4 hours after various doses of total body irradiation. Black bars: phosphate uptake in μmol/mg N ; *white bars: oxygen uptake in* μatoms/mg N. *Each experiment consists of 4 groups including a control.*

mitochondria have been described previously (VAN BEKKUM *et al*[6]) and were applied with minor modifications to the study of mitochondria from thymus. Mitochondria were prepared from the pooled tissues of at least 2 rats in case of spleen and of 4 rats in case of thymus. Oxidative phosphorylation of these preparations was estimated in duplicate. Variation of activity between and to a less extent within batches of normal rats necessitated the inclusion of control animals in every experiment. Some of the results are depicted in *Figures 1–3.*

It is apparent that in the case of spleen mitochondria, oxygen uptake is usually much less affected than phosphorylation, while in the case of mitochondria from thymus this difference is not so outspoken, although P/O ratios are depressed after irradiation in most experiments. The minimal dose of radiation which was capable of inducing a decrease of mitochondrial phosphorylation was found to be between 50 r and 100 r for both tissues.

Microscopical examination of the tissues used in these experiments revealed extensive cellular destruction at 4 hours after doses of 300 r and more. In

the spleen after a dose of 50 r, some cell débris is clearly recognizable in most of the lymph follicles, while in an occasional one very little if any sign of damage is to be found. After 100 r, a greater number of nuclear fragments

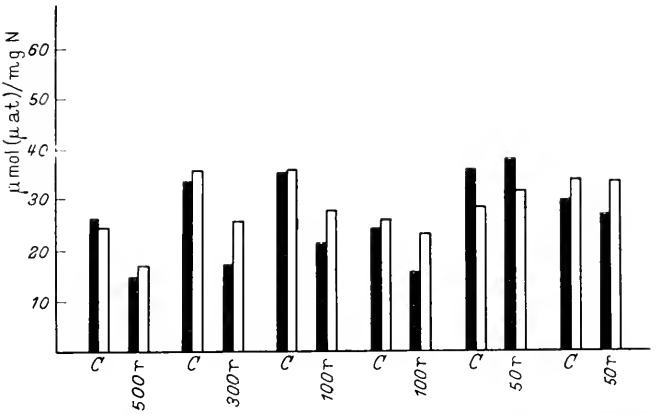


Figure 2. Oxidative phosphorylation of rat spleen mitochondria at 4 hours after various doses of total body irradiation. Black bars : phosphate uptake in μmol/mg N ; *white bars : oxygen uptake in* μatoms/mg N

and pycnotic nuclei have been observed, but the damage is markedly less than after the larger doses of radiation.

In thymus slices only a small amount of nuclear fragmentation could be observed at 4 hours after 50 r and mitotic figures were still present. After

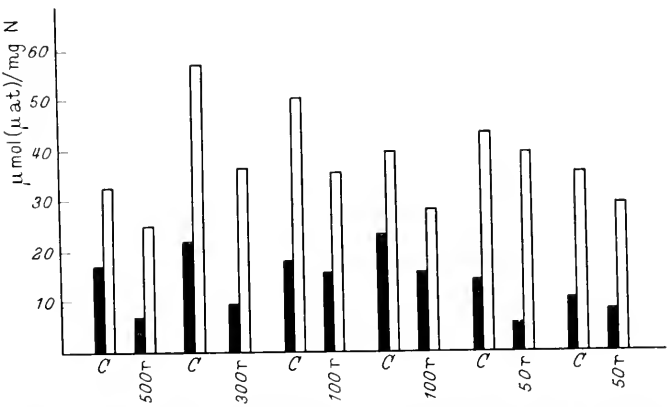


Figure 3. Oxidative phosphorylation of rat thymus mitochondria at 4 hours after various doses of total body irradiation. Black bars : phosphate uptake in μmol/mg N ; *white bars : oxygen uptake in* μatoms/mg N.

100 r the signs of destruction were more extensive, although many apparently normal nuclei and a few mitotic figures remained. Although no accurate estimation of the amount of visible damage has been attempted, it appears that the minimal doses of total body irradiation required to produce morphological evidence of nuclear damage in a sizable number of cells and those

necessary for the biochemical change under discussion to become measurable do not differ widely if at all.

TIME-EFFECT

Since the process of oxidative phosphorylation has thus been found to be highly radiosensitive, it seemed of interest to determine more accurately the earliest time after irradiation at which this lesion becomes discernable. These experiments were performed with rat spleen mitochondria at various times up to 4 hours after total body irradiation with a dose of 700 r. The time-effect curve shown in *Figure 4* reveals a latent period of more than 1 hour after the irradiation before the oxidative phosphorylation becomes clearly impaired. From most of the tissues used in this series, samples were taken for histological study with the object to investigate whether the disturbance of oxidative phosphorylation precedes the morphological changes in the nuclei or otherwise.

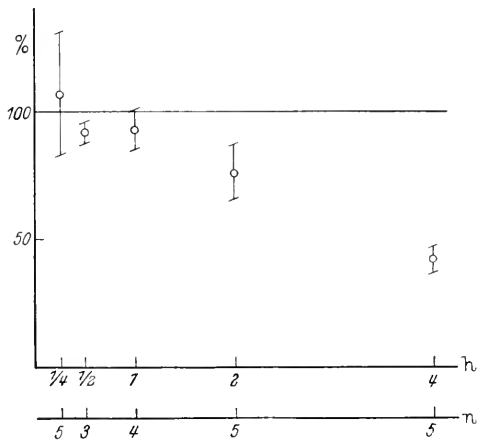


Figure 4. Phosphate uptake of rat spleen mitochondria at various periods after total body irradiation with a dose of 700 r. *Abscissae: hours after irradiation, ordinates: percentage of corresponding control value; white circles with attached vertical lines: mean ± S.E.; n: number of experiments*

The first definite signs of damage, which consisted mainly of nuclear vacuolization, were found at 1 hour after irradiation, but the number of nuclei affected was very small at this time. At 2 hours after 700 r the degeneration was more pronounced with some pycnotic nuclei in most of the follicles. At this time the picture resembled that found at 4 hours after 50–100 r in the previous series. The slices obtained at 4 hours after 700 r showed a domination of pycnosis and fragmentation in all of the follicles and a number of cells in the pulpa was involved as well. After that period degeneration progresses still further and removal of dead material by macrophages begins. In general this pattern closely resembles the observations made by Trowell[1] on lymph nodes after *in vivo* irradiation. The limited number of our results does not allow a definite conclusion as to the sequence in which the two effects develop after irradiation. It is therefore at present not possible to decide if a causal relationship between the two phenomena exists but the approximately simultaneous appearance of both the cytoplasmic and the nuclear changes seems to warrant further investigation.

In this connection it is perhaps of interest to mention that we have not been able to find any interference with the oxidative phosphorylation of mitochondria isolated under similar conditions from the livers of irradiated rats. Even a dose of 5,000 r administered to the liver region failed to produce an effect as observed in the case of spleen and thymus mitochondria. Since the liver is generally held to be a radio-resistant organ, these observations suggest a relation between the disturbance of oxidative phosphorylation and the radiosensitivity of the cells from which the mitochondria are derived.

Because of the well known sensitivity of the spleen to various forms of stress, it was considered essential to investigate the effect of radiation on the

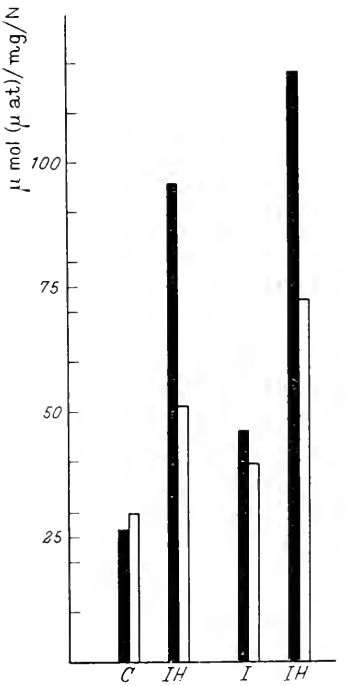


Figure 5. Effect of intermittent hypoxia on oxidative phosphorylation of spleen mitochondria. Black bars : phosphate uptake in μmol/mg N ; *white bars : oxygen uptake in* μatoms/mg N ; *C : controls ; IH : intermittent hypoxia*

spleen only. To this end the spleen was exteriorized during irradiation while the rest of the animal was being shielded. In this series the controls were subjected to a sham-procedure. The depression of oxidative phosphorylation of the mitochondria isolated from the irradiated spleens was found to be comparable to that observed after total body irradiation. The interference with phosphorylating activity after total body exposure is therefore at least for the greater part the result of the action of X-rays on the spleen tissue itself.

In vitro irradiation of isolated spleen mitochondria has been performed both in the inactive state at 0° C and during incubation in the presence of substrates and oxygen at 38° C. Following the irradiation the mitochondria were kept at 0° C in the presence of versene for 4 hours and reincubated

for the estimation of oxidative phosphorylation. No effect of irradiation even with doses of 20,000r has been found, which is in accordance with results of POTTER *et al*[3]. From this it should not be concluded that the mitochondrial defect is necessarily secondary to some radiation-induced aberration in other parts of the cell. For one thing, it has not been possible to keep the mitochondria *in vitro* in a state of active metabolism for a period of 2 hours, and this might well be essential for the lesion to develop, as has been observed with other forms of radiation-induced damage. The results obtained with rat spleen do not allow an evaluation of the radio-sensitivity of the mitochondria in the various haematopoietic cells, since this organ contains beside lymphoid elements, also variable quantities of erythropoietic and myelopoietic cells. Our results with thymus mitochondria indicate that the mitochondria of lymphoid cells are affected by total body irradiation. In order to assess the sensitivity of mitochondria from erythropoietic cells in

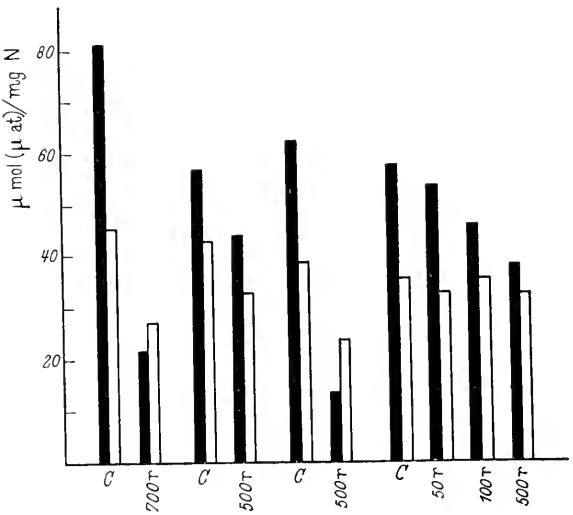


Figure 6. Oxidative phosphorylation of spleen mitochondria at 4 hours after various doses of total body irradiation of rats subjected to intermittent hypoxia for a period of 3–4 days. Black bars : phosphate uptake/mg N ; *white bars : oxygen uptake*/mg N

this respect, an increase of the red cell forming elements has been induced by exposure of the animals to intermittent hypoxia for a period of 3 to 4 days. Mitochondrial preparations from the spleens of these pretreated rats were found to exhibit an increased rate of oxidative phosphorylation, with a concomitant increase of P/O ratios to nearly double the control values (*Figure 5*). This activity is also severely depressed after total body irradiation (*Figure 6*), which indicates that the activity of mitochondria from erythropoietic cells is also susceptible to irradiation.

INTERPRETATION

The work so far summarized has been mainly of a descriptive kind. The significance of the disturbance of oxidative phosphorylation with regard to the mechanism of radiation injury to the cell is not known. Also the nature of the derangement of oxidative phosphorylation is still obscure. There have been suggestions that it might be secondary to the increased ATP breakdown which occurs in spleen homogenates following total body

irradiation. In 1952 Ashwell and Hickman[7] reported a threefold increase of ATP dephosphorylating activity (to be denominated ATP-ase activity hereafter) of mouse spleen homogenates at 1 to 11 days after total body irradiation with lethal doses of X-rays and in 1953 more details on this subject were published (Ashwell and Hickman[8]). The authors conclude that this rise could be best explained by the premise that a large amount of inert cellular material had been destroyed after irradiation, while the particular enzyme systems remained unaffected. More recently Dubois *et al*[9] showed that the increase of ATP-ase activity could be observed in rat spleen and thymus homogenates even after total body irradiation with doses as small as 25–50 r and they suggested the possible significance of this effect with regard to the maintenance of energy requiring reactions after irradiation. Previously it had been pointed out by Maxwell *et al*[5] that the increase

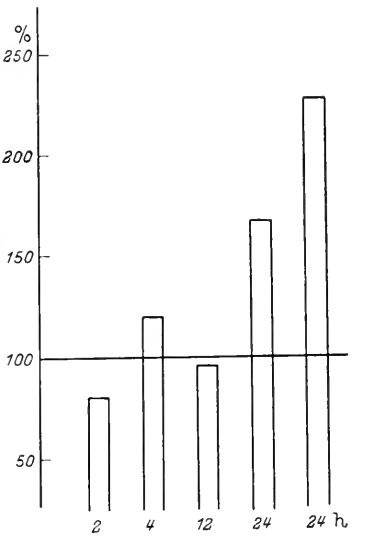


Figure 7. ATP-ase activity of rat spleen homogenates at various periods after total body irradiation with a dose of 1,100 r. *Values are expressed as a percentage of corresponding controls*

of ATP-ase activity cannot be the cause of the depressed phosphorylation of spleen mitochondria, because ATP-ase activity had been blocked by NaF in the system used for the measurement of oxidative phosphorylation. However in our hands the amount of NaF employed by Maxwell *et al* left a small part of the ATP-ase activity uninhibited and we found this remaining activity in homogenates to be proportional to the values obtained in the absence of NaF. Therefore a more extensive investigation of the possible role of ATP-ase in the disturbance of oxidative phosphorylation has been carried out, using mouse and rat spleen preparations. The results confirm the conclusion of Maxwell *et al* and may be summarized as follows :

(1) The ATP-ase activity of rat spleen homogenates has been estimated at various intervals after irradiation and the results have been compared with the time-effect curve obtained in the experiments on oxidative phosphorylation. It has been found that the increase of ATP-ase activity of the homogenates is not apparent within a few hours after irradiation, when the disturbance of oxidative phosphorylation is already well developed (*Figure 7*).

(2) The ATP-ase activity of isolated spleen mitochondria after total body irradiation has been found to be normal even at 24 hours after irradiation, in the presence of a severely impaired phosphorylating capacity which was observed in samples of the same preparation (*Figure 8*).

We have also investigated if the decrease of anaerobic glycolysis which has been described in mouse spleen homogenates at one day and longer

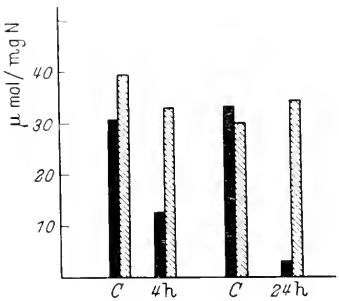
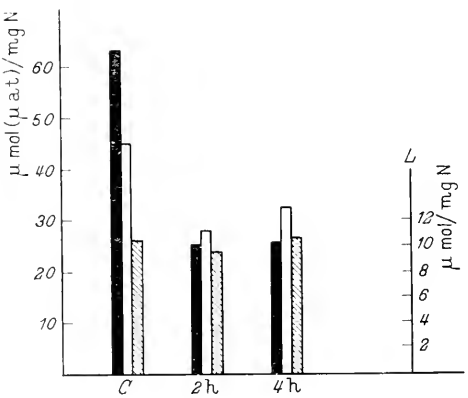


Figure 8. Phosphate uptake and ATP-ase activity of mouse spleen mitochondria at various periods after total body irradiation with a dose of 1,100 r. *Black bars : phosphate uptake in* µmol/mg N *; hatched bars : ATP-ase activity in* µmol/mg N *of phosphate formed*

after irradiation (HICKMAN and ASHWELL[10]), has any relation to the disturbance of oxidative phosphorylation. The fact that a normal rate of glycolysis was observed in the presence of a decreased oxidative phosphorylation (*Figure 9*) indicates that different mechanisms are probably involved in the development of these two types of radiation damage.

In conclusion there is as yet no evidence of the depression of oxidative phosphorylation being secondary to some other known radiation-induced

Figure 9. Oxidative phosphorylation of spleen mitochondria and anaerobic glycolysis of spleen homogenates after total body irradiation with a dose of 700 r. *Black bars : phosphate uptake in* µmol/mg N (*left ordinate*) *; white bars: oxygen uptake in* µatoms/mg N (*left ordinate*) *; hatched bars : lactate formation in* µmol/mg N (*right ordinate*)



biochemical alteration. Therefore the effect must be ascribed to some block in the oxidation-coupled phosphorylating reactions, the exact nature of which is at present little understood.

SUMMARY

The depression of oxidative phosphorylation of mitochondria isolated from spleen or thymus has been found to occur after doses as low as 50 r. This biochemical change develops within a few hours after irradiation and a close parallelism with the appearance of morphological changes in the nuclei

has been observed. The effect has also been found after irradiation of the exteriorized spleen only, but could not be produced by *in vitro* irradiation of mitochondria.

The decrease of phosphorylation is not secondary to the increased ATP-ase activity and develops prior to the depression of anaerobic glycolysis, which are observed in spleen homogenates after total body irradiation.

The sensitivity of oxidative phosphorylation as well as its relatively rapid impairment after irradiation suggest that this cytoplasmic lesion may be intimately connected with the primary radiation injury.

REFERENCES

[1] Trowell, O. A. *J. Path. Bact.* 1952, **64** 687.
[2] Mole, R. H. *Brit. J. Radiol.* 1953, **26** 234.
[3] Potter, R. L. and Bethel, F. H. *Federation Proc.* 1952, **11** 270.
[4] van Bekkum, D. W., Jongepier, H. J., Nieuwerkerk, H. T. M. and Cohen, J. A. *Trans. Faraday Soc.* 1953, **49** 329.
[5] Maxwell, E. and Ashwell, G. *Arch. Biochem. Biophys.* 1953, **43** 389.
[6] van Bekkum, D. W., Jongepier, H. J., Nieuwerkerk, H. T. M. and Cohen, J. A. *Brit. J. Radiol.* 1954, **27** 127.
[7] Ashwell, G. and Hickman, J. *Proc. Soc. exp. Biol. Med.* 1952, **80** 407.
[8] Ashwell, G. and Hickman, J. *J. Biol. Chem.* 1953, **201** 651.
[9] Dubois, K. P. and Petersen, D. F. *Amer. J. Physiol.* 1954, **176** 282.
[10] Hickman, J. and Ashwell, G. *J. Biol. Chem.* 1953, **205** 651.

DISCUSSION

R. H. Mole : I am sure van Bekkum is right in deciding to look for biochemical changes in the first few hours after irradiation. As he points out, at a time when there is visible cell death in the tissue examined, biochemical changes must be occurring from the fact of cell death alone. But in the light of this I wonder why he chose as long an interval as 4 hours since by that time cell death in the spleen is quite evident.

I would also like to suggest that changes in oxidative phosphorylation by mitochondria may be analogous to the morphological changes in cell nuclei which follow irradiation, and may be a consequence of lethal damage to the cell rather than the cause of it. Just as nuclear pycnosis occurs in radio-sensitive organs and not radio-resistant organs like muscle, so oxidative phosphorylation is altered in the spleen, but not in the muscle (as Laser mentioned). If there is one thing clear about the effects of whole body irradiation it is that some organs are radiosensitive and some are resistant. If there is a biochemical lesson due to irradiation—and a biochemical lesson there surely must be—then it seems to me it must be sought in the specific biochemical activities which distinguish radio-sensitive from radio-resistant tissues, not in the general metabolic properties which are common to all cells.

ACTION D'UNE DOSE UNIQUE DE RAYONS X SUR QUELQUES FRACTIONS DE PHOSPHORE ACIDO-SOLUBLE ET SUR LA RESPIRATION DE LA PEAU CHEZ LE RAT*

P. Mandel, Ch. Gros et J. Rodesch

Institut de Chimie Biologique, Faculté de Médecine, Strasbourg

L'importance du métabolisme énergétique dans les synthèses cellulaires et dans le renouvellement des constituants tissulaires, a incité divers auteurs à rechercher les modifications des composés phosphorés acido-solubles de la rate[9, 11], de la moelle osseuse[9] ou de bactéries entières[2] sous l'effet des rayons X. Il nous a paru intéressant d'examiner l'action de ces rayons sur le phosphore acido-soluble de la peau et ceci pour des multiples raisons. Tout d'abord il est aisé de comparer l'effet d'une irradiation de l'animal entier et d'une irradiation locale directe au niveau de la peau. D'autre part, tout en étant sensible aux rayons X, du fait des proliférations de la couche basale, la peau subit cependant sous l'effet des irradiations moins de destructions cellulaires que la rate, la moelle osseuse ou les cultures bactériennes. On ne sera donc pas astreint à tenir compte des disparitions de cellules pour l'interprétation des résultats dans le cas de la peau dans la même mesure que lorsqu'il s'agit de la rate ou de la moelle osseuse. Enfin il est à peine nécessaire de rappeler l'importance des lésions cutanées sous l'effet d'irradiations, objet de préoccupations de nombreux radiobiologistes.

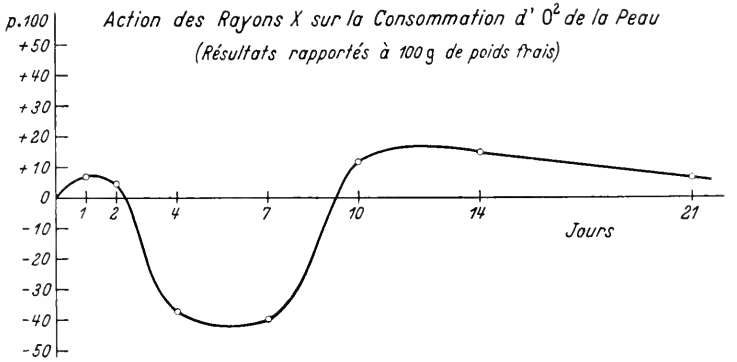
Nous rapportons ici nos résultats concernant l'action d'une irradiation totale sur diverses fractions de phosphore acido-soluble de la peau en même temps que les répercussions sur la consommation d'oxygène de ce tissu. Rappelons que l'étude des composés phosphorés acido-solubles tels l'A.T.P., le phosphagène et les esters phosphoriques fournissent des indications à la fois sur les disponibilités en énergie et sur le catabolisme des glucides. Il ne saurait être question, dans le cadre de cet exposé de rappeler la bibliographie concernant les effets métaboliques des radiations et en particulier leur action sur le métabolisme des glucides. On trouvera des indications dans de nombreux articles, monographies et mises au point[1, 12, 16, 8].

Nos essais ont porté sur un total de 120 rats Wistar, d'un poids variant de 150 à 250 g. Soixante de ces rats ont servi de témoins, les 60 autres ont subi une irradiation corporelle totale. (Dose : 700 r ; tension : 180 kv ; Filtre : 1 Cu ; Distance foyer-rat : 50 cm ; Débit : 25 r/mn.)

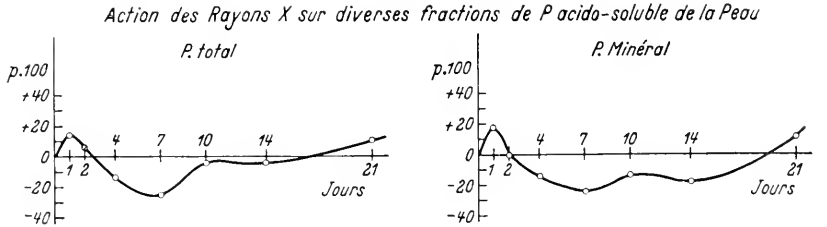
Les animaux groupés en lots homogènes, du même sexe, issus d'une même portée et descendant de croisements répétés entre frères et soeurs à travers plusieurs générations, ont été sacrifiés par saignée 24 heures, 48 heures, 4 jours, 7 jours, 10 jours, 14 jours et 21 jours après l'irradiation. En vue

* Travail effectué avec le concours de l'Institut National d'Hygiène.

des dosages de phosphore acido-soluble, la peau prélevée rapidement dans la région comprise entre les omoplates en haut, les crêtes iliaques en bas et limitée latéralement par la ligne axillaire, a été congelée dans un mélange d'acétone et de neige carbonique. Un jour avant le sacrifice la peau qui devait être prélevée, a été rasée. Le tissu cutané a été ensuite extrait par de l'acide trichloracétique à 10% en chambre froide à 0°. Sur l'extrait ainsi obtenu nous avons dosé le P total selon BRIGGS, le P minéral à l'état de phosphate ammoniaco-magnésien et le P du phosphagène selon LOHMANN[7].



Le reste de l'extrait a été fractionné par précipitation à la baryte conformément à la technique décrite par SACHS[14], que nous avons modifiée[10] compte tenu du procédé de LEPAGE[6]. Nous avons ainsi déterminé dans la fraction des composés baryum-insolubles : le P labile de l'A.T.P. et les esters phosphoriques ; dans la fraction baryum-soluble, alcool insoluble : les esters glucose-1-phosphoriques, glucose-6-phosphoriques et les trioses-phosphates.



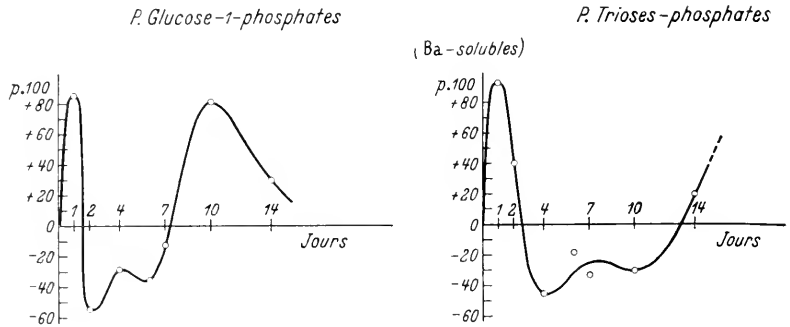
Dans le résidu, après délipidation, nous avons déterminé le P de l'acide désoxyribonucléique (A.D.N.) après séparation selon la technique de SCHMIDT et TANNHAUSER[15] légèrement modifiée, suivie de dosages de phosphore et de désoxypentoses.

La consommation d'oxygène a été mesurée à l'appareil de WARBURG selon la technique classique en milieu de KREBS glucosé et à 37°5. La peau a été soigneusement rasée sous anesthésie générale 3 heures avant le sacrifice des animaux et a été rapidement prélevée à l'aide d'un dermatome dans la même région que les échantillons utilisés pour les dosages de P acido-soluble. Des examens histologiques ont montré que la partie prélevée comprenait

l'épiderme et le derme, à l'exclusion de toute formation musculaire. Les résultats de nos essais consignés dans les graphiques ci-contre :

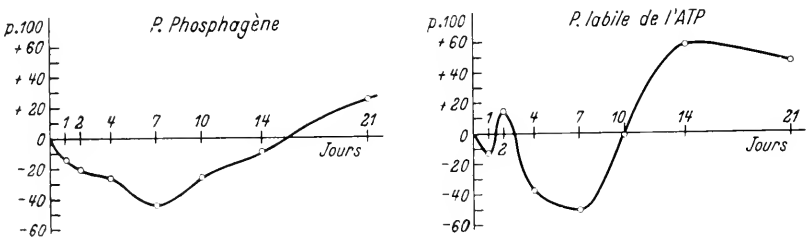
Graphiques

Nous y avons représenté les variations des valeurs en p. 100 par rapport aux témoins non irradiés. Chaque point de la courbe représente une expérience comportant un minimum de 4 témoins et de 4 animaux irradiés.



En ce qui concerne le *P acido-soluble total* qui est de 29,3 mg chez le rat normal on remarque après un accroissement léger dans les premières 48 heures, une baisse de sa valeur qui atteint 23 p. 100 aux environs du 7ème jour.

Le *P minéral* (valeur normale 14,2 mg en moyenne) accuse également un accroissement au bout de 24 heures approximativement du même ordre de grandeur que celui du P total. On note par la suite une réduction qui se maintient plus longtemps que celle du P total, puisque le retour à la normale survient seulement après 14 jours.



Le phosphagène dont le taux normal est de 3,33 mg environ accuse une baisse notable dans la période qui s'étale entre le 1er et le 13ème jour après l'irradiation ; la valeur la plus basse (—40 p. 100) est enrégistrée entre le 6ème et le 7ème jour. Il convient d'insister sur l'augmentation du phosphagène après le 14ème jour qui s'accentue encore jusqu'au 21ème jour.

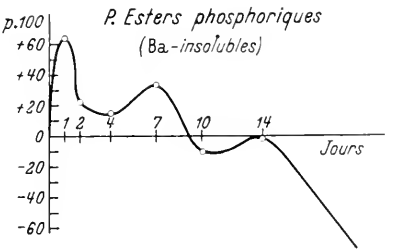
L'A.T.P. dont la valeur normale est de 3,75 mg, montre une oscillation tantôt dans le sens négatif, tantôt dans le sens positif au cours des premières 48 heures. Par la suite on relève une réduction considérable ; les valeurs les plus basses se situent aux environs du 7ème jour. Après le 10ème jour on assiste à une augmentation notable de l'A.T.P.

Parmi les composés phosphorés intermédiaires du catabolisme des glucides, nous retiendrons l'évolution des esters phosphoriques baryum-insoluble et du glucose-1-phosphate de la fraction baryum-soluble. *Les esters phosphoriques* baryum-insolubles présentent une augmentation jusqu'au 14ème jour alors que l'on note une réduction après ces délais. *Les glucose-1-phosphates* par contre se trouvent régulièrement abaissés à partir du 2ème et ceci jusqu'au 10ème jour. Notons que les trioses-phosphates montrent également une baisse régulière à partir du 4ème jour : (45 p. 100 le 4ème jour, 30 p. 100 le 10ème jour).

Pour ce qui est de la consommation d'oxygène on relève un accroissement de celle-ci dans les premières 24 heures suivie d'une baisse avec retour à la normale aux environs du 10ème jour.

Discussion des Resultats

L'accroissement du P minéral comme celui du P total dans les premières 24 heures est a rapprocher de l'augmentation de la consommation d'oxygène de la peau à la même époque. Une telle augmentation de l'oxygène consommé été signalée en ce qui concerne la moëlle osseuse[13]. Nous ne



saurions expliquer d'une façon valable ce phénomène avec les données dont nous disposons actuellement.

La réduction sensiblement parallèle du P minéral et du P total jusqu'au 7ème jour, montre que la teneur en P organique qui englobe à la fois les composés intermédiaires du métabolisme des glucides et les constituants riches en énergie, n'a pas varié d'une façon sensible. Après le 7ème jour le maintien d'un phosphore minéral abaissé avec un phosphore total qui revient à la normale, traduit un accroissement global des composés phosphorés organiques acido-solubles. Comme par ailleurs nous constatons durant la même période qui s'étale du 2ème au 10ème jour une baisse très accentuée des composés riches en énergie, A.T.P. et phosphagène, on peut admettre une accumulation des composés phosphorés intermédiaires du métabolisme à la suite des blocages enzymatiques. Les blocages portent d'une façon inégale sur les divers enzymes. Ceci nous explique l'augmentation des esters phosphoriques de la fraction baryum-insoluble et la diminution du glucose-1-phosphate à partir du 2ème jour de même que la baisse régulière des trioses-phosphates à partir du 4ème jour. Il convient d'insister sur l'accroissement des esters phosphoriques de la fraction Ba-insoluble durant la période de baisse de l'A.T.P. Elle peut s'interpréter comme le reflet d'un blocage du catabolisme au stade de ces esters phosphoriques, dont la conséquence évidente est une réduction de la formation de l'A.T.P.

Au moment où l'A.T.P. revient à la normale et atteint même des valeurs supérieures à celles-ci, le blocage se trouve levé et le taux des esters phosphoriques Ba-insolubles se trouve naturellement abaissé.

Pour ce qui est du glucose-1-phosphate, stade initial de la dégradation du glycogène, on constate une réduction après 48 heures qui parle en faveur d'un blocage de la phosphorylase.

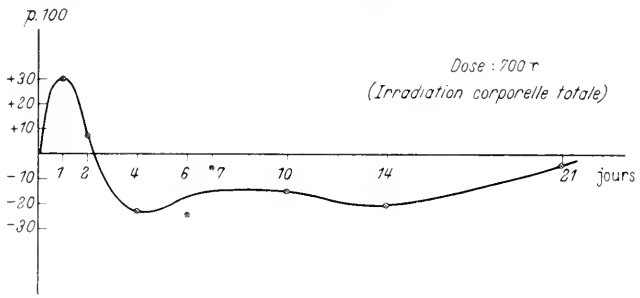
On pourrait se demander si les modifications observées en rapportant les résultats au poids frais, ne sont pas en relation avec des modifications éventuelles de l'état d'hydratation. Nous avons de ce fait déterminé la teneur en protéines des échantillons de peau analysés et recalculé les valeurs en les rapportant à ces protéines. Les conclusions qui se dégagent se trouvent en parfait accord avec ce qui vient d'être dit plus haut. Il en est de même dans les grandes lignes si l'on considère les valeurs rapportées à l'A.D.N. Cependant, dans ce dernier cas, à la suite de la destruction d'un certain nombre de noyaux, que reflète la diminution de l'A.D.N., les réductions de l'A.T.P. et du phosphagène sont plus atténuées. Il n'en reste pas moins vrai que la chute des composés phosphorés riches en énergie ne peut être mise sur le compte de la disparition d'un certain nombre de cellules. La réduction de l'A.T.P. et du phosphagène que l'on retrouve en rapportant les valeurs à l'A.D.N., traduit un appauvrissement des cellules en ces composés.

Addendum

Nous avons étudié comparativement les effets d'une irradiation de l'animal entier et d'une irradiation locale superficielle sur le Phosphore acido-soluble de la peau. Dans les deux cas la même dose de 700r a été administrée.

Si l'on compare les résultats que nous venons de discuter avec ce que nous avons observé à la suite d'une irradiation locale, superficielle, il apparait que dans ce dernier cas les réactions sont bien plus atténuées. En effet, après l'irradiation locale, les réductions du P total, du P minéral, de l'A.T.P. et du Phosphagène de même que l'accroissement des esters phosphoriques baryum-insolubles sont nettement plus faibles que dans le cas de l'irradiation

Action des rayons X sur l'acide desoxypentose nucleique de la Peau



de l'organisme entier. De plus l'accroissement réactionnel de l'A.T.P. et du phosphagène au 21ème jour analogue à celui des acides nucléiques de la moëlle osseuse et de la rate rapportés antérieurement est pratiquement inexistant après une irradiation locale.

Conclusion

De l'ensemble de ces résultats se dégage la notion d'une chute importante des composés phosphorés riches en énergie dans les premiers jours qui suivent l'irradiation. Ce fait est sans doute la conséquence d'un trouble du catabolisme des glucides dont un autre aspect apparaît à l'examen de l'évolution des esters hexoses-phosphoriques. Dans ce cadre il convient de noter le blocage des diverses activités enzymatiques provoquant la baisse de certains constituants (glucose-1-phosphates, trioses-phosphates, glucose-6-phosphates) et l'accroissement de certains autres (esters phosphoriques Ba-insolubles). A partir du 7ème jour on assiste à un retour à la normale des taux de l'A.T.P. d'abord, du phosphagène ensuite. Il est intéressant de noter qu'au 21ème jour on enrégistre des valeurs d'A.T.P. nettement supérieures à la normale. Pareil phénomène a déjà été observé pour l'acide ribonucléique et de l'acide désoxyribonucléique de la rate[3] et la moelle osseuse[4] après irradiation. Il semble ainsi que dans la période éloignée de l'irradiation on observe à la fois un effet stimulant sur la synthèse des acides nucléiques et le métabolisme glucidique. Il importe encore de souligner que les modifications rapportées ne sont pas dues seulement à une destruction de tissus avec disparition d'un certain nombre de cellules mais à des variations au niveau des cellules restantes. Si l'on oppose aux conséquences de l'irradiation totale ceux observées à la suite d'une irradiation locale superficielle on constate que dans ce dernier cas les troubles sont beaucoup plus atténués.

REFERENCES

1 Bacq, Z. M., Lecomte, J. et Herve, A. *Arch. int. Physiol.* 1949, **57** 142.
2 Billen, D., Strehler, B. L., Stapleton, G. E. et Brigham, E. *Arch. Biochem. Biophys.* 1953, **43** 1.
3 Gros, Ch. M. et Mandel, P. *J. belge Radiol.* 1952, **35** 357.
4 Gros, Ch. M., Mandel, P., Metais, P. et Voegtlin, R. *C.R. Acad. Sci., Paris*, 1951, **233** 1685.
5 Krebs, H. A. *Biochim. biophys. Acta*, 1950, **4** 1249.
6 Lepage, G. A. In *Manometric Techniques and Tissue Metabolism*. Burgess Publishing Co., Minneapolis, 1949, **1** 227.
7 Lohmann, K. *Biochem. Z.* 1928, **194** 306.
8 Loureau, M. et Lartique. *Experientia*, 1950, **6** 25 ; *Arch. Sci. physiol.* 1950, **4** 197 ; *Arch. Sci. physiol.* 1951, **5** 83 ; *J. Physiol.* 1951, **43** 593 ; *C.R. Acad. Sci., Paris*, 1952, **234** 2022.
9 Lutwak, —, Mann, C. *Biochem. J.* 1951, **49** 300 ; *ibid*, 1952, **52** 356.
10 Mandel, P., Bieth, R. et Weill, J. D. *Bull. Soc. Chim. biol., Paris*, 1953, **35** 973.
11 Maxwell, E. et Ashwell, G. *Arch. Biochem.* 1953, **43** 389.
12 Ord, M. G. et Stocken, L. A. *Physiol. Rev.* 1953, **33** 357.
13 Richmond, J. E., Altmann, K. I. et Salomon, K. *Science*, 1951, **113** 404.
14 Sacks, J. *J. biol. Chem.* 1949, **181** 655.
15 Schmidt, G. et Thannhauser, J. S. *ibid*, 1945, **161** 83.
16 *Symposium of Radiology*, Vol. 1, p. 465, John Wiley, New York, Chapman & Hall, London.

DNA SYNTHESIS IN BONE MARROW STUDIED BY AUTORADIOGRAPHY

L. G. Lajtha, R. Oliver and F. Ellis

Department of Radiotherapy, Oxford

The purpose of this paper is to report on experiments on the incorporation of ^{32}P orthophosphate, adenine ^{14}C, and formate ^{14}C into deoxyribonucleic acid (DNA) by human bone marrow cells *in vitro*, and on the effect of X-irradiation and aminopterin on the synthesis of deoxyribonucleic acid.

METHODS

Cell suspensions from human bone marrows were cultured in a medium containing 80 per cent fresh human serum and 20 per cent balanced salt solution. The method of culture has been described in detail elsewhere (Lajtha[1]). The labelled compounds were added to the culture medium : 5 μc/ml medium ^{32}P orthophosphate (with less than 0·1 μg inactive PO_4

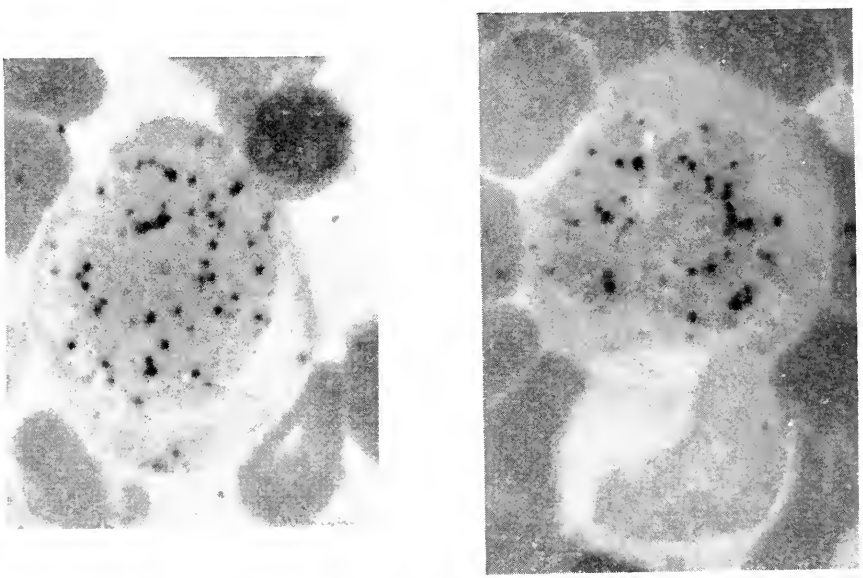


Figure 1. Localization of formate ^{14}C *in human bone marrow cells* in vitro. *Stained autoradiographs.* × 2000.

buffer) : 0·5 μc/ml medium adenine ^{14}C (specific activity 10 μc/mg) : 1 μc/ml medium formate ^{14}C (specific activity 15 μc/mg). The cultures were incubated at 37° C for 2–48 hours, then smears were made, fixed in 95 per cent methylalcohol and hydrolysed in N HCl at 60° C for 6 minutes.

This hydrolysis was found to remove all non DNA phosphorus or adenine from the cells while leaving DNA phosphorus and adenine behind (LAJTHA[2]). After hydrolysis autoradiographs were prepared using the stripping film technique, and were exposed in light-tight boxes in a refrigerator. After processing, the slides were stained without removing the photographic emulsion (LAJTHA[3, 4]). The stained autoradiographs thus obtained allow differential and grain counting *(see Figure 1)*. The number of cells showing positive autoradiographs can be expressed as a percentage of the total counted. Grain counting over individual nuclei is also carried out and the maximum activity is expressed as 'upper average' grain count (the average grain count of 10–15 cells judged visually to show maximum activity).

RESULTS

(*a*) *The cell cycle*—If the cells synthesized DNA throughout the entire intermitotic period, then, after a few hours of culture in the presence of the isotope all the cells should contain labelled DNA. This, however, was found not to be the case. Experiments with ^{32}P or adenine ^{14}C indicated that when cells were cultured for progressively greater lengths of time, a gradually increasing percentage of the cells showed DNA autoradiographs (per cent positive nuclei). The grain counting (upper average grain count per nucleus) demonstrated that the amount of labelled DNA per nucleus also increased with time, until a maximum was reached in about 15–18 hours. These findings indicate that DNA synthesis takes place during a limited period in the cell cycle.

LAJTHA, OLIVER and ELLIS[5] showed that labelling of the DNA indicated a length of period of DNA synthesis (S period) of the order of 12–15 hours, and a total cell cycle time (intermitotic period) of about 40–45 hours. When only the mitotic figures were counted on the smears (metaphase and anaphase) it appeared that, while in a 2–4 hours' culture with ^{32}P the majority of the mitotic figures did not show autoradiographs, in a 6 hours' culture most of the mitoses already contained labelled DNA. This observation suggests that immediately prior to mitosis there is a 3–4 hours' stage of the cell cycle (G_2 period) during which no DNA synthesis takes place. Prior to this second gap is the period of DNA synthesis (S), which is preceded by the long first gap (G_1) during which again no DNA synthesis takes place. The cell cycle related to DNA synthesis measured by the incorporation of ^{32}P or adenine ^{14}C into DNA :

This timing, in principle, agrees well with that found in the bean root by HOWARD and PELC[6].

Effect of X-irradiation—Large doses of X-rays (5,000 r in 15 minutes, 140 kV 1 mm Al filter) exerted a marked inhibition on DNA synthesis. When in a 0–6 hour culture in isotope, radiation was delivered in the third hour (0–3x–6) then both the number of cells showing DNA autoradiographs and the grain count over the nuclei give similar counts to those in a 3-hour culture (0–3). This suggests that cells in the period of DNA synthesis are immediately and completely prevented from synthesizing DNA.

Radiation delivered at the beginning of the culture period in isotope (0x–6 : 0x–24) also markedly inhibited DNA synthesis. Since the grain counts indicated that subsequent to irradiation no cells, even with long culture times, synthesized more DNA than a 2–3 hours' non-irradiated culture would, it was suspected that the cells in the G_1 period suffered a latent damage. If the isotope was added to the cultures three or more hours after irradiation (x–3–24) hardly any DNA synthesis was observed, as opposed to those cultures in which the isotope was added immediately following the irradiation (0x–24). These observations suggest that cells in the G_1 period, immediately before the S period receive a latent damage from a dose of 5,000 r. During the first 3 hours following radiation these cells may enter the S period ; after about 3 hours, however, even these cells will be incapable of DNA synthesis.

All G_1 cells are damaged by a dose of 5,000 r. The grain counts indicate that the difference between 6 and 24 hour cultures after radiation is produced by some G_1 cells entering the S period for a limited time. The radiation damage in these cells becomes apparent in that although they may enter the S period, they cannot synthesize more than 2–3 hours' worth of DNA as compared with the normal 12–15 hours' worth. The effect of irradiation on the G_2 period was indicated by the fact that no mitoses were observed in any of the irradiated cultures.

Formate ^{14}C *uptake*—The incorporation of adenine or phosphorus is not necessarily the true picture of DNA synthesis, therefore in a series of experiments the uptake of formate ^{14}C as a purine and pyrimidine precursor was investigated. Formate ^{14}C as a one-carbon compound takes part in many of the reactions in the cytoplasm of the cell, and therefore it is surprising that most of the labelled carbon taken up by the cells was localized in the nucleus, even on the non-hydrolysed autoradiographs. This suggests a preferential uptake of formate into DNA and RNA and also the central role of the nucleus (? nucleolus) in RNA synthesis.

On the acid hydrolysed preparations a quantitative assessment of the formate ^{14}C into DNA was possible. The maximum uptake per nucleus was of the order of $1 \cdot 2 \times 10^7$ atoms DNA ^{14}C (50 grains in 10 days' exposure, 2 grains/electron) which is only slightly greater than that found by previous adenine ^{14}C experiments. (The concentration of adenine ^{14}C in the cultures was $0 \cdot 5\,\mu c$/ml (specific activity $1 \cdot 56$ mc/mM) *i.e.* $0 \cdot 3$ millimolar. The concentration of the formate ^{14}C : $2 \cdot 0\,\mu c$/ml (specific activity $1 \cdot 04$ mc/ml) *i.e.* $2 \cdot 0$ millimolar.) The adenine stock was labelled in the ratio of 1 : 45 and the formate stock in the ratio of 1 : 75. Therefore, if a cell took up 1,000 molecules of adenine or formate they would include 22 molecules adenine ^{14}C or 13 molecules formate ^{14}C. Further, since corresponding cultures contained 1,000 adenine molecules or 6,000 formate molecules in equal volumes of the medium, if the uptake was parallel to the concentration, then for each 22 labelled adenine molecules 78 labelled formate molecules (*i.e.* over 3x) should have been taken up. This in fact was not the case. It was considered that the concentrations used were in excess of those permitting maximum uptake. Subsequent experiments have confirmed this.

Effect of Aminopterin—As can be seen from *Table I*, aminopterin, in a concentration of $0 \cdot 5\,\mu g$/ml medium markedly inhibited the incorporation of

formate ^{14}C into DNA, while showing only slight inhibition on the uptake of adenine ^{14}C into DNA. It appears therefore that folic acid antagonists, unlike X-rays, do not affect significantly the assembly of the DNA molecule

Table I. Effect of Aminopterin on uptake of ^{14}C into DNA on formate ^{14}C uptake into DNA

Exp. No.	*Culture time*	*Control*		0·5 μg ml *aminopterin*	
		% + nuclei	*Upper av. grain count*	*% + nuclei*	*Upper av. grain count*
Formate ^{14}C					
200	18 hr	74	80	48	30
205	18 hr	33	30	11	5
207	18 hr	62	45	14	5
210	20 hr	55	40	12	10
217	20 hr	65	50	34	30
224*	21 hr	74	50	60	50
Adenine ^{14}C					
205	18 hr	35	25	27	20
207	18 hr	58	25	39	20
210	20 hr	64	50	47	40
217	20 hr	52	40	48	40
224*	21 hr	50	50	50	50

* Old aminopterin solution

from its constituents (purines and pyrimidines, desoxyribose, phosphate) but inhibit the synthesis of pyrimidines, and, probably to a lesser extent, that of the purines. Citrovorum factor, in a concentration of 75 μg ml medium reverses the inhibiting effect of aminopterin.

This work is part of a project supported by the British Empire Cancer Campaign.

REFERENCES

[1] Lajtha, L. G. *J. Clin. Path.* 1952, **5** 67.
[2] Lajtha, L. G. *Nature, Lond.* 1954, **174** 587.
[3] Lajtha, L. G. *Exp. Cell. Res.* 1952, **3** 696.
[4] Lajtha, L. G. *J. Photogr. Sci.* 1954, in press.
[5] Lajtha, L. G., Oliver, R. and Ellis, F. *Brit. J. Cancer*, 1954, in press.
[6] Howard, A. and Pelc, S. R. *Isotopes in Biochem.* Ciba Conf. 1951, Churchill, London.

DISCUSSION

R. Oliver : It has been shown that radiation prevents the incorporation into DNA of adenine added to the culture. However, it is not at present clear whether this is due to interference with the synthesis of the other necessary purines or with the assembly of the DNA molecule. As an extension of the work reported here it is suggested that ^{14}C labelled adenine be added to the irradiated culture together with the other three purines required. If then it is only the purine synthesis which is inhibited DNA synthesis should be able to proceed as indicated by incorporation of the labelled adenine. The radiation effect on each purine synthesis could be investigated in this way.

THE INHIBITION OF DNA SYNTHESIS BY IRRADIATION WITH SPECIAL PREFERENCE TO IRRADIATION IN THE EARLY STAGES OF LIVER REGENERATION

Barbara E. Holmes and Lorna K. Mee

Department of Radiotherapy, University of Cambridge

It has been noticed by Hevesy[1], by Vermund *et al*[2], by Skipper and Mitchell[3] and by ourselves[4] and various other authors that irradiation with a considerable range of X-ray doses will reduce the rate of deoxyribonucleic acid formation in growing tissues to about 50 per cent of normal. Provided that the technique of irradiation is so arranged that the animal suffers no undue shock from pain or fear or constricting strapping, the dose may be as high[2] as 9,000r and still not reduce the DNA formation below this level. This fact may be of interest in a general consideration of mitotic process, since it has been suggested by Von Euler and Hevesy[5] and later by other authors, that the reconstitution of the DNA already present, as well as the formation of new DNA takes place in dividing cells and that the new formation is sensitive to irradiation whereas the reconstitution is not. These views receive support from the work of Hevesy[1] and of Stevens, Dauost and Leblond[6] who show that the rate of DNA formation, as indicated by isotope uptake, is double the amount required for the formation of new cells actually appearing. The number of new cells appearing is judged sometimes by the increase in weight of an organ or tumour and sometimes by mitotic counts after colchicine.

Most of these estimations of rate of DNA synthesis were based on ^{32}P uptake and it was therefore possible that only the phosphate of the resting DNA was being replaced. We have, however, carried out some double labelling experiments with regenerating liver (using glycine or adenine containing ^{14}C and injecting it at the same time into the same animal as the ^{32}P), which suggest that the new formation of the basic part of the nucleic acid is proceeding at the same rate as the new formation of the phosphate. These experiments are still incomplete, but as far as we can see, if the DNA of the cell is indeed broken down and rebuilt during the mitotic cycle, it is the whole molecule which must be broken down and rebuilt. This may be difficult to reconcile with a template theory of DNA synthesis.

Since the work of Howard and Pelc[7] it has been realized, as Lajtha has pointed out in this symposium, that synthesis of DNA is complete some hours before mitosis and we cannot, therefore, suppose that the breakdown of the original DNA is some part of the chromosome division and general rearrangement of mitosis. In our material, Howard has been able to show that the synthesis, as measured by adenine incorporation, is complete about

7 hours before mitosis. In the very early stages of rat liver regeneration, just before the first mitoses take place, enlarged cells with nuclei carrying their double loads of DNA are found. Since the total uptake of isotopes into the DNA up to this point can be measured and the number of enlarged cells counted, it should be possible to decide here also whether double the amount of isotope needed for new synthesis has been taken up.

It is possible that this apparent doubling is due to an occurrence at actual mitosis. Various authors have been led to believe that there is a doubling of DNA content at mitosis or directly after. It can also be pointed out, though this is not meant as a serious suggestion, that a throwing out of old, unlabelled DNA at mitosis might occur and would reduce the actual amount of DNA present while causing a sudden rise in the specific activity of the DNA now extractable from the cells. In our curves we find a sudden rise in the specific activity of DNA phosphorus just before the first burst of mitoses in the tissue, though no new ^{32}P is now entering the DNA.

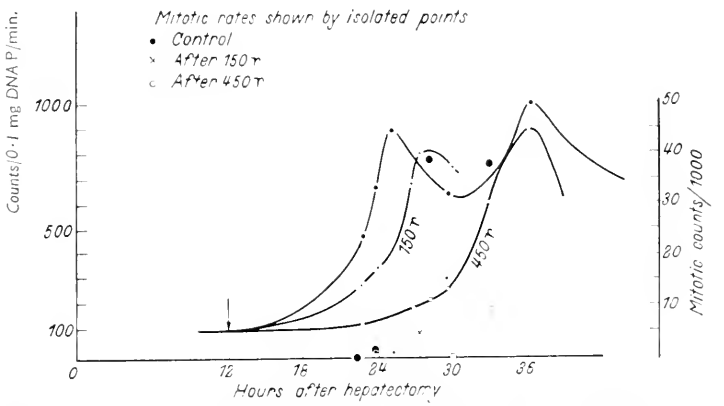


Figure 1. Effect of 150 r *and* 450 r *X-ray doses on rate of formation of DNA* (^{32}P *used*)

Another possible way, and quite a different one, of accounting for the X-ray resistant 50 per cent of DNA synthesis was brought out by the work of PELC and HOWARD[8]. They showed that DNA synthesis could be inhibited for some hours by very small doses of X-rays given shortly before the synthesis began. During the period of actual DNA synthesis even considerably larger doses had no effect on it. It therefore seemed possible that, when a growing tissue containing cells in every stage of the mitotic cycle was irradiated, a proportion of them would be in an insensitive stage and a proportion in a sensitive stage as regards DNA synthesis. Thus it might happen that only part of the synthesis would be inhibited.

The early stages of rat liver regeneration provide very easy and straightforward material for investigating this possibility. In fact KELLY[9] has already shown that the formation of DNA can be inhibited by total body irradiation in the pre-synthesis period much more easily than by irradiation in the synthesizing period in this material also. It can be seen from *Figure 1* that no synthesis of DNA takes place in the remaining lobe during the first 12 or 15 hours after partial hepatectomy. Synthesis then begins and the

rate of synthesis increases rapidly up to about 24 or 27 hours after hepatectomy. A fast rate is maintained for some hours ; at about 42 hours, however, the rate of synthesis and the rate of mitosis are lower than before, but remain at a fairly constant level. During this later period, the tissue can probably be regarded as an ordinary growing tissue containing all stages of the mitotic cycle in an established proportion.

In the following experiments rate of synthesis has usually been measured by uptake of ^{32}P, but many confirmatory experiments have been done with glycine ^{14}C as marker. If irradiation is given at 12 hours, no cells will yet be in the synthetic stage. It is easy to show the sensitivity of DNA synthesis to irradiation at 12 hours, since 450r will delay synthesis for many hours and some delaying action can be seen with as small a dose as 150r. This small dose will also cause a delay of a few hours in the appearance of the first mitoses and 450r will cause a longer delay (9 hours). This early-stage irradiation with small doses can also cause chromosome breakage ; Koller has seen about 10 per cent of breaks about 15 hours after irradiation with 150r, in the first mitoses to appear ; about 20 hours after 450r he has found chromosome breakage in up to 100 per cent of the first dividing cells.

Twelve or fifteen hours after irradiation (that is, 24–27 hours after the partial hepatectomy) all these effects are very apparent. Mitosis is suppressed and the DNA synthesis, which is very rapid in the controls, is scarcely beginning. The period from about 15 to about 24 hours after the hepatectomy is one of DNA synthesis, but still without mitosis ; this begins at about 24 hours but is much more active at 27 or 30 hours, when from 3 per cent to 6 or even 10 per cent of cells may be found to be in mitosis. It is once more very easy to show, thus confirming other authors, that irradiation with doses of 450r has no immediate effect on DNA synthesis if delivered during this period of active synthesis. It must be emphasized, however, that mitosis can still be greatly delayed by this irradiation and it seems that we must believe that inhibition of some other mechanism than DNA synthesis must here be the cause of the temporary inhibition of mitosis.

If we consider the case of a growing tissue, containing cells in all stages of mitosis, we can see that the 50 per cent inhibition of deoxynucleic acid synthesis produced by fairly large doses of X-rays could not be produced if only the cells in the pre-synthetic stage were affected. The 50 per cent inhibition is an immediate effect, whereas some hours must elapse to allow the pre-synthesis inhibition to demonstrate itself at all strikingly. An immediately visible inhibition must be due to an immediate inhibition in the synthetic period. Lajtha has described experiments in which this inhibition can be brought about by 5,000r. In a confirmatory experiment (only one so far) we have found that 2,200r will cause an immediate 50 per cent inhibition of DNA synthesis during the synthetic period ; this is a dose sufficient to cause regression in at least 50 per cent of various implanted tumours and would probably cause the death of the liver lobe. The effect of 450r on DNA synthesis is temporary only ; the effect of 2,200r is probably irreversible (*see Table I*). When X-radiation is acting on a dividing tissue with cells in all stages (46 hours), 450r is not sufficient to produce an immediate reduction in rate of DNA synthesis, whereas 2,200r is sufficient. If on the other hand, the period from 9–12 hours after the irradiation is chosen

(48–60 hours, ^{32}P injected at 57 hours) the effect of the low dose is obvious. Several other examples of the lack of immediate effect and the marked delayed effect of the low dose are shown in *Table I.*

It seems that the immediate 50 per cent reduction in synthesis seen after clinical doses of X-rays is not accounted for by the action on cells in the sensitive state only. There is still a possibility that the new synthesis of DNA is stopped but that some sort of renewal of old DNA is taking place

Table I.—Immediate and delayed effects of 450 r *and* 2,200 r *Rate of DNA Synthesis Expressed as Percentage of Controls* ^{32}P *used and injected 3 hours before killing*

Time after hepatectomy			*Mitotic counts per mille*		
Irradiation		*Killing*	*Rate of DNA Synthesis*	*Control*	*Irradiated*
150 r	12 hours	24 hours	30	0·75*	0
450 r	12 hours	24 hours	20	10·0*	0
450 r	12 hours	27 hours	30	64	0·3
450 r	17 hours	29 hours	30	23·4	0·5
450 r	19 hours	22 hours	100	0	0
450 r	19 hours	23 hours	96	4·8*	0
2,000 r	19 hours	23 hours	50		0
450 r	24 hours	27 hours	95	26·8	0
2,200 r	24 hours	27 hours	37		0
450 r	21 hours	27 hours	100	46	6
450 r	24 hours	36 hours	29	15	6
450 r	24 hours	36 hours	30	16	8·7
450 r	36 hours	48 hours	26	6	0·5
450 r	36 hours	39 hours	100	4	0
450 r	46 hours	49 hours	92	19·3	0·2
2,200 r	46 hours	49 hours	50		0
450 r	48 hours	60 hours	20	2·7	1
2,200 r	48 hours	60 hours	20		0

* Mitotic counts irregular from 23–26 hours, but always high from 27–33 hours

at the same time and is not inhibited. If the renewal of the old DNA is taking place without much expenditure of energy, by exchanges of base and phosphate with free base and phosphate in the medium for instance, this could well be true. To reconcile this idea with the template theory (and particularly if we find that the synthesis of the new and the renewal of the old takes place at the same time) we have to suppose that the components of the old DNA become more mobile at the moment when the new DNA is being formed, or has just been formed, upon it.

REFERENCES

[1] Hevesy, G. *Radioactive Indicators.* Interscience Publishers, New York. 1948.

[2] Vermund, H., Barnum, C. P., Huseby, R. A. and Stenström, K. W. *Cancer Res.* 1953, **13** 633.

[3] Skipper, H. E. and Mitchell, J. H. *Cancer*, 1951, **4** 363.
[4] Holmes, B. E. and Mee, L. K. *Brit. J. Radiol.* 1952, **25** 273.
[5] Von Euler, H. and Hevesy, G. *Arkiv. Kemi, Mineral. geol.* 1944, A17, No. 30.
[6] Stevens, C. E., Daoust, R. and Leblond, C. P. *J. Biol. Chem.* 1953, **202** 177.
[7] Howard, A. and Pelc, S. R. *Heredity*, 1953, **6** 261.
[8] Pelc, S. R. and Howard, A. *Aarhus Conference of Radiobiology*, 1953.
[9] Kelly, L. S. *Proc. Amer. Ass. Cancer Res.* 1954, **1** 24.

DISCUSSION

Z. M. Bacq : If I have clearly understood, Mrs. Holmes has observed several times after irradiation that DNA synthesis may be normal when there is no mitotic activity.

Is it possible that mitosis and DNA synthesis are unrelated events ?

A. Howard : It is obvious that a cell must synthesize DNA if it is to divide, but DNA synthesis does not always lead to division. The existence of many cells having tetraploid or higher amounts of DNA in tissues with low rates of division indicates this clearly, and is one of the grounds for criticizing Leblond's conclusion that the high rate of incorporation of ^{32}P relative to mitotic rate means that existing DNA is being broken down. In view of the clear separation in time of the two events, it would, I think, be surprising if DNA synthesis were inevitably followed by mitosis.

INCORPORATION DU ^{14}C DANS LE GLYCOGÈNE DU FOIE APRÈS IRRADIATION*

Mme Lourau-Pitres

Institut de Biologie Physico-chimique, Paris

UN grand nombre d'observations[1] montrent qu'une irradiation générale par les rayons X provoque un trouble du métabolisme des glucides. On sait par exemple qu'au cours du jeune on trouve toujours plus de glycogène hépatique chez les animaux qui viennent d'être exposés aux rayons que chez des témoins jeunant depuis le même temps[2]. C'est la signification de ce fait qui a été examinée dans ce travail : l'irradiation augmente-t-elle la synthèse du glycogène—et dans ce cas est-ce à partir de molécules privilégiées—ou bien ralentit-elle sa vitesse de renouvellement ?

La méthode utilisée est celle des indicateurs radioactifs. L'animal d'expérience, qui est la souris, reçoit, en injections, des métabolites, marqués par du ^{14}C, qui sont incorporés dans le glycogène du foie : glucose, bicarbonate. Au bout de temps variables, les animaux sont sacrifiés, le glycogène isolé et purifié et sa radioactivité mesurée sur un compteur de Geiger–Muller à fenêtre mince. Dans ces expériences préliminaires les doses de rayons X étaient largement léthales (2.000 et 2.500 r) et la période d'observation limitée aux 24 premières heures qui suivent l'irradiation.

Les expériences réalisées jusqu'à ce jour peuvent être réparties en 3 groupes, 2 d'entre eux destinés à suivre la synthèse du glycogène, le troisième plus spécialement conçu pour déterminer sa vitesse de renouvellement.

I. INCORPORATION DANS LE GLYCOGÈNE DU ^{14}C CONTENU DANS LE GLUCOSE CIRCULANT

Ces expériences[3] montrent que l'irradiation modifie l'incorporation, dans le glycogène, du ^{14}C fourni par le glucose circulant. Lorsque le glucose marqué est administré en une seule injection, l'activité spécifique du glycogène atteint très rapidement son maximum, au bout de 40 à 50 minutes, puis décroit. L'irradiation ne déplace pas la position de ce maximum mais elle augmente très fortement sa valeur, d'au moins trois fois ; aussi les courbes représentant les variations de l'activité spécifique en fonction du temps ont-elles une pente beaucoup plus forte chez les irradiés que chez les témoins.

Des contrôles expérimentaux ont montré que cette augmentation d'activité est réelle, en particulier qu'elle n'est due ni à une impureté radioactive entrainée avec le glycogène, ni à une accéleration de la résorption du glucose marqué (*cf.*[5]), ni à une baisse de la glycémie. Toutefois une hypothèse méritait d'être envisagée et discutée, c'est que l'irradiation accélère le

* (Travail du Laboratoire Pasteur a l'Institut du Radium, réalisé avec une subvention du Commissariat a l'Energie Atomique.)

catabolisme des glucides. On sait en effet[4] que des accélérateurs du métabolisme modifient le profil des courbes : activités spécifiques/temps dans le sens que nous avons constaté, en augmentant leur pente. Mais on sait aussi que cet effet persiste dans la période d'activité décroissante de sorte que les courbes données par des animaux à métabolisme accéléré coupent toujours celles des témoins dans leur branche descendante. Or les expériences de ce premier groupe ne permettaient pas d'envisager cet aspect du problème car les activités spécifiques développées par une seule injection de glucose marqué sont trop faibles pour qu'il soit possible de suivre leur décroissance et d'y relever des différences significatives. Un plan différent a donc été recherché pour les expériences du deuxième groupe.

II. VITESSE DE RENOUVELLEMENT DU GLYCOGÈNE

Le plan adopté est basé sur les observations résumées dans la *Figure 1*. Le métabolite porteur du ^{14}C est du bicarbonate ; on favorise son incorporation dans le glycogène en faisant disparaître le glycogène inactif par un jeune

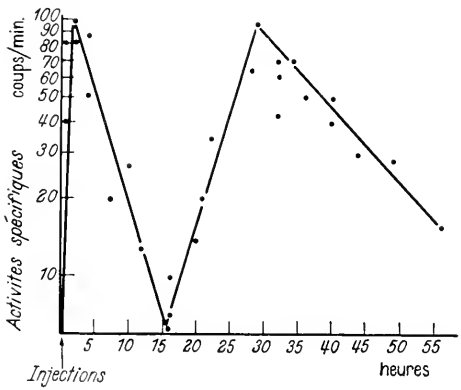


Figure 1. Activités spécifiques du glycogène extrait du foie de souris normales ayant reçu temps 0, une injection de glucose inactif et une injection de bicarbonate de sodium marqué par du ^{14}C.

de quelques heures, puis en reconstituant les réserves au moyen de glucose inactif injecté, en petites quantités, quelques milligrammes, en même temps que le bicarbonate. (Activité injectée environ 1,7 microcurie.) On voit dans la figure que le ^{14}C du bicarbonate est rapidement incorporé et utilisé. Mais le phénomène qui a attiré notre attention est l'apparition, 28 à 30 heures après les injections, d'un deuxième maximum aussi élevé que le premier, excellent point de départ pour établir une courbe de décroissance. Ce deuxième maximum est donc choisi comme origine des expériences ; l'irradiation est faite à ce moment là, les deux groupes d'animaux, témoins et irradiés, ayant à leur disposition un matériel marqué identique.

La *Figure 2* donne un exemple des résultats, qui sont extrèmement nets : il n'y a aucune différence dans la décroissance de l'activité spécifique des témoins et des irradiés, la première observation étant faite 5 heures après l'irradiation et la dernière 17 heures après. Avec une échelle semi-logarithmique les valeurs expérimentales se disposent suivant une droite et celle-ci a la même pente.

L'irradiation ne modifie pas la vitesse de renouvellement du glycogène du foie et les résultats donnés par les expériences exposées dans le premier

paragraphe semblent indiquer que l'irradiation accélère la synthèse du glycogène à partir du glucose circulant.

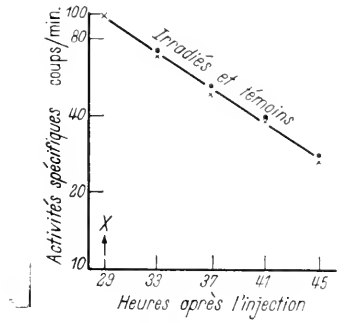


Figure 2. Activités spécifiques du glycogène extrait du foie de souris normales et de souris irradiées, préparées au temps 0 comme dans la Figure 1. L'irradiation a été faite au moment indiqué par la flèche, 29 heures après les injections de glucose et de bicarbonate.

III. SYNTHÈSE DU GLYCOGÈNE A PARTIR DE MOLÉCULES MARQUÉES PAR LE ^{14}C DU BICARBONATE

Le dernier dispositif expérimental a été repris pour étudier la synthèse du glycogène à partir de molécules marquées par le ^{14}C du bicarbonate que l'organisme a synthétisées avant d'être irradié. Ce problème est différent de celui qui a été traité dans le premier paragraphe. En effet le glycogène n'est pas seulement formé à partir du glucose circulant, mais aussi, avec une égale importance, à partir de molécules plus petites formées au dépend des protides et des lipides aussi bien que des glucides. La radioactivité qui apparaît dans le glycogène 30 heures après l'injection de bicarbonate marqué est due, selon toute vraisemblance, à l'incorporation de ces petites molécules, et il était intéressant de savoir si l'irradiation perturbe aussi cette synthèse.

Les animaux sont préparés comme en II, mais l'irradiation a été faite avant le deuxième maximum, dans la deuxième branche ascendante et même dans la première branche descendante de la courbe n° 1 et les examens ont été faits de 1 à 10 heures après l'irradiation.

Quatre expériences de ce genre ont été faites. Les figures obtenues sont assez différentes, plus complexes que les précédentes. Toutefois elles ont

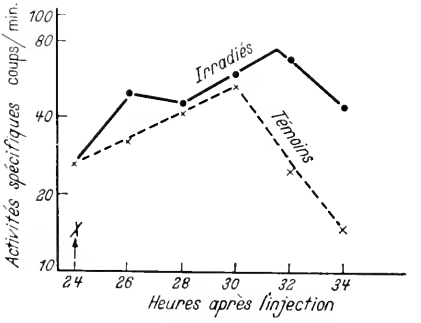


Figure 3. Activités spécifiques du glycogène extrait du foie de souris normales et de souris irradiées préparées au temps 0 comme dans la Figure 1. L'irradiation a été faite au moment indiqué par la flèche, 24 heures après les injections de glucose et de bicarbonate.

en commun une perturbation très nette de l'incorporation dans le glycogène des métabolites marqués. Chez les témoins, la courbe représentative est toujours une droite, et quand il y a un point d'inflexion celui-ci est toujours unique. Au contraire chez les irradiés il y a toujours plusieurs points

d'inflexion et les activités spécifiques sont généralement supérieures à celles des témoins (*Figure 3*).

RÉSUMÉ ET CONCLUSIONS

(*1*) Une irradiation générale par des doses de rayons X largement léthales augmente l'incorporation dans le glycogène du foie du ^{14}C contenu dans le glucose circulant et dans divers métabolites qui ont fixé le ^{14}C fourni à l'organisme sous forme de bicarbonate.

(*2*) En revanche l'irradiation ne modifie pas l'utilisation du glycogène dont la vitesse de renouvellement n'est pas modifiée dans les 24 heures qui suivent l'irradiation.

REFERENCES

[1] ORD, M. G. et STOCKEN, L. A. *Physiol. Rev.* 1953, **33** 357.
[2] NORTH, N. et NIMS, L. F. *Fed. Proc.* 1949, **8** 119.
NIMS, L. F. *J. chim. Phys.* 1951, **48** 280.
ROSS, M. H. et ELY, J. O. *J. cell. comp. Physiol.* 1951, **37** 163.
STOCKEN, L. A. *Chem. Ind.* 1951, **32** 116.
MACKEE, K. W. *Fed. Proc.* 1952, **11** 256.
LOURAU, M. *C.R. Acad. Sci.* 1953, **236** 422.
NIMS, L. F. et SUTTON, E. *Amer. J. Physiol.* 1954, **177** 51.
[3] LOURAU, M. *Arkiv f. Kemi*, 1954, **7** 211.
[4] BEECKMANS, M. L., CAISER, H. et HEVESY, G. *Arch. Int. Pharmacodyn*, 1951, **186** 33.
[5] HEVESY, G. et FORSSBERG, A. Ce symposium.

DISCUSSION

P. MANDEL : Je pense que des expériences négatives concernant le renouvellement du glycogène ne permettent pas à eux seules de conclure à l'absence d'un trouble du métabolisme des glucides après une irradiation aux rayons X.

E. H. BETZ : Les recherches que Lourau-Pitres vient de nous exposer montrent l'existence d'une augmentation précoce du glycogène hépatique chez l'animal irradié. Une augmentation similaire s'observe au cours des jours qui suivent l'irradiation. Le taux du glycogène hépatique semble se modifier parallèlement à l'évolution de l'activité surrénalienne telle que Patt l'a décrite. Les modifications du glycogène hépatique semblent bien être le reflet de l'activité cortico-surrénalienne puisqu'elles sont inhibées par la surrénalectomie, ainsi que l'a montré Fischer.

Mme LOURAU-PITRES : Les expériences de Fischer et celles de Nims et Sutton sont en effet favorables à l'interprétation de Betz. On devait s'attendre à ce que l'irradiation diminuât la vitesse de renouvellement du glycogène, puisque, selon l'opinion admise, les hormones corticales préservent le glycogène du foie en diminuant les oxydations périphériques. Or ce n'est pas ce que j'ai observé. Mais peut être les hormones corticales agissent-elles par un autre mécanisme ? Il faut aussi noter qu'aux doses de rayons que j'ai utilisées (2.000 r), Hevesy et Forssberg observent des effets qui s'expliquent mieux par une déficience d'hormone surrénale que par un excès.

OBSERVATIONS MADE ON THE HUMAN RESPONSE TO A SINGLE DOSE OF X-RAYS—THE LATENT PERIOD

W. M. Court Brown and John D. Abbatt

Medical Research Council, London

It is clearly desirable that human material should be used for radiobiological investigations. This can only be done if a reproducible response to irradiation occurs, of which at least some of the governing factors are known. The length of time which elapses from the commencement of irradiation to the onset of the symptoms of radiation sickness, the latent period, seems such a response. Experience with more than 150 patients has shown that provided a large enough single dose of irradiation is given to a large enough,

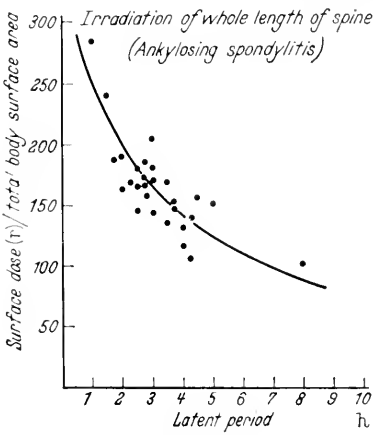


Figure 1

volume of tissue in the trunk, or to the whole body, a symptomatic disturbance occurs which is sudden in onset, and that the time required to initiate this disturbance can be measured with reasonable accuracy. Experience with control sham irradiations has indicated that the symptoms which terminate this latent period are the genuine symptoms of radiation sickness ; these symptoms are acute and sudden nausea, and the development of uncontrollable and often persistent vomiting. Observations on the latent period have been made in two classes of patients : those irradiated with medium kilovoltage X-rays and those given large doses of ^{131}I. This period and the ensuing symptomatic disturbance, together with such physiological changes that occur, appears to correspond to the initial phase of the General Radiation Syndrome in man and may well be the human equivalent of the first phase of response in the rat as described by Lamerton and his co-workers[1].

Three known factors influence the length of the latent period, the dose of radiation, the body size and the particular anatomical site irradiated. It has been found that for a given anatomical site of irradiation, *i.e.* the whole

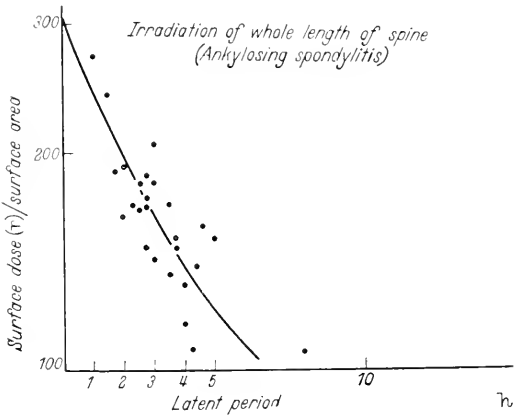


Figure 2

length of the spine, or the whole abdomen, or the whole pelvis, a distinct relationship exists between the dose of radiation expressed in terms of body size and the length of the latent period, and that this relationship is a curvilinear one (*Figure 1*). If the log dose per unit of body size is plotted against the latent period, the relationship is linear provided only that a restricted range of time is used (*Figure 2*). By plotting the dose against the reciprocal

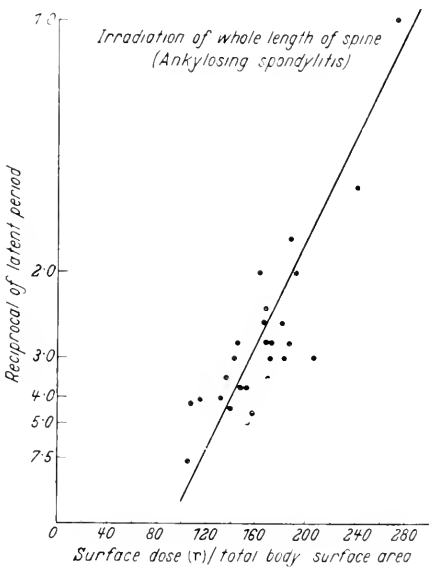


Figure 3

of the latent period, the relationship is a linear one over the whole range of observed values (*Figure 3*). This behaviour is in keeping with the usual pharmacological dose response relationships[2] and the reasons for viewing

this response from this aspect are reinforced by the obvious inverse relationship existing between the length of the latent period and the approximate duration of the acute symptoms (*Figure 4*). These relationships appear to hold not only for the cases subjected to medium voltage X-rays but also for those given large doses of ^{131}I. In this latter group the whole body dose corrected for renal and thyroid clearance has been calculated according to principles laid down by MAYNEORD and SINCLAIR[3]. In this instance the mean dose rate per hour has been plotted against the latent period.

No correlation has been found between the length of the latent period and whole body integral dose in either the cases treated with X-rays or with ^{131}I. This is not surprising as the implication of such a correlation would have been that energy absorbed in all tissues would be equally effective in the production of this syndrome.

A consideration of the X-ray dose per unit of body size required to produce latent periods of comparable length gives some indication of the radio-sensitivity of differing anatomical sites. Thus it can be shown that to

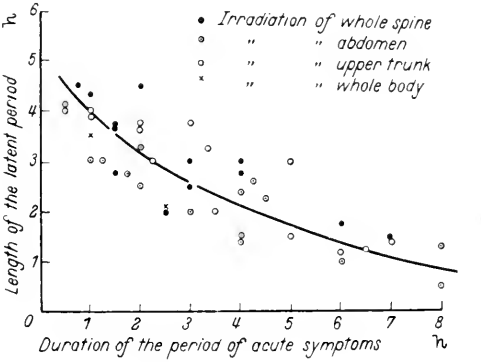


Figure 4. Relationship of the length of the latent period to the duration of the period of acute symptoms (44 cases)

produce latent periods of similar duration following irradiation of the pelvis alone or of the whole abdomen, the X-ray dose to the pelvis has to be about five times greater than that to the abdomen.

The influence of body size and the relationship that exists between the length of the latent period and the period of acute symptoms suggests that the production of symptoms is related to the concentration of some circulating compound. On this hypothesis it would be expected that a given X-ray dose would produce a constant amount of this substance, which on passing out of the irradiated volume of tissue would become diluted in some fluid compartment of the body. An interesting piece of indirect evidence in favour of this hypothesis has been noted in some of the patients given large doses of ^{131}I. A number of these patients have been in congestive cardiac failure with obvious oedema. In these patients the latent periods have been found to be considerably longer than would be expected from the observations made on cases not in cardiac failure.

This dose response system provides a convenient method of assessing either the protective or therapeutic efficacy of a drug for this particular phase of the radiation syndrome. Provided sufficient material is available, it is possible to carry out properly planned clinical trials. In this way the

compound β-mercaptoethylamine has been tested on patients given whole abdominal irradiation. It was found that with intravenous doses in the range from 160–300 mg, given either immediately before the commencement of X-irradiation or within an hour of its completion, the measured latent periods did not differ significantly from those computed.

REFERENCES

[1] Lamerton, L. F., Elson, L. A., Harris, E. B. and Christensen, W. R. *Brit. J. Radiol.* 1953, **26** 510.

[2] Gaddum, J. H. *Pharmacolog. Rev.* 1953, **5** 87.

[3] Mayneord, W. V. and Sinclair, W. K. 'The Dosimetry of Artificial Radioactive Isotopes.' *Advances in Medical and Biological Physics*, Vol. III, New York, Academic Press, 1953.

DISCUSSION

Z. M. Bacq : It seems that unfortunately Court-Brown's experimental technique is not suitable to show an action of cysteamine. It is during the first hours after irradiation that the action of cysteamine is most difficult to show (see *Figure 1*, page 229, for instance).

We know that the beneficial effects of cysteamine in radiation sickness are not observed rapidly, and that often the administration of cysteamine must be repeated two or three times in order to obtain the therapeutic result.

A. Herve : Les tests décrits par Court-Brown présentent un interêt certain. On peut cependant se demander s'ils donnent toujours une idée exacte du degré de protection ou de l'effet thérapeutique des substances étudiées.

Je discuterai deux points.

(*1*) Les conditions expérimentales cliniques utilisées par l'auteur s'éloignent des données radiothérapiques habituelles. Une dose forte et unique distribuée sur un très large champ est, dans la majorité des cas, très mal supportée et ne correspond à aucune nécessité thérapeutique.

Le fractionnement de la dose associé à la réduction du 'volume-dose' (champs étroits) donne au mal des rayons un autre caractère. J'ai pu montrer que la détermination de l'énergie absorbée permettait de mettre en évidence pour chaque segment corporel une dose intégrale seuil au delà de laquelle apparaissait, chez les patients prédisposés, la symptomatologie du mal des rayons.

Les troubles apparaissent : 1° sur l'abdomen après une à trois irradiations quotidiennes d'environ 0,5 mégagrammes (en moyenne après 24 heures et 1 megagramme); 2° sur le thorax après dix à quinze irradiations quotidiennes d'environ 0,6 mégagrammes (en moyenne après 12 jours et 0, à 8 mégagrammes).

Dans ces conditions, plus habituelles en clinique, les phénomènes sont plus tardifs et de ce fait plus complexes, de nombreux mécanismes indirects entrant en jeu. Il n'est pas impossible que l'effet thérapeutique de certaines substances puissent se manifester dans ces secondes conditions de travail et non dans celles de Court-Brown.

L'interprétation est aussi plus difficile.

(*2*) Si l'on adopte les tests proposés (apparition des premiers symptômes après 2 à 3 heures), doit-on admettre que ces réactions très précoces seront nécessairement en rapport avec l'éventuelle action protectrice ou thérapeutique d'une substance déterminée ?

Dans le cas particulier de la cystéamine ou de ses dérivés on peut en douter. Plusieurs tests chimiques, biologiques ou cliniques nous ont montré que les réactions immédiates étaient identiques chez les animaux protégés et chez les témoins. Le comportement des protégés (dont la survie sera voisine de 90 pour cent) et des

contrôles (qui mourront tous) ne se dissocie qu'après un ou plusieurs jours (voir notamment Bacq et coll.*).

La cystéamine par voie intra-péritonéale ou la cystamine *per os* administrée *immédiatement avant* l'irradiation protège 80 à 100 pour cent des souris irradiées à dose mortelle de rayons X. Ces substances n'empêchent cependant pas l'anorexie et la chute de poids initiale chez les protégés.

Administrées *après* l'irradiation, ces substances n'ont plus aucun effet sur la survie. L'anorexie et la chute du poids sont identiques à celles du contrôle.

A titre expérimental, une série de cas de mal des rayons observés au Service Universitaire de Radiothérapie, a été traitée par la cystéamine ou la cystamine.

Chez tous les patients nous avons attendu l'apparition de la symptomatologie (il ne s'agit donc pas de prévention) et nous avons administré 200 mg. de cystéamine par voie intraveineuse ou 600 mg de cystamine *per os*, après l'irradiation afin

Tableau I

Mal des rayons resultats %	Cystéamine (1573 L) I.V.		Cystéamine (1573 L) per os		Cystamine (1591 L) per os		Ensemble	
	Graves	Légers	Graves	Légers	Graves	Légers	Graves	Légers
Bons	63	82	42	80	30	61	53	77
Incomplets	20	6,5	36	4	50	24	28	9
Echecs	17	11,5	22	16	20	15	19	14
Nombre de cas (171)	49	46	19	26	10	21	78	93

d'éviter un éventuel effet protecteur. La thérapeutique a été poursuivie un à quatre jours.

Les résultats sont résumés dans le *Tableau I*.

Dans un grand nombre de cas, la cystéamine et ses dérivés ont donc un effet favourable sur la régression des symptômes du mal des rayons, en clinique, bien que préventivement chez l'animal elle ne semble guère empêcher l'apparition des troubles.

L'amélioration, lorsqu'elle survient, se marque habituellement après 24 ou 48 heures. Si aucun effet n'est observé après 3 jours, l'échec est presque certain.

Dans le *Tableau II*, nous comparons l'effet de la cystéamine et de quatre substances étudiées par Ellis sur quatre symptômes provoqués par l'irradiation.

Les nausées, les vomissements, symptômes assez caractéristiques, sont très favorablement influencés. L'anorexie, par contre, et moins encore l'asthénie ne répondent guére mieux à la cystéamine qu'aux autres thérapeutiques.

Dans notre esprit, l'action de la cystéamine et de la cystamine (administrée après l'irradiation) dans le mal des rayons n'a aucun rapport avec leur action protectrice, d'ordre physico-chimique liée vraisemblablement à une compétition pour les radicaux libres formés pendant l'irradiation et dont la durée de vie est infiniment brève. Il

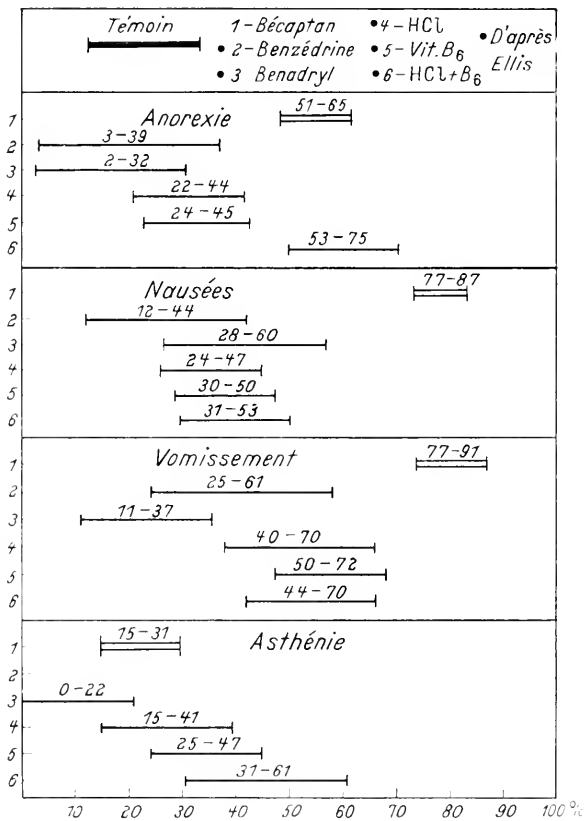
* *Science*, 1953, **117** 633.

est probable que ce sont les actions métaboliques de ces amines qui sont responsables de ces effets thérapeutiques.

Le mal des rayons est un syndrome généralisé, témoin de troubles à distance provoqués en dehors du foyer irradié. Notre ignorance de la pathogenie de ces troubles ne permet pas d'étudier avec plus de précision l'action de la cystéamine dans cette affection.

Le fait que cette substance intervient en hâtant la disparition des symptômes et que son action ne se marque qu'après 24 ou 48 heures nous fait penser que l'effet

Tableau II



thérapeutique (comme l'effet protecteur) serait difficilement mis en évidence par les tests décrits par Court-Brown.

W. M. Court Brown : In answer to Herve's claims for the efficacy of β-mercaptoethylamine in the treatment of radiation sickness the following points were made : (*1*) The reported observations in the development of radiation sickness following a single dose of X-rays have been made because of our lack of knowledge of the effects of a single acute exposure such as may occur in future industrial accidents or during the explosion of nuclear weapons. The condition described as radiation sickness in clinical radiotherapy is not considered to be a serious problem. (*2*) From previous experience of attempts to assess the value of therapeutic agents on patients being treated in a routine hospital radiotherapy department it is extremely difficult to obtain results which are amenable to subsequent statistical analysis.

COMPARISON OF THE PHYSIOLOGICAL RESPONSE TO RADIATION AND TO RADIOMIMETIC CHEMICALS

L. A. Elson*

Chester Beatty Research Institute, Royal Cancer Hospital, London

In a large amount of the experimental work on the physiological effects of radiation, and the modification of these effects by chemical 'protective' agents or other means the criterion used has been the mean lethal dose. Although this criterion provides some quantitative measure of the effect of radiations it suffers from serious limitations, one of the most important of which is that it gives little information on the various phases of radiation response except insofar as 'grouping' of deaths in time is concerned.

A study of the phases of radiation response of the rat to uniform whole body irradiation has been made by Lamerton, Elson and Christensen[1] and to non-uniform irradiation by Lamerton, Elson and Harris[2]. In these studies an attempt was made to use the weight changes of young growing rats as a measure of severity of physiological response to radiation, and to relate these weight changes to effects on the circulating blood cells. The changes in the growth curves were found to indicate clearly different stages of the radiation response.

Based on these results and some more recent evidence it is now possible to attempt in *Figure 1* to provide a convenient reference diagram relating the blood changes to the various phases of radiation response as indicated by the weight response curves. This is necessarily only an approximation as naturally considerable variations from this pattern are found in the behaviour of individual animals, but it does represent a useful diagrammatic picture of the average response of rats to whole body X-irradiation with doses of the order of 400–600r.

It is convenient to distinguish four phases of response based on the growth curves. *Phase I* is the initial weight loss phase and lasts for about 2 to 4 days immediately following irradiation. The animal loses weight steadily during this phase and a measure of the amount of weight lost is conveniently given by the parameter L_1. The *lymphocytes* fall rapidly during phase I and reach their minimum value usually by the end of this phase. *Phase II* is the first recovery phase. An abrupt change in the weight curve occurs and the animal resumes its normal growth rate, and continues to grow normally until about the twelfth day after irradiation. During phase II the *neutrophils* which start to fall in phase I continue to fall after a slight recovery and reach a minimum at about the end of the phase. Lymphocytes begin a gradual recovery during phase II. *Phase III* is the second weight drop phase. This second weight drop occurs frequently in rats receiving

* British Empire Cancer Campaign Research Fellow.

400–600 r but less often with smaller doses of X-radiation. From about the twelfth day after treatment the animal loses weight fairly steadily for a varying period often extending to about the twentieth day after irradiation. The most important blood effect connected with phase III is the fall in *red cells*. This fall commences about the middle of phase II but the minimum is reached at about the end of phase III. There is a correlation between the fall in red cells and the second weight drop, and the severity of the anaemia is probably the main factor in determining the magnitude (L_2) of this

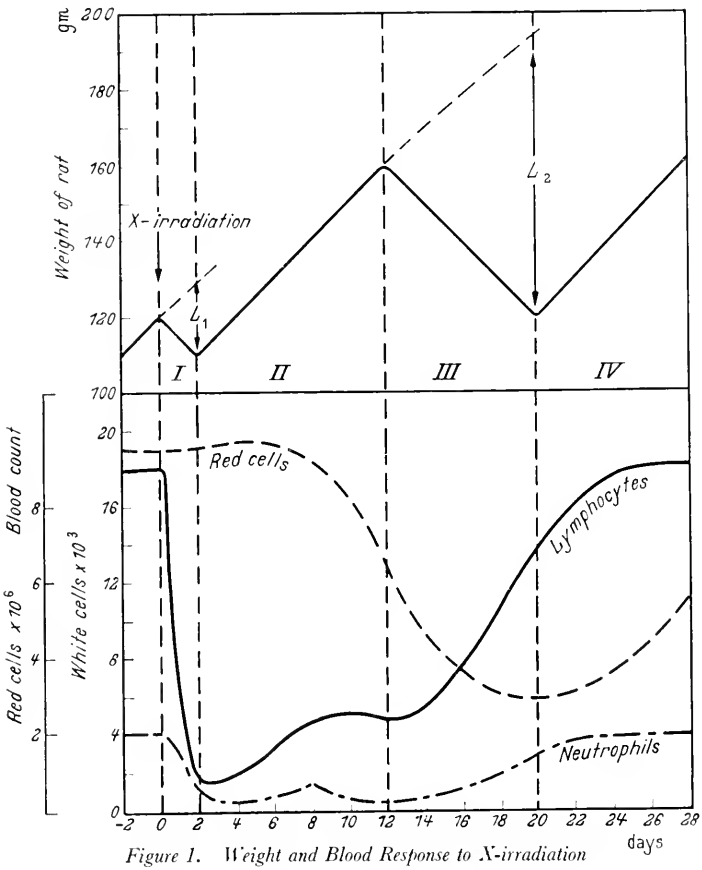


Figure 1. Weight and Blood Response to X-irradiation

weight loss. The relation of the anaemia to the fall in platelets which occurs during phases II and III has been discussed by LAMERTON and BAXTER[3]. During phase III the lymphocytes and neutrophils continue their rise towards normal values. *Phase IV* is the final recovery phase. The animal resumes its normal growth at the same rate as before irradiation. Red cells recover steadily and lymphocytes and neutrophils soon regain their normal values.

With toxic doses of radiation death occurs either during phase I or phase III. Examples of growth curves of individual rats showing variations of pattern which may occur are given in *Figure 2*.

'*Radiomimetic*' *Chemicals*—The inhibitory effect of certain carcinogenic chemicals on the growth of rats was first described by HADDOW, SCOTT and SCOTT[4] and compared with the growth inhibition produced by X-radiation. Following up the tumour-inhibiting action also shown by these substances, a large number of different types of chemicals has been examined and compounds have been developed with much more powerful growth-inhibitory

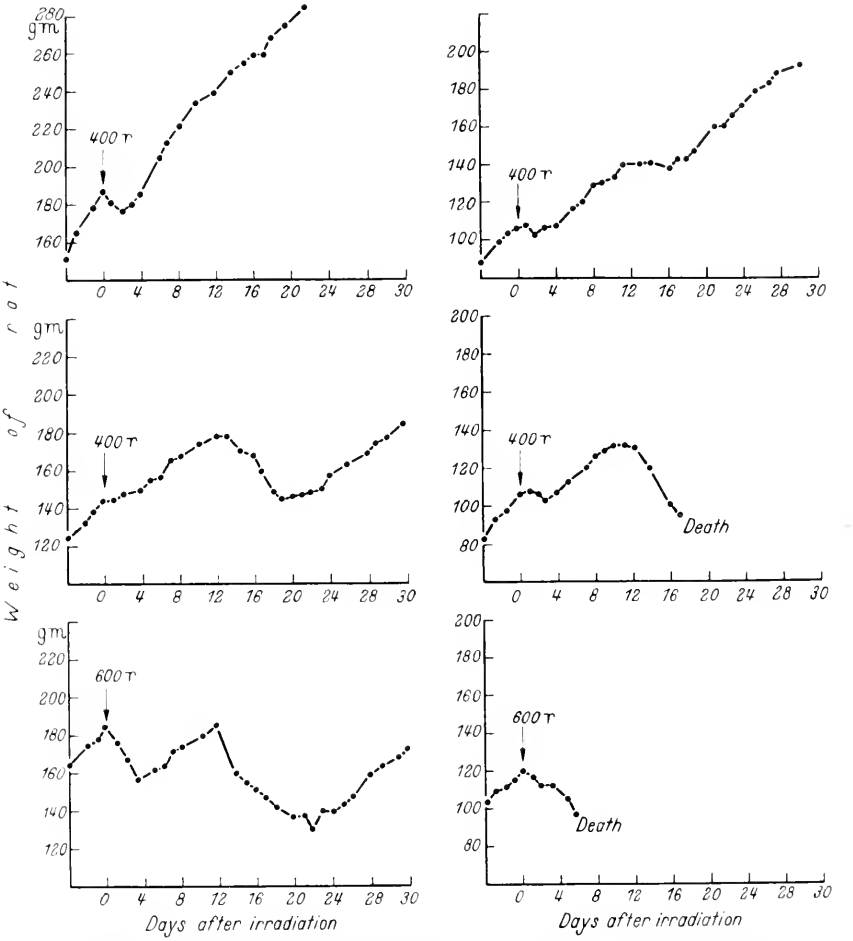


Figure 2. Effect of whole body X-irradiation on growth rate. Selected examples

properties than the original carcinogens. One class of these is related to the 'nitrogen mustards' which are often described as radiomimetic because of their radiation-like effects on dividing cells and on the haematopoietic system. Of these a series of water-soluble nitrogen mustards (EVERETT, ROBERTS and ROSS[5]) were found to have less general toxic properties than nitrogen mustard itself HN_2 formula $CH_3.N(CH_2CH_2Cl)_2$ and one member CB 1348 N.N-dichloroethyl-p-aminophenyl butyric acid p.$COOH(CH_2)_3.C_6H_4$-$N(CH_2CH_2Cl)_2$ is undergoing clinical trials in cases of lymphoma and lymphatic leukaemia.

Another type of compound Myleran 1 : 4-dimethanesulphenyloxybutane $CH_3SO_2.O(CH_2)_4O.SO_2CH_3$ (Haddow and Timmis[6]; Galton[7]) has already proved of very considerable interest in the clinical treatment of myeloid leukaemia. Compounds of both these types are being studied as part of a general investigation of the growth-inhibitory action of carcinogens (Elson and Warren[8]; Elson[9]) and the growth curves of rats treated with Myleran and other members of the series were found to resemble in many respects the curves shown in response to whole body X-irradiation. An investigation

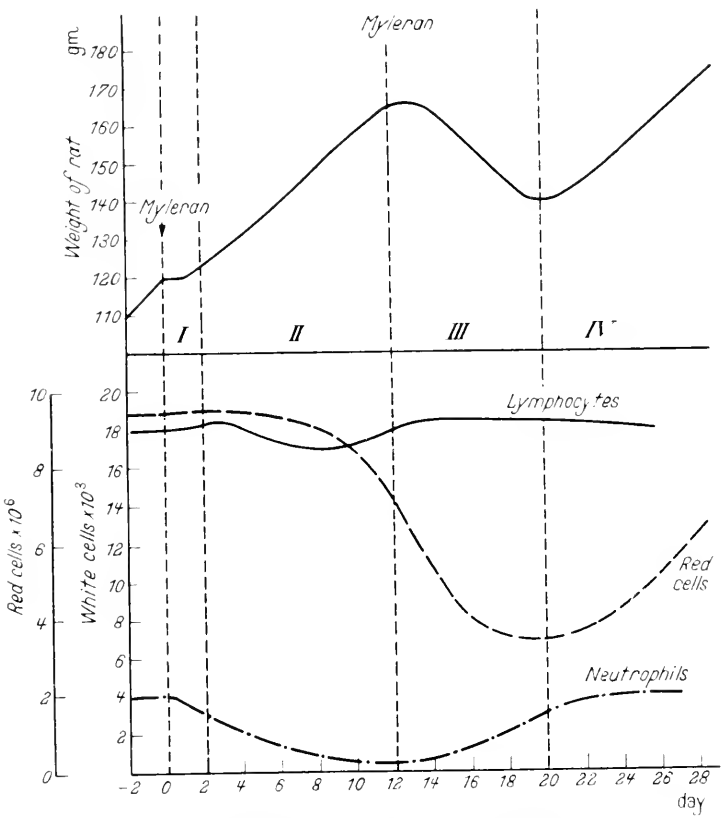
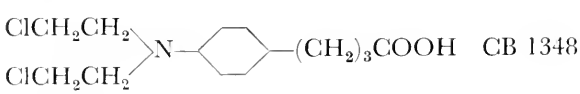


Figure 3. Weight and Blood Response to Myleran

of the blood changes in relation to the phases of weight response, similar to that described for X-radiation, has therefore been carried out for Myleran and for the nitrogen mustard derivative CB 1348 (Elson[10]).



CB 1348

Myleran—In studying the weight response to Myleran the striking similarity in behaviour of some rats to that often observed after X-irradiation was first noticed in the delayed weight drop which occurred at about 12 days

after treatment. A general picture of the weight and blood response to Myleran is given in *Figure 3*. There is very little initial weight drop in response to Myleran and little effect on lymphocytes. In fact, *Phase I* can be considered as practically non-existent compared with X-irradiation (*Figure 1*) and the response to Myleran over this phase is quite unlike that to X-irradiation. There is practically no initial weight loss and no fall in lymphocytes. Neutrophils commence a steady fall but not nearly as rapidly as after X-irradiation. In *Phase II* the neutrophils continue to fall and as with X-radiation reach their minimum at about the end of this phase. The behaviour of the red cells during phase II is also very similar to that following X-irradiation. With doses of Myleran which cause a second weight drop a rapid fall of red cells takes place starting from about the eighth day after treatment. From this time a general haemorrhagic state is usually observed and can easily be demonstrated by plucking a small area of fur from the animal's chest, when petechial haemorrhages appear almost immediately. This haemorrhagic state usually persists until at least 20 days after the original dose of Myleran. Lympocytes may show a slight fall during phase II, but owing to the large variations which occur in the number of these cells in normal rats the fall is often barely significant. *Phase III* in response to Myleran closely resembles that of X-irradiation except, of course, in behaviour of lymphocytes. In animals showing a second weight drop, red cells continue to fall and reach their minimum at the end of this phase. Neutrophils show a steady recovery throughout the phase. *Phase IV*, the final recovery phase, again corresponds closely with that of X-radiation. With toxic doses of Myleran, deaths always occur during phase III, never in phase I. For example, in one experiment, 5 rats treated with 20 mg kg Myleran all died between 10 and 12 days after treatment. In all cases death was caused by massive haemorrhage, usually in the stomach.

Myleran thus shows mainly the myeloid effect of X-radiation and has little effect on lymphoid tissue. Thus, referring to *Figure 1*, phase I which may be considered associated with lymphoid effects, is not shown by Myleran. Phase III, however, with its associations with red cell and neutrophil depression is strongly represented by Myleran.

Nitrogen Mustard CB 1348 (Figure 4)—In contrast to Myleran *Phase I* of the response to CB 1348 is practically identical to that shown by X-radiation. The initial weight drop and fall in lymphocytes may be slightly more prolonged but resembles very closely the pattern followed after X-irradiation. With toxic doses of CB 1348 deaths nearly all occur during phase I. *Phase II*, however, shows considerable differences from X-radiation, mainly in the very marked neutrophilia which occurs as the neutrophils recover from their minimum fall at about the end of phase I. The neutrophilia is at its maximum at about 10 days after treatment at which time the number of circulating neutrophils may be three or four times the normal value. A slight rise in the neutrophil curves at about 8 to 10 days after X-irradiation has often been observed and this, as now seen, probably corresponds to the peak of the neutrophilia in response to nitrogen mustard (*see Figure 1*). No sharp fall in red cells in response to CB 1348 occurs during phase II but there is often a slight fall with a minimum at about the time of the maximum degree of neutrophilia. Thus phase II of the response to CB 1348 does not at all

resemble that of X-radiation with regard to red cells or neutrophils. The behaviour of lymphocytes is, however, very similar to that following X-irradiation throughout all four phases. With CB 1348 practically all blood cells have returned to normal by the end of *Phase III*. A few exceptions to this, however, have been noticed, but these are rare. In these, after the neutrophilia, the neutrophils, instead of returning to normal values and remaining normal, continue to fall. The animal then shows a second weight drop preceded by a general haemorrhagic state as with X-irradiation.

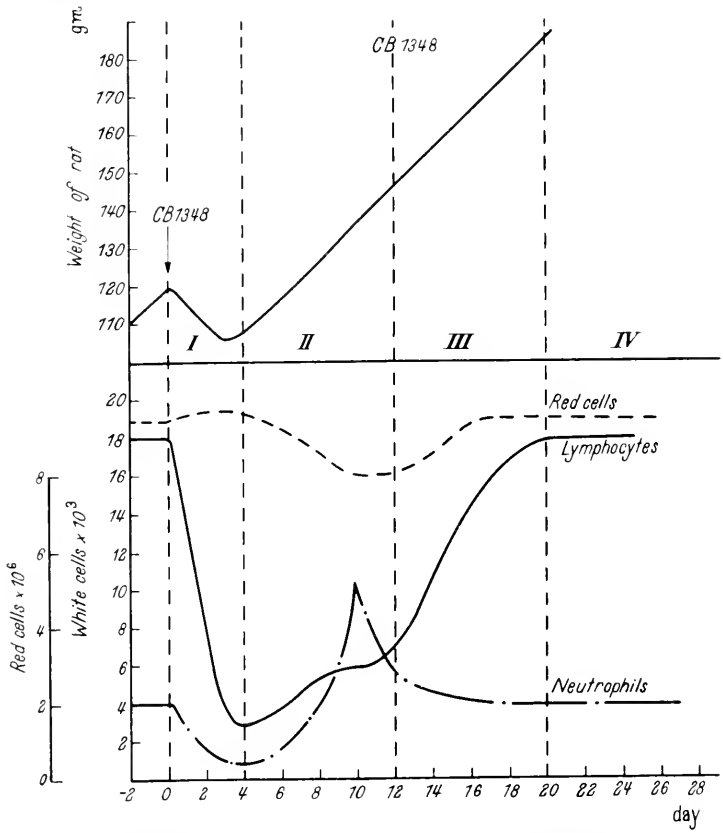


Figure 4. Weight and Blood Response to nitrogen mustard derivative CB 1348

It appears, therefore, that both Myleran and CB 1348 are each only partially radiomimetic, the former showing mainly the myeloid effects and the latter mainly the lymphoid effects of X-radiation. By combining the two chemicals it should be possible to produce a much more complete imitation of the response to X-radiation. This has been tested using a dose of CB 1348 of 12·5 mg/kg given by intraperitoneal injection and a dose of Myleran of 12·5 mg/kg given by mouth. The response is shown in *Figure 5* and although this dosage was found to correspond with about 250 r of X-radiation the resemblance of the response to that pictured in *Figure 1* (corresponding to 400–600 r X-radiation) can be seen clearly. The dose

of each chemical was rather more than half the toxic dose so that, had the toxic effects been additive, the animals would not have survived the combined treatment. No animals died, however, so that as far as total toxic effects are concerned the substances act largely independently of each other. The curves for combined effects on the blood, however, are almost exactly what one would expect by superimposing the curves for each chemical. For instance the neutrophilia resulting from CB 1348 is largely neutralized by the neutrophil-depressing action of Myleran and the resulting flattened

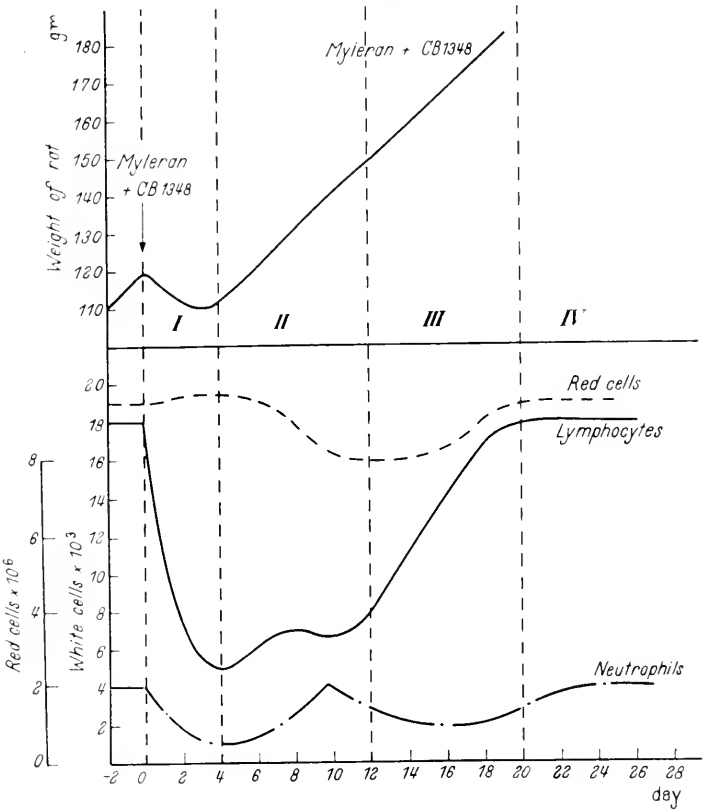


Figure 5. Weight and Blood Response to Myleran + CB 1348

hump in the curve (*Figure 5*) could easily correspond to the slight hump observed in the radiation response curves (*Figure 1*).

Further work on the combined effects of chemicals of this type is being carried out and effects on the bone marrow are being investigated. The results obtained so far on the bone marrow confirm the idea of the partially radiomimetic effect of Myleran and CB 1348 alone and the much more complete resemblance to radiation shown by a combination of the two.

In view of these results it is tempting to speculate on the possibility as to whether the effects of radiation might not be the result of two distinct chemical reactions, possibly occurring simultaneously, provoked by the

action of the radiation on the tissues. In this connection it would be interesting to know the effect of radiation protective agents such as cysteamine on the different phases of the radiation response as it is possible that such agents may have more effect on one reaction than the other. One would of course also want to know how these protective agents influence the response to radiomimetic chemicals such as Myleran and CB 1348.

With regard to the mechanism of action of the radiomimetic chemicals both Myleran and CB 1348 show the same type of chemical reactivity 'alkylation' but whilst the nitrogen mustard CB 1348 acts by means of an Sn_1-type of reaction mechanism (Ross[11]) Myleran acts mainly by the slower Sn_2-type mechanism. Both compounds could therefore have cytotoxic properties related to their common ability to alkylate, but the different type of reaction mechanism of Myleran could result in more specific biological properties, since, as pointed out by Ross[11], the degree of reactivity of a substance by the Sn_2 mechanism is controlled by the concentration of reacting groups with which the chemical comes in contact. It seems possible that a high concentration of active groups could occur during certain stages in cell division, so that compounds of the Myleran type might tend to have some degree of selective action on dividing rather than on 'resting' cells.

ACKNOWLEDGEMENTS

This investigation has been supported by grants to the Chester Beatty Research Institute, from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institution of Health, U.S. Public Health Service.

REFERENCES

[1] Lamerton, L. F., Elson, L. A., and Christensen, W. R. *Brit. J. Radiol.* 1953, **26** 510.
[2] Lamerton, L. F., Elson, L. A. and Harris, E. B. *ibid*, 1953, **26** 568.
[3] Lamerton, L. F. and Baxter, C. F. *ibid*, 1954, in press.
[4] Haddow, A., Scott, C. M. and Scott, J. D. *Proc. Roy. Soc. B*, 1937, **122** 477.
[5] Everett, J. L., Roberts, J. J. and Ross, W. C. J. *J. Chem. Soc.* 1953, **486** 2386.
[6] Haddow, A. and Timmis, G. M. *Lancet*, 1953, **264** 207.
[7] Galton, D. A. G. *ibid*, 1953, **264** 208.
[8] Elson, L. A. and Warren, F. L. *Brit. J. Cancer*, 1947, **1** 86.
[9] Elson, L. A. *Acta Un. internat. Cancer*, 1948, **6** 396.
[10] Elson, L. A. *Brit. J. Haematol*, 1954, in press.
[11] Ross, W. C. J. *Advances in Cancer Research*, **I** 397. New York, N.Y., Academic Press, 1953.

A NEW HYPOTHESIS FOR 'CHROMATID' CHANGES

S. H. Revell

Chester Beatty Research Institute, Royal Cancer Hospital, London

In this paper a new hypothesis is suggested for structural chromosome changes of the 'chromatid' class (Catcheside, Lea and Thoday[1]). The hypothesis has been evolved during a comparative study of the effects of X-rays and certain chemical agents, and is intended to apply to both. But on this occasion only the radiation side of the matter will be discussed.

So far as the author can judge, the scheme proposed resembles a 'contact' hypothesis—that is, in the 'contact first' versus 'breakage first' sense—and he is well aware that the revival of anything like the rejected view in this controversy may be regarded as conflicting with evidence pointing in the opposite direction. An attempt will be made, however, to show how this apparent conflict might be resolved.

It should be emphasized that the hypothesis is at present only advanced to explain what are usually called 'post-split' or 'chromatid' aberrations. However, an important part of the general theory for chromosome changes has been derived from a study of changes of this particular category (largely in *Tradescantia* pollen grains; Sax[2, 3]; Catcheside *et al*[1, 4]). Their incidence has also been used as an index in testing the relative efficiencies of different types of ionizing radiation, and in testing radiation sensitivity under different conditions. But the hypothesis is not now concerned directly with the explanation of other important types of aberration. Thus no attempt will be made to explain 'chromosome' changes in the restricted sense ('pre-split' changes), or changes resulting from anaphase breakage, or changes inducible in *Drosophila* reproductive cells, although this should not be taken as implying that there is necessarily an essential distinction between these other aberrations and those under consideration. It should also be borne in mind that this short paper allows only a very cursory discussion of most of the problems which the proposed hypothesis raises: some of these will be dealt with in more detail in other publications.

CRITICAL ACCOUNT OF THE HYPOTHESIS

Vicia faba root tip cells have been used throughout the work, which, following on the observations of Ford[5], was originally undertaken with the object of comparing the distribution of visible aberrations induced by various chemical agents with those induced by X-rays. But, as this work went on, the orthodox interpretation was found increasingly unsatisfactory, not only for the chemically induced changes but for the X-ray ones as well. This was firstly because it did not seem to account plausibly for all the chromosome changes that were occurring (this will be amplified below), and

secondly because an unexpectedly high proportion of exchanges seemed to be homologous—that is to say, structurally similar to meiotic chiasmata. For example, seventeen out of a series of forty X-ray interchanges between the two long chromosomes in *Vicia* were found, so far as could be judged, to be exactly homologous. This seemed to suggest that a close somatic pairing must be involved in their formation.

As is usual in this kind of material, it was also found that all exchanges, whether homologous or not, were reciprocal. This last observation can by itself be explained without difficulty by the orthodox theory, but, in conjunction with the other evidence, raised doubts as to whether this theory was after all entirely sound.

The question of whether or not chromosomes might have to be functionally related in some way for exchange to occur has been the subject of much experimentation and some controversy (see MULLER[6]). 'Two opposite views of the mechanism were conceivable : either that breakage of the chromonemas occurred first—presumably independently in the different places—to be followed by fusion as a later consequence, or that the process of breakage was dependent on the prior approximation and perhaps fusion of the chromonemas—the different breaks concerned being in this case results of a common initiating process.'

However, there is already an assumption implicit in this statement of the problem : that a process of *breakage* is necessarily involved. This is also an important premise involved in the actual scoring of visible changes at metaphase. Described without prejudice these changes consist of *discontinuities* (LOVELESS[7]) and rearrangements. The classical theory assumes that these discontinuities are breaks ; that these breaks are the primary aberrations that were induced ; and that such breaks are the components—by their reunion—of all the rearrangements. It is thus assumed that the changes which can be seen at metaphase are of two kinds : the primary breaks and the secondary rearrangements.

Now the fact that breaks were found to rise about linearly and interchanges to rise as the square of the X-ray dose (under certain conditions) often seems to be taken as proof that each interchange involves two independent breaks. Also, data from experiments where the X-ray dose was separated into two halves by varying times, or where the dose intensity was varied, have been used to estimate the mean time which is supposed to elapse between breakage and reunion. But actually it is clear that this kind of evidence does not itself indicate that two *breaks* are the two events induced because it cannot by itself give any information about the nature of the changes in the two chromosomes which cause them to exchange.

However, the orthodox theory, as already pointed out, does assume that 'chromatid breaks and chromatid exchanges represent different ultimate fates of originally the same event, namely a chromatid break' (CATCHESIDE[8]). Thus the two independent events which are separable in this type of X-ray experiment are identified as chromatid breaks. It does seem to the author that the fact that this is an assumption is sometimes overlooked. Actually there is no direct evidence that the aberrations interpreted as breaks at metaphase were the potential components of the rearrangements, or that they are breaks at all.

But more is known about exchanges than this. Although they rise as about the square of the X-ray dose, and although this is taken as ruling out a 'contact' mechanism, nevertheless several different kinds of evidence all indicate that two chromosome points must be both close together in space and must both be affected within a very short time if they are to exchange. This is conventionally interpreted as meaning that restitution usually occurs quickly after breakage. Therefore only breaks induced very close together (the 1μ and $3\frac{1}{2}$ minutes of LEA[9]) have much chance of reuniting because restitution otherwise supervenes and makes them unavailable to one another.

But after all, the original 'contact first' problem was essentially a biological problem, not of how many ionization columns are required to cause an exchange, but whether or not its initiation is dependent on there being first a functional proximity of some kind. It was only afterwards suggested that a study of dose-interchange relations might give an answer to the question.

Now in the case of the present work, it seemed to the author that the occurrence of the homologous exchanges raised the possibility that there might be an association involved which had a biological capacity for exchange, as distinct from there being just a spatial proximity which favoured exchange as the outcome of an essentially random breakage and reunion.

In considering the possibility of an exchange *process* for interchanges, the question at once arises of what the single breaks might otherwise be if it turned out that they were not the components of the interchanges. Now the difficulty of scoring certain changes in *Vicia*, which has already been mentioned, was concerned with the single breaks. Isochromatid breaks were easily observed but it was difficult to have confidence in the chromatid break scores because, although some of the latter were clear enough, they seemed to grade imperceptibly into a whole complex of other anomalous changes which, as a group, could not reasonably be described as chromatid breaks at all. Part of this difficulty was concerned with numerous small unstained regions. These occur in many places. For instance, in *inter*changes they seem to mark the points where the breaks and reunions might be supposed to have occurred (*Figure 1a*), but other apparently identical unstained regions may also be observed in single chromosomes which have not interchanged, and these might perhaps be conventionally interpreted as detected restitutions (MORRISON[10]). This, however, did not appear to be a completely convincing explanation because, at least in *Vicia*, these regions so often occur in chromosomes with unequal chromatids and, frequently, associated minutes as well. Altogether, it seemed difficult to justify the arbitrary separation of the typical chromatid breaks (*e.g. Figure 2c*) from all these others (*Figure 1b*).

In one type out of this group of anomalies it seems clear that part of one chromatid has been intercalated into the other. The simplest way in which this could occur is by chromatid exchange in a small loop (*Figure 1c*, type 1). Such *intra*changes are well known in *Tradescantia* and *Trillium*, there being four possible types depending on which pair of chromatids exchange (*Figure 1c*, types 1–4; see also Figure 17 of Catcheside, Lea and Thoday[1], and Figure 10 of DARLINGTON and LA COUR[11]). It was at first difficult to accept this interpretation for *Vicia* because the three other *intra*changes seemed to be missing (*Figure 1c*, types 2–4) each of

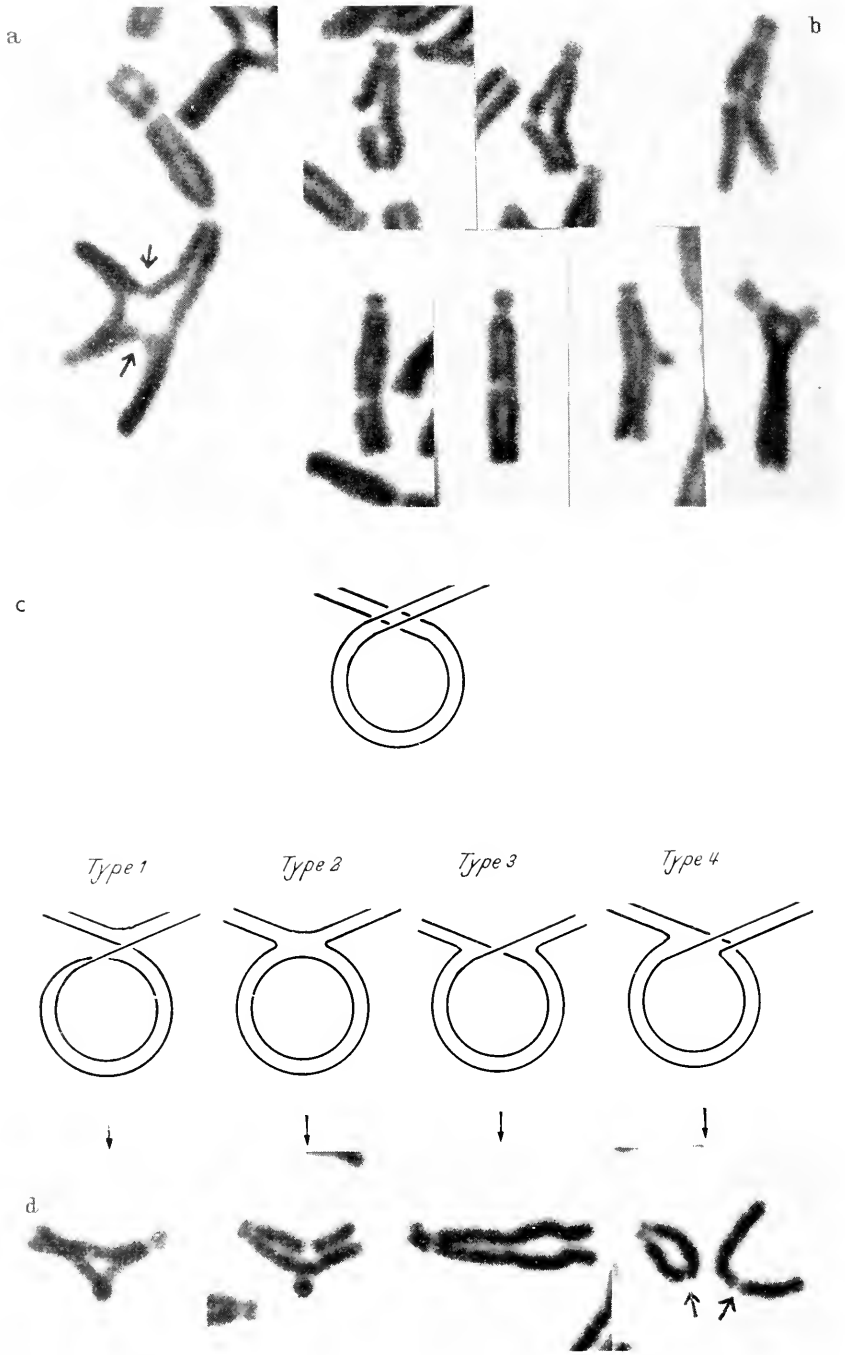


Figure 1. *Photomicrographs of* Vicia faba *chromosomes in Feulgen squashes of root tip mitoses (all colchicine treated), after treatment with X-rays. All × 3,000.*

(a) Chromatid inter*change between long and short chromosomes. Note the short achromatic regions at points of exchange.*

(b) Examples of anomalous changes which are difficult to distinguish from chromatid breaks. Probable chromatid break at top-left.

(c) and (d) The four types of proposed intra*change, with explanatory diagram.*

which should be occurring with a frequency equal to the duplication-deletion (type 1).

However, the resolution of this problem could depend on one assumption : that if the loops of these *intra*changes were always small enough in *Vicia* then, as the whole configuration shortened to metaphase, the original chromatid relations would be lost. Type 2 would then yield the bent chromosome with minute juxtaposed ; type 3 would probably be difficult to see at all since it is merely a small inversion ; and type 4 would appear as an isochromatid break with sister union (*Figure 1d*). Carried a stage further, this interpretation could account for a proportion of chromatid breaks : if, like *inter*changes, these *intra*changes may also be incomplete—that is, with one of the two pairs of chromatid ends not joined up in the exchanged relation (*Figure 2a* ; see Catcheside *et al*[1]), then types 1 to 3 could yield 'chromatid breaks' (compare *Figures 2b* and *2c*) and type 4 could give failed 'sister union'.

This interpretation is supported by three observations.

(1) If the aberrations in question are really collapsed *intra*changes—that is to say, chromatid exchanges like the *inter*changes—then some of the small unstained regions already mentioned which were evidently associated with these aberrations may now be rationally explained (no matter what they actually are) as marking the points of exchange, just as they are seen to do in the case of *inter*changes (*Figure 1a*). For example, if 'sister unions' are really collapsed *intra*changes then the points of chromatid exchange must be to one side of the 'end' of each fragment. It is frequently possible to confirm this by observation (*Figure 1d*, type 4 photomicrograph). Such achromatic points may also be observed on the other presumed *intra*changes (*e.g. Figure 1b*). Previous to the development of this hypothesis, the author had interpreted many of these points as chromatid breaks.

(2) In contrast to *Trillium* and *Tradescantia*, no clear intra-arm *intra*changes are observed at metaphase in *Vicia*. However, such *intra*changes are demonstrable at prophase although they are always relatively much smaller than those in the former species (*Figure 2d*). Therefore these *intra*changes must reach metaphase in a disguised form, and it seems that the most rational explanation available is that they are represented by the aberrations already described* (*Figure 1d*).

(3) As was observed by Lewitsky and Araratian[12], the related parts of affected chromosomes are frequently closely juxtaposed at metaphase. Thus the fragments from an isochromatid break are often very close together and minutes are usually close to the chromosomes from which they are presumably deleted (*Figure 1d*). (A similar phenomenon can be observed in the case of 'triradials' and certain other types of *inter*change—see below.) This observation is not readily accounted for by the orthodox hypothesis since breakage and reunion are assumed to have occurred at the time of treatment, there being no subsequent relation between the separated chromosome parts. It can, however, be plausibly explained by the hypothesis now suggested, which postulates that the chromatids of the minute loops which

* The same explanation could be advanced for these aberrations in *Trillium* and *Tradescantia*, although in these species the largest *intra*changes retain their form even at metaphase and are therefore recognized as such.

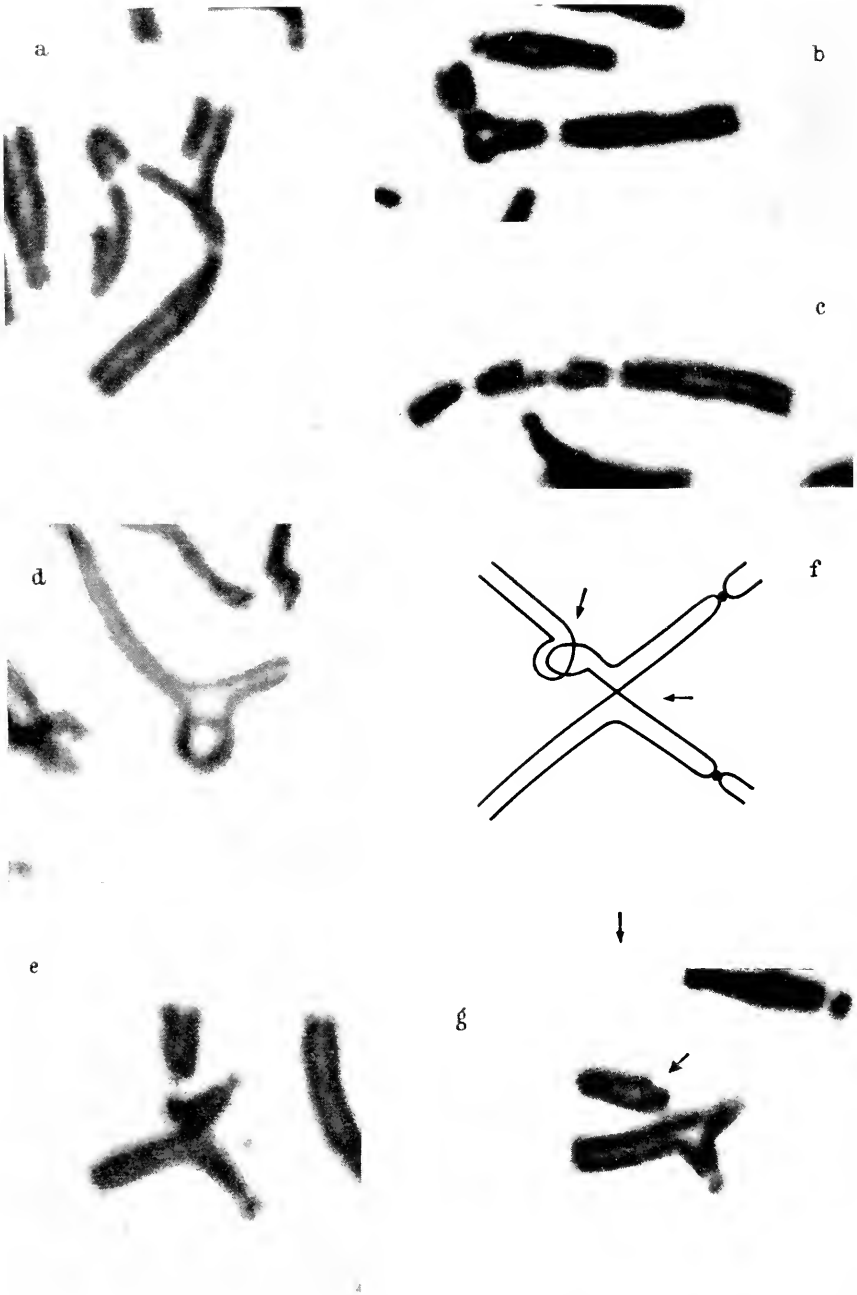


Figure 2. Photomicrographs of Vicia faba *chromosomes in Feulgen squashes of root tip mitoses (all colchicine treated), after treatment with X-rays. All × 3,000.*

(*a*) *Incomplete* inter*change between long and short chromosomes.*
(*b*) *Complete* intra*change in long chromosome.*
(*c*) *Proposed incomplete* intra*change in long chromosome (= chromatid 'break').*
(*d*) Intra*change at prophase.*
(*e*) Inter*change with isochromatid break in one arm (= 'near triradial').*
(*f*) *and* (*g*) *Triradial (= chromatid-isochromatid* inter*change) with diagram of suggested interpretation.*

undergo rearrangement only lose their paired relation during prophase. If this were correct then the two fragments from an 'isochromatid break' might still remain close together at metaphase because the association between them had only just lapsed (compare with Figure 17b of Catcheside *et al*[1]).

In addition to these three items of supporting evidence, it should also be pointed out that the new interpretation gives a satisfactory explanation of the original difficulty encountered in scoring chromatid breaks. All the aberrations which previously were difficult to distinguish would now be assumed to be equivalent : all are either complete or incomplete small *intra*changes. But only one out of the four types—that which gives an 'isochromatid break' (type 4)—would be observed and discriminated efficiently. The others, owing to their small size, would frequently be missed or else confused with one another, and it would moreover be difficult in some cases to decide whether they were incomplete (that is, whether or not they constituted 'chromatid breaks'). The hypothesis also accounts for the occurrence of chromatid and isochromatid breaks in the same nucleus.

A large proportion of the whole class of 'chromatid' aberrations as defined in the orthodox theory—both chromatid and isochromatid breaks, chromatid *inter*changes and *intra*changes—may thus be interpreted in terms of one unit of change : a chromatid exchange. But it will be noted that there is still an important group of *inter*changes which remains unaccounted for ; namely those which are conventionally interpreted as involving reunion of one or more isochromatid breaks. It is not obvious how this type of *inter*change could arise by chromatid exchange. Nevertheless, certain facts suggest that they might be formed in essentially the same way.

The most frequent of this last group of aberrations is the chromatid-isochromatid *inter*change ($\equiv$ triradial) which, according to the orthodox interpretation, results from reunion between one isochromatid fragment and a single chromatid break, the other isochromatid fragment commonly undergoing sister union. It is often the case (*a*) that this latter fragment remains close to its related configuration at metaphase (see paragraph (*3*) and *Figure 2g*) ; and (*b*) that the short achromatic region is eccentrically placed as it is in sister unions of non-*inter*changed chromosomes (see paragraph (*1*) and *Figure 2g*). These two facts strongly suggest that the sister union event, whatever it is, which occurs in a triradial is the same as that which results in ordinary isochromatid fragments.

Now it is occasionally evident that a sister union event can occur close to a chromatid *inter*change (*Figure 2e*). In view of this, the items of evidence (*a*) and (*b*) above suggest that a triradial could consist of two chromatid exchanges very close together—one *inter*change and one *intra*change (*Figure 2f*) : the chromatid relations would be obscured during contraction, the fragment thus separating from the main configuration by the time of metaphase (*Figure 2g*). Similar interpretations in terms of two or more exchanges near together may be given of other less common types of 'interchange'.

Thus it is possible, at least qualitatively, to interpret all recognized 'chromatid' aberrations in terms of one or more chromatid exchanges. It is evident that since incomplete *inter*changes, failed 'sister unions' and chromatid 'breaks' are all assumed to be incomplete exchanges their numbers should be positively correlated. There is, of course, some evidence that

this is so (Catcheside *et al*[1]), the conventional explanation being that there is a certain likelihood that any break may remain open until metaphase. The quantitative aspects of the exchange hypothesis given above, and especially the question of whether it can account for all changes conventionally scored as chromatid breaks, will be discussed in detail in another paper.

SURVEY

It must be borne in mind very clearly that the hypothesis which has been proposed above does not rest on any completely critical evidence. It is, like the breakage-and-reunion hypothesis, a scheme for the *interpretation* of visible changes in metaphase chromosome structure, and through which they may be related to the quantitative data. Although these changes are observed at metaphase they are, of course, only the consequence of much earlier alterations in chromosome development. Obviously, a knowledge of this development is essential for a detailed understanding of the nature of its aberrations. At present this knowledge is almost entirely lacking, and it therefore seems unlikely that the examination of the proposed exchanges at the time they reach metaphase could alone throw much light on the process of their formation (Loveless[7]).

It is for this reason that the new hypothesis, as it now stands, only attempts to account for the observed aberrations in terms of a chromatid exchange, as being the least biological entity resolvable by the technique employed—that of metaphase analysis.

Now if the exchange and the breakage-and-reunion hypotheses alone are considered, then it seems to the author that three main possibilities may be distinguished. Thus, the exchange hypothesis might be totally incorrect. Or it might be only partially correct : this could mean that the breakage-and-reunion interpretation was not essentially incorrect, but had been inaccurate insofar as it had failed to recognize that certain 'breaks' were actually *intra*changes ; or it could mean that aberrations happening to resemble one another in some respects were arising by the two quite different mechanisms. Or, lastly, the new hypothesis might be entirely correct : in this case all the 'chromatid' group of aberrations would be correctly interpreted as exchanges (complete or incomplete) which occurred either between points on different chromosomes or else between points close together on the same chromosome.

In the author's opinion the items of evidence numbered *1* to *3* on pages 247–249 suggest rather strongly that the new hypothesis is at least partially correct. However, it seems impossible at present to be more definite than this, although it should be pointed out that the observations as a whole provide no critical objections to its being wholly correct. It must therefore be emphasized that the object of the ensuing discussion is to consider briefly what would follow if the new hypothesis *were* correct, and not what necessarily follows from its having been proved correct.

If all defined 'chromatid' aberrations were chromatid exchanges then would it be possible to say anything at all about the exchange process ?

The conventional explanation would be that each exchange is due to two chromatid breaks and reunions. The complete acceptance of the new hypothesis would implicitly question this view because all the 'breaks' seen

at metaphase would themselves be the result of exchange : hence the observed ' breaks ' would not represent the residue of primary aberrations, the rest of which had either reunited in pairs as exchanges or else restituted. Interpreted in this way, the visible changes thus provide no evidence of ' breakage first ', or indeed of breakage at all. Of course, it might still be postulated that both *intra-* and *inter-*changes arise by breakage and reunion, but this would now be an entirely separate question connected with the general problem of whether resting-stage ' chromosomes ' are actually permanent structures.

If the new hypothesis were correct, then, for the reason already given, it could hardly be expected at present that much could be deduced about the exchange process, except in the most general terms. But it is clear that, for exchange to occur, the loci involved must come close together at some stage ; also, it seems difficult to conceive of such an exchange as other than an organized process. At least for the author, it is difficult to see how exchange could be the result of any random damage and repair, even if such events were guided by a proximity of 1 μ. Nor does it seem to the author that the incomplete exchanges themselves constitute evidence that a breakage process is involved : these should be regarded without prejudice simply as *imperfect* exchanges. The case for considering the exchange as a developmental entity, until more is known about it, is greatly strengthened by the fact that exchange does actually occur as a physiologically determined process at meiosis, where it seems to be connected essentially with chromosome doubling. It would obviously be tempting to regard the aberrations as induced heterologous exchanges of the same developmental type : at present, however, such a comparison must only be considered with caution.

Thus the question which was posed earlier in this paper in connection with the homologous exchanges alone now arises again with a much wider application. Are there *inter-* and *intra-*chromosomal associations in somatic cells which may be defined by their capacity to exchange in response to stimuli such as ionizing radiations ?

It seems to the author that, whatever may be the general view of this hypothesis, there need be no essential conflict between it and the observed dose-*inter*change relation for X-rays, although at first sight this might appear to be so. It is agreed that all types of ' chromatid ' aberration increase linearly with doses of the densely ionizing radiations (Giles[13], Thoday[14, 15] ; Kotval and Gray[16]), and because of this and for other reasons it has been deduced that chromosome loci have to be very close together (*ca.* 1 μ, Lea [9]) before exchange is possible between them, and that this is so even with X-rays where two electrons are necessary. This last fact, however, does not by itself appear at all to exclude the possibility that the chromosomes may be in ' contact '—if this means their being functionally associated so that they can exchange—*unless ' contact ' is also specifically defined as capacity to respond to a single X-ray hit.* Now this is, of course, exactly the definition which has been adopted, and it is possible that it might be a misleading one since it has always been so closely bound up with the assumption that it is two breaks which are the two primary events associated with the two ionization paths. It is just this assumption which is now in question.

So far as the author can judge, it may equally well be proposed that an exchange process is initiated at the chromosome association, but that there

is not always a one-to-one correspondence between ionization paths and exchanges. The properties of the associations where *intra-* and *inter-*changes may occur differ in some way which happens to be critical for the different radiations. Thus, in the case of small *intra*changes, either the two loci involved are always close enough together, or other conditions at the critical region of proximity are always such that one ionizing particle is enough to involve both chromosomes in the exchange. But the properties of *inter*-chromosomal associations cause them to react differently to the different radiations : the amount of ionization—or the distribution of its effects, or the precise chemical changes induced—along the tracks of recoil protons and α-particles is such that they are singly effective in causing *inter-* as well as *intra*-changes. In the case of X-rays, the passage of a mean of two primary electrons is required for *inter*changes, and these passages must occur within a very short time to effect an exchange. If this were correct, then there would be in this respect a primary event—an unstable effect of some kind—corresponding to what is conventionally visualized as breakage, its decay being 'restitution'. A successful exchange initiation, on the other hand, would correspond to 'reunion'. It should be stressed that whatever actual processes it is they represent, these two stages need not be the only ones connecting the release of the dose energy with the final metaphase exchange : it could merely happen that they are the only two stages which are *separable* by the experimental method of varying the amount of the X-ray dose or its intensity. In similar neutron or α-ray experiments they are not separable at all.

In distinguishing the new hypothesis from the breakage-and-reunion hypothesis, the present problem is that the data do not reveal anything about the nature of the 'association', as it has been conveniently called in this paper. Thus, although it seems certain that the two chromosomes are close together (and that they are sometimes even homologously close together), it is not known whether it is this closeness itself which alone confers sensitivity or whether there are specific physiological conditions necessary for exchange induction which exist only at these associations. Now whether this hypothesis is really a 'contact' one seems to depend definitively on the answer to the question of whether or not the primary event—whatever it is, which corresponds to 'breakage'—can be induced at loci other than those in associations. The orthodox theory necessarily assumes that this may happen because the premise is that chromatid breaks are the primary events induced and that two go to make an exchange : therefore it must follow that all single breaks which are actually observed as such are primary events which have not participated in *inter*changes and hence were not in 'contact' by definition. *This is an assumption which in no way depends on the statistical relation between interchanges and X-ray dose.* It also follows that, if all parts of the chromosomes are equally liable to breakage, there is about ten times the observed number of breaks which disappear without trace, due to restitution. Both these postulates appear to depend on the initial assumption that the observed discontinuities are breaks and that they are visible examples of the primary units of change.

On the other hand, in terms of the interpretation presented in this paper, there is no visible evidence that the primary events occur anywhere

other than at the associations, although of course this is by itself quite inconclusive. If the exchange interpretation were correct then the nature of the primary events would have to be known, and a method would have to be devised which could detect them wherever they occurred, before the 'contact' question could be decided conclusively.

It will be clear that the present exchange hypothesis is much less unlike the breakage-and-reunion hypothesis of Sax, Catcheside and Lea than the somewhat different version of this hypothesis proposed by Darlington and others. It may be pointed out that the exchange hypothesis disposes of those arguments for delayed reunion which are based on the observation of chromatid and chromosome 'breaks' in the same cell, and on the observation of certain rearrangements such as triradials.

In conclusion, the reservation made in the introduction to this paper must be repeated: the present hypothesis is only suggested as an explanation of the aberrations which characteristically occur in cells about to undergo mitosis, namely 'chromatid' changes. Such a restriction may seem arbitrary, for it has been customary to consider observations on all types of chromosome change together, and in general to seek a common explanation for them all. If all this information were to be considered now, much of it could certainly not be reconciled with the present scheme. In spite of these discrepancies, the author considers that there is enough evidence in favour of an exchange hypothesis for 'chromatid' changes to justify its being regarded as a possible alternative to the orthodox hypothesis.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. L. H. Gray, to Dr. L. F. Lamerton, and to Dr. A. Loveless for their advice and for criticism of the ideas presented in this paper.

This investigation has been supported by grants to the Royal Cancer Hospital and Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Services.

REFERENCES

[1] Catcheside, D. G., Lea, D. E. and Thoday, J. M. *J. Genet.* 1946, **47** 113.
[2] Sax, K. *Genetics*, 1938, **23** 494.
[3] Sax, K. *ibid.* 1940, **25** 41.
[4] Catcheside, D. G., Lea, D. E. and Thoday, J. M. *J. Genet.* 1946, **47** 137.
[5] Ford, C. E. Proc. 8th Intern. Cong. Genet. Suppl. to *Hereditas*, 1948, 570.
[6] Muller, H. J. *J. Genet.* 1940, **40** 1.
[7] Loveless, A. Suppl. to *Heredity*, 1953, **6** 293.
[8] Catcheside, D. G. *Advanc. Genet.* 1948, **2** 271.
[9] Lea, D. E. *Actions of radiations on living cells.* Cambridge University Press, 1946.
[10] Morrison, J. W. Suppl. to *Heredity*, 1953, **6** 83.
[11] Darlington, C. D. and La Cour, L. F. *J. Genet.* 1945, **46** 180.
[12] Lewitsky, G. A. and Araratian, A. G. *Bull. appl. Bot. Genet. Pl.-Breed.* 1931, **27** part 1 (U.S.S.R.), 265.
[13] Giles, N. H. *Proc. nat. Acad. Sci. Wash.* 1940, **26** 567.
[14] Thoday, J. M. *J. Genet.* 1942, **43** 189.
[15] Thoday, J. M. *Brit. J. Radiol.* 1951, **24** 572, 622.
[16] Kotval, J. P. and Gray, L. H. *J. Genet.* 1947, **48** 135.

EFFECT OF OXYGEN TENSION ON THE PRODUCTION OF CHROMOSOME BREAKAGE BY IONIZING RADIATIONS: AN INTERPRETATION *

C. P. Swanson

The Johns Hopkins University, Baltimore, Maryland, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

It was first demonstrated by Thoday and Read[1] (1947) that anoxia reduces the effectiveness of any given dose of X-rays. This is strikingly illustrated when the frequency of chromosome aberrations is used as the criterion of effect, but it should be recognized that the protective action of anoxia extends to other phenomena as well: survival of organisms, delay in cell division, and the stickiness of chromosomes. The time is not yet ripe, however, for an integrated interpretation of these multiple effects even if it is presumed that they are related to a common mechanism. It has been recognized that the sensitivity of cells to X-rays, as regards breakage, stickiness, and delay in cell division, is greatest at late prophase and very early metaphase, which suggests possible interrelations. This discussion will, however, be confined to chromosome breakage in the microspores of *Tradescantia*, and a more extensive interpretation will be attempted in the light of new evidence. Firstly, it will be well to recount those circumstances which have been established with some certainty, and then to reinforce and extend them with additional observations made recently.

All other things being equal, it is the oxygen tension of the cell which determines, in part, the frequency of detectable chromosome aberrations. The oxygen-dependent portion of the total frequency will vary in magnitude with different ionizing radiations, but that oxygen is indeed part of a reactive system which influences breakage can be considered established by the studies of Giles and his co-workers. They have irradiated cells in hydrogen, helium, and in vacuum, and the results are essentially similar. Clearly, then, the oxygen tension of the cell is an important factor in the breakage of chromosomes, a finding reinforced by the observation that it is the oxygen tension during irradiation that is important, post-treatment changes in oxygen tension being without detectable effect. Gray[2] (1953) has adequately discussed these aspects of chromosome breakage in terms of radiochemistry, and it is apparent that a more meaningful picture is beginning to emerge.

Secondly, it is now quite clear that the degree of reduction resulting from anoxic irradiation is not the same for all types of aberrations even when the quality of radiation is the same. This was first demonstrated by Riley, Giles and Beatty[3] (1952), who showed that chromatid deletions were less

* Work performed under Contracts No. W-7405-eng-26 and No. AT-(30-1)-851, U.S. Atomic Energy Commission.

affected by the absence of oxygen than isochromatid deletions and chromatid exchanges. If the reduction in frequency of aberrations is expressed as an air/nitrogen ratio, then that for chromatid deletions was about 1·4 as compared to 2·6 for isochromatid deletions and exchanges. These observations have been confirmed (Swanson and Schwartz[4], 1953), and in addition it was possible to show that the air/nitrogen ratios varied with the stage of cell division. Dominant lethals and translocations in *Drosophila* also exhibit different air/nitrogen ratios with the same quality of radiation (Baker and Edington[5], 1952; Baker and Von Halle[6], 1953), therefore any hypothesis dealing with the relation of oxygen tension to radiation effect must be flexible enough to account for differential reductions among the possible aberration types.

Table I. Chromatid Aberrations Produced in Tradescantia *by Four Qualities of Radiation in Air and in Nitrogen.* 150 r *at* 8·9 r/minute

Radiation	*Atmosphere*	*Aberrations per* 100 *cells*		
		Chromatid deletions	*Isochromatid deletions*	*Exchanges*
X-rays–50 kvp unfiltered	Air	49·3	84·6	35·3
	N_2	98·2	15·0	9·5
	Air/N_2 ratio	0·50	5·6	1·9
X-rays–100 kvp 1 mm of Al	Air	66·0	62·5	30·5
	N_2	79·3	14·0	7·3
	Air/N_2 ratio	0·83	3·9	2·0
X-rays–250 kvp 4 mm of Cu	Air	78·0	44·0	28·0
	N_2	63·5	16·6	6·0
	Air/N_2 ratio	1·25	2·7	2·3
Gamma rays 1·1–1·3 MeV	Air	96·2	32·5	26·0
	N_2	50·5	14·0	5·0
	Air/N_2 ratio	1·9	2·3	2·3

Thirdly, it must also be recognized that the influence of oxygen tension on the frequency of breakage is closely linked to the type of radiation employed. Thoday and Read[7] (1949) have demonstrated that the oxygen effect is much less with alpha rays than with X-rays, and that neutrons appear to occupy an intermediate position (Giles, Beatty and Riley[8], 1952). The ion density of the radiation will therefore be a determining factor in governing the magnitude of the oxygen effect. Additional information has now been obtained from *Tradescantia* studies in which the effects from three qualities of X-rays, together with 1·1- to 1·3-MeV gamma rays from a cobalt-60 facility, have been compared (Swanson[9]). The results of one of four similar experiments are given in *Table I.*

If we first consider the frequencies of aberrations obtained in air, it is apparent that the chromatid deletions increase in frequency as the ion density of the radiation decreases but that the reverse holds true for isochromatid deletions. In fact, this shift in frequency of the two types of deletions

is a compensatory one, for the total frequency of deletions does not vary appreciably with quality of radiation. In view of the results of KIRBY-SMITH and DANIELS[10] (1953), who have demonstrated a decrease in total frequency with decreasing ion density of the radiation, these findings are somewhat surprising. However, since four rather extensive and essentially identical experiments have yielded almost identical results, it appears that the compensatory shift in type of deletion is a real one.

The relation of chromatid exchanges to quality of radiation in air is similar to that found for isochromatid deletions, and thus agrees with the earlier data of Kirby-Smith and Daniels.

Irradiation under anoxia yielded isochromatid deletions which, although greatly reduced in frequency, appeared to show no relation to quality of radiation. This, of course, leads to air/nitrogen ratios which are large with 50-kvp X-rays, and which become progressively smaller as the ion density of the radiation decreases. The chromatid deletions, on the other hand, now exhibit an opposite trend to that found after irradiation in air. They increase rather than decrease as the ion density of the radiation increases, and this leads to air/nitrogen ratios which are largest in value with gamma rays and smallest with 50-kvp X-rays. With 50- and 100-kvp X-rays, there is, in fact, an actual increase in the absolute frequency of chromatid deletions obtained in nitrogen as compared to air, and the air/nitrogen ratios, as a consequence, have values less than 1. The chromatid exchanges, like the isochromatid deletions, are much reduced in nitrogen, but the air/nitrogen ratios follow a trend comparable to that for chromatid deletions, as might be expected.

As pointed out in an earlier account of this work (Swanson[9]), it is believed that the differential air/nitrogen ratios for the several types of chromatid aberrations can be accounted for by assuming that there is a shift, in nitrogen, of one type of aberration into another. Thus, if we consider that the formation of isochromatid deletions and exchanges involves two broken chromatids, the repair or restitution of one of these but not of the other would essentially transform potential isochromatid deletions and exchanges into chromatid deletions. It is difficult to account in any other way for the increases in chromatid deletions at 50 and 100 kvp in nitrogen.

If we now convert the aberrations obtained in air and in nitrogen into terms of total breakage, *i.e.* by scoring chromatid and isochromatid deletions as single events, and exchanges as resulting from two independent breaks—it can be demonstrated that the inverse relation of ion density of the radiation to magnitude of the oxygen effect holds very well (*Table II*), as Thoday and Read[7] (1949) and Giles, Beatty and Riley[8] (1952) pointed out earlier.

An interpretation

Two hypotheses have been advanced to explain the reduction in frequency of aberrations obtained when irradiation is carried out under conditions of anoxia. Giles and his co-workers[8] proposed a hypothesis in which the principal effect of oxygen during irradiation is considered to be on the breakage mechanism, but they do not specify in detail how this is accomplished. SCHWARTZ[11] (1952) considers that oxygen affects the processes

Table II. Percentage Reduction in Total Breakage in Tradescantia *as the Result of Exposure in the Absence of Oxygen*

Radiation	*Experiment C*	*Miscellaneous**
Gamma rays		
1·1–1·3 Mev	59	—
X-rays–250 kvp	48	58
X-rays–100 kvp	43	—
X-rays–50 kvp	36	—
Neutrons	—	33
Alpha rays	—	0

* Reduction of 58 per cent at 250-kvp X-radiation was extracted from Riley, Giles and Beatty (1952, Table 1 at 150 r). The neutron and alpha ray reductions were derived from data in Giles, Beatty and Riley (1952, Table 2 at 10 n). Thoday and Read (1949) indicate that some reduction is obtained with alpha rays when exposure is made in nitrogen.

of restitution in such a manner that restitution, which follows the actual breakage of chromosomes, is favoured by anoxia. As will be pointed out, neither hypothesis is satisfactory in its present form. If we adopt the point of view of Gray[2] (1953) that a variety of primary events are produced in or near the chromosome by ionizing radiations and that the relative importance of these events, when translated in terms of breakage, is determined by the physiological condition of the cell during irradiation and up to the time of final rupture of the chromatin strands, it becomes clear that breakage and restitution can be effected in a variety of ways and at various levels. In what follows, a more detailed analysis of possible events will be made, and the two schemes to be presented (*Figures 1* and *2*), although obviously tentative, will at least serve a useful purpose as points of departure for discussion and possibly for future studies.

Before proceeding with an explanation of *Figures 1* and *2*, it will be well to point out why the two previous hypotheses are inadequate. Insofar as the breakage hypothesis is concerned, increases in any type of aberration—for example, the chromatid deletions induced by 50-kvp X-rays in nitrogen as compared with air (*Table 1*)—cannot arise by a reduction in breakage alone. Partial repair or restitution, which converts one type of aberration into another, as explained earlier, must be postulated as an additional process. Also, if breakage alone is affected by oxygen tension, it becomes equally difficult to explain how aberrations such as chromatid and isochromatid deletions, which have somewhat similar relations to dose, vary quite differently with stage of division (Swanson and Schwartz[4], 1953).

Similar awkwardness is encountered if an attempt is made to explain the data in *Table 1* in terms of the restitution hypothesis. It can be seen that as the ion density of the radiation increases the chromatid deletion/isochromatid ratio decreases. With the more densely ionizing particulate radiations it would be expected, on the restitution hypothesis, that this ratio would be even lower in value than that obtained with 50-kvp X-rays, and that the values for the air/nitrogen ratios would be lower for chromatid deletions and higher for isochromatid deletions than those obtained with 50-kvp X-rays. That this expectation has not been realized has been

demonstrated by Thoday and Read[7] (1949) for alpha rays and by Giles, Beatty and Riley[8] (1952) for neutrons. It must therefore be considered that restitution as defined by SCHWARTZ[11] (1952) cannot be the process principally affected by oxygen tension in *Tradescantia*.

Figure 1 is an operational scheme adopted, in modified form, from THODAY[12] (1953), and applicable only to the data obtained from the study of chromatid aberrations in *Tradescantia*. In agreement with Gray[2] (1953) that a variety of initial events can take place in the chromosome as the result of exposure to ionizing radiations, it is suggested that both potential (latent)

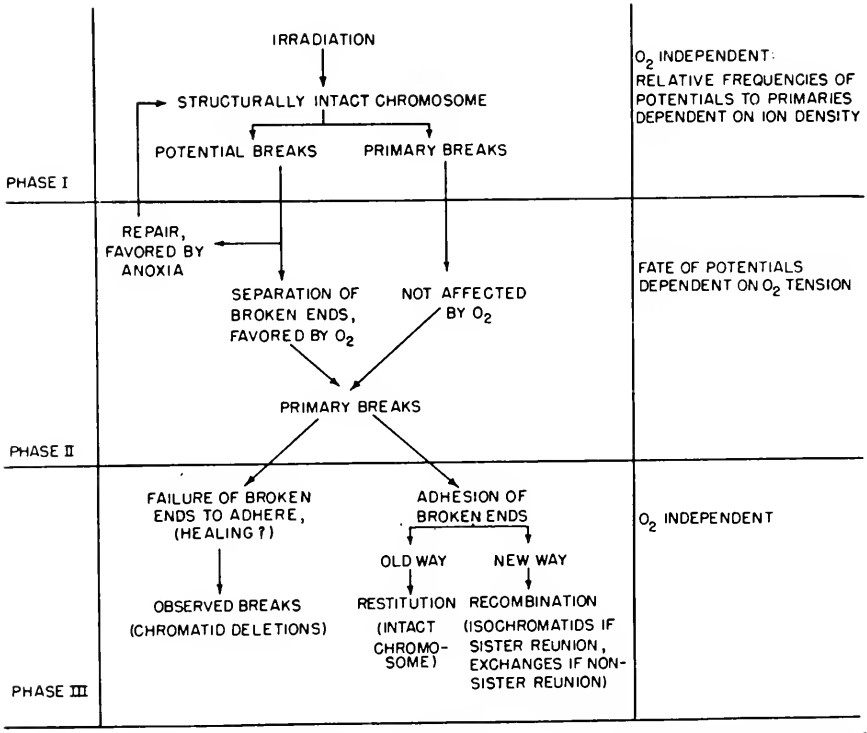


Figure 1. A schematic diagram representing in brief the major events taking place in the chromosomes of Tradescantia *during and after radiation, with suggestions as to the most likely manner by which oxygen affects the frequency of aberrations.*

and primary breaks are formed, and that in Phase I their formation is independent of oxygen tension. Valid objections to the terminology employed may be raised, but these are terms of convenience and may perhaps best be considered as the termini of a continuous spectrum of damage inflicted in the chromosome, the primary breaks being sites of irretrievable damage and of subsequent rupture, and the potential breaks, lesions or sites of lesser damage capable of repair or of complete disruption, as the cellular circumstances might determine. Furthermore, it is proposed that the relative frequencies of the two types of breaks would be a function of ion density, for it is inconceivable that radiations of such quantitatively different ionization characteristics as gamma and alpha rays could produce the same

spectrum of initial events. Since there is little or no oxygen dependence with alpha rays, and since the probability of a single alpha particle producing a break is close to unity (Gray[2], 1953), the initial events induced by alpha rays would probably lie far to the right of the spectrum, *i.e.* they are principally primary breaks. Their transformation into actual breaks with separated broken ends may be immediate or delayed—this we cannot establish, and the time factor may be different with different organisms—but complete rupture of the chromatids takes place regardless of the oxygen tension (Phase II).

The less densely ionizing gamma and X-rays would produce changes more to the left of the spectrum—neutrons would be intermediate in this respect—although some primary breaks would still be produced. The fate of the potential breaks is determined by the available oxygen in the cell in such a fashion as to favour their complete disruption in the presence or their repair in its absence. Also, the less the damage, the greater the dependence on oxygen for complete transformation into actual breaks. If these changes induced by radiation are in single-strand chromosomes, only a reduction in total frequency of aberrations can be expected if anoxia exists during irradiation, and rings and dicentrics, the customary types of aberrations scored, would be reduced to the same degree. Giles and his co-workers[8] have shown that this expectation is realized. However, if double-strand chromosomes are irradiated in the absence of oxygen, repair of one or two damaged chromatids becomes possible, and a situation is therefore available for the transformation of latent isochromatid deletions and exchanges into actual chromatid deletions. Variable air/nitrogen ratios among chromatid aberrations are therefore possible, and the extent of variability will be determined not only by the ratio of potential to primary breaks in Phase I, but also by the ratio of single- to double-strand lesions. Since both ratios are dependent on the ion density of the radiation, a complex situation exists, but there can be no doubt that there is a correlation between oxygen dependence and ion density.

The data obtained to date provide no reason for believing that oxygen tension exerts an influence once the actual breaks are formed (Phase III).

The scheme presented appears to be consistent with the biological data derived from the study of irradiated chromosomes of *Tradescantia* as well as with the physical facts of radiation. To what degree it corresponds to actual events taking place in irradiated chromosomes remains to be demonstrated for it is becoming increasingly evident that the final answers lie in the area of radiochemistry (Gray[2], 1953). The work of Lea and others has provided strong support for the belief that individual chromosome breaks are produced by individual ionizing particles, but the role of oxygen suggests that both direct and indirect energy transfer to the molecular bonds within the chromosome can lead to breakage. Presumably, the indirect energy transfer is through the medium of reactive substance produced by the radiation in or near the chromosome. With this in mind, the scheme in *Figure 2* provides a more general approach to the problem of how oxygen may affect breakage.

Several possibilities exist, and for convenience they may be listed as follows :

(1) Phase I—At this level the physiological conditions of the cell or the surrounding medium can have an influence on the amount of indirect

energy available for transfer to chromosomal substances. Thus bacteria irradiated in water and in broth have different survival rates even though the oxygen tension in the cells may be similar. So far as breakage in *Tradescantia* is concerned, it is likely that this is an important step because it is here that the relative amounts of direct versus indirect energy are determined.

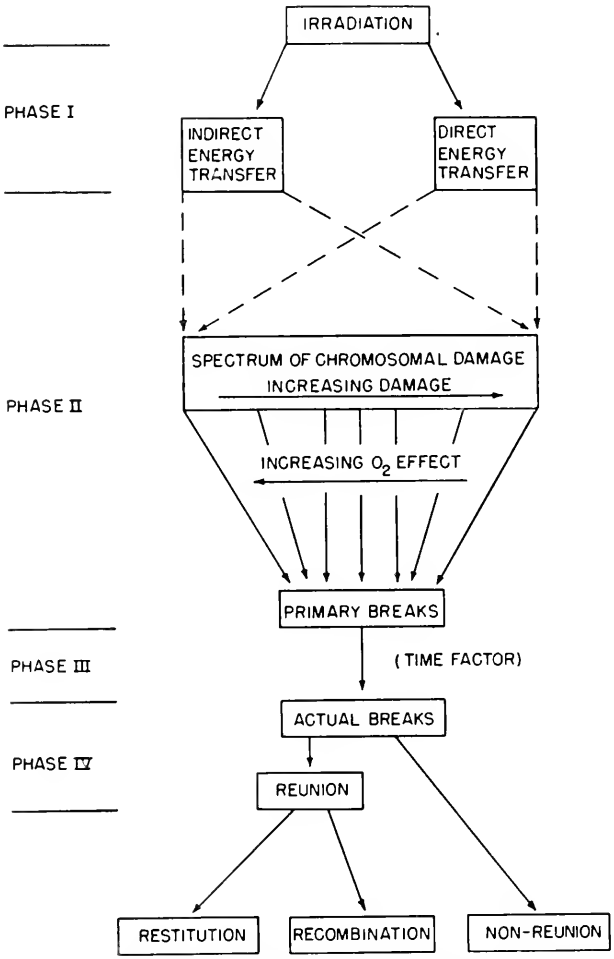


Figure 2. A schematic representation in more general form of the possible roles of radiation and oxygen tension in inducing chromosomal aberrations. (Prepared in collaboration with Dr. K. G. Lüning, Institute of Genetics, University of Stockholm, and published with his kind permission.)

(2) *Phase II*—Several steps can be involved here, but at present there is no way of distinguishing between them experimentally. Oxygen, through the medium of indirect energy transfer, can contribute to the spectrum of chromosomal damage, or it can enhance the damage already induced by direct ionization. Anoxia, therefore, would tend to repair damage created by direct energy transfer or to lessen the amount of damage created by

indirect energy transfer. Phase II consequently corresponds to Phase II of *Figure 1* where the fate of the potential breaks is considered to be determined, and the influence of oxygen is greater at the left than at the right end of the spectrum of chromosomal damage, as demanded by the data in *Table II.*

(3) Phase III—Primary breaks are distinguished from actual breaks only by a time factor, and the transformation may be immediate or only after an interval of time as indicated. The *Tradescantia* data suggest that oxygen is ineffective at this level, but we cannot be certain that this is true for breakage in other organisms.

(4) Phase IV—The *Tradescantia* data again suggest that the fate of actual breaks is not influenced by oxygen, but the possibility is included since it may be important in other organisms.

ACKNOWLEDGEMENTS

This paper was written at the Institute of Genetics, University of Stockholm, where the author was in residence as a Guggenheim Fellow. The author wishes to acknowledge his indebtedness to Professor Gert Bonnier for the facilities placed at his disposal, and for the many courtesies extended. Figure 2 *and the discussions revolving around it are to be considered part of a collaboration with Dr. K. G. Lüning, and they are included here with his kind permission.*

REFERENCES

[1] Thoday, J. M. and Read, J. *Nature, Lond.* 1947, **160** 608.
[2] Gray, L. H. *Brit. J. Radiol.* 1953, **26** 609.
[3] Riley, H. P., Giles, N. H. and Beatty, A. V. *Amer. J. Bot.* 1952, **39** 592.
[4] Swanson, C. P. and Schwartz, D. *Proc. Nat. Acad. Sci. U.S.* 1953, **39** 1241
[5] Baker, W. K. and Edington, C. W. *Genetics*, 1952, **37** 665.
[6] Baker, W. K. and Von Halle, E. S. *Proc. Nat. Acad. Sci. U.S.* 1953, **39** 152.
[7] Thoday, J. M. and Read, J. *Nature, Lond.* 1949, **163** 133.
[8] Giles, N. H., Beatty, A. V. and Riley, H. P. *Genetics*, 1952, **37** 641.
[9] Swanson, C. P. *ibid*, in press.
[10] Kirby-Smith, J. S. and Daniels, D. S. *ibid*, 1953, **38** 375.
[11] Schwartz, D. *Proc. Nat. Acad. Sci. U.S.* 1952, **38** 490.
[12] Thoday, J. M. *Heredity* (Symp. on Chromosome Breakage, Springfield, Ill., Thomas), 1953, **6** 299.

THE RELATIVE EFFECTIVENESS OF VARIOUS IONIZING RADIATIONS ON CHROMOSOME BREAKAGE IN *TRADESCANTIA*

J. S. Kirby-Smith, C. W. Sheppard and Doris L. Craig

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

A determination of the relative biological effectiveness (RBE) of X-rays of mean energies approximately 60 and 200 kV respectively, mixed 1·17- and 1·33-MeV gamma rays from ^{60}Co and high-energy beta rays from ^{32}P on *Tradescantia* pollen has been reported recently by Kirby-Smith and Daniels[1]. These studies have shown medium-energy X-rays in the 60-kV range (0·20 A wavelength) to be twice as effective as 1-MeV gamma rays or beta rays of approximately 500-kV mean energy in producing chromosome aberrations in this material. The effectiveness of 200-kV X-rays was found to be midway between that for 60-kV X-rays and 1-MeV gamma rays. This work now has been extended to cover the effects of fast neutrons on dry pollen and inflorescences of *Tradescantia*. In the course of these later investigations, the twofold increase in biological effectiveness in 60-kV X-rays over 1-MeV gamma rays has been confirmed for both of these biological materials.

The source of fast neutrons in the present studies has been the Oak Ridge National Laboratory 86-inch, 22-MeV proton cyclotron with the reaction of protons on an internal beryllium target. Measurements of the fast neutron flux and dosage have been made by means of proportional counter dosimeters and tissue-equivalent ion chambers. A comparison of these two different methods, as well as an independent determination of the stray gamma rays present with neutron insensitive ion chambers, has shown the gamma-ray contamination to be approximately 10–15 per cent.

In order to reduce the gamma-ray contamination to this figure, a biological exposure facility consisting of a lead box with 1-inch-thick walls was constructed outside the dee chamber. The neutron energy spectrum within this facility from the proton reaction includes considerably lower energies than the well-known curve for deuterons on beryllium, and there is also the degradation in neutron energies and scattering produced by the lead and by the other metal structures of the cyclotron. The neutron energy spectrum within the exposure facility has been determined approximately by means of various threshold and fission detectors. As is to be expected, it is a much degraded spectrum, with a broad maximum in the 1-MeV range and an appreciable thermal component.

Both *Tradescantia* dry pollen and inflorescences were exposed to fast neutrons in the lead-walled facility described. Following irradiation, the pollen was cultured after the methods of Bishop[2] and Conger[3]. Aberrations were scored at metaphase in the pollen tube division. In the inflorescences, four-day chromosome aberrations were scored. Results of the

pollen irradiations are shown in *Table I* together with aberration frequencies for X-ray and gamma ray treated samples of the same material for comparison. Data for the neutron-irradiated inflorescences, both hydrated and

Table I.—Effect of fast neutrons, medium-energy X-ray, and ^{60}Co gamma rays on Tradescantia pollen

Treatment	*Dose*	*No. of cells scored*	*Percentage normal*	*No. of Aberrations per cell*		
				Cd	Iso	Ex
Fast neutrons	24·7 rep	400	46	0·15	0·44	0·17
	49·3 rep	300	21	0·28	0·91	0·35
	73·8 rep	300	12	0·39	1·31	0·46
	98·8 rep	150	8	0·41	1·56	0·55
X-rays (250 kVp 3-mm Al)	200 r	300	12	0·34	0·99	0·42
Gamma rays (^{60}Co)	200	300	38	0·17	0·49	0·23

desiccated, as well as for an additional independent experiment in which normal buds were used, are summarized in *Table II*. These data show no differences in aberration frequencies for the wet and dry material and thus indicate that variations in the degree of hydration normally encountered in the flower buds do not affect their neutron sensitivity.

Table II.—Effect of fast neutrons on hydrated and nonhydrated Tradescantia inflorescences

	Dose (rep)	*No. of cells scored*	*Percentage normal*	*No. of Aberrations per cell*	
				Exchanges	*Deletions*
Wet buds	24·7	200	60	0·23	0·28
	49·4	200	44	0·44	0·44
	73·8	150	27	0·74	0·61
	98·3	100	14	1·02	0·89
Dry buds	24·7	140	63	0·22	0·20
	49·4	350	39	0·52	0·45
Normal buds	24·7	200	63	0·24	0·22
	49·3	200	42	0·44	0·45
	73·8	150	26	0·65	0·69

The RBE of fast neutrons with respect to cobalt gamma rays and 60-kV X-rays is presented in *Table III*. The results of previous work[1] on the relative effectiveness of X-, gamma and beta rays are also included in this table. Owing to the variation in biological effectiveness over the X-ray region, the effectiveness of 1-MeV gamma rays in producing the biological effects of chromosome breakage is assumed to be unity. This convention, which differs from previous usage in which X-rays are made the reference

Table III.—Relative biological effectiveness of various ionizing radiations

Type of Radiation	*Relative Biological Effectiveness*	
	Tradescantia pollen	*Tradescantia inflorescences*
^{60}Co gamma rays (1·17 and 1·33 MeV) ..	1	1
^{32}P beta rays (mean energy ~ 500 kV)	1	1
200-kV mean energy X-rays (0·06 Å)	1·5	1·5
60-kV mean energy X-rays (0·20 Å)	2·0	2·0
Fast neutrons (1-MeV mean energy)	8·0	10·0

index, gives an unambiguous reference point for comparing the effects of the various radiations.

These studies indicate the type of rough quantitative radiobiology possible with fast neutrons from a large internal target cyclotron source. Certain physical difficulties continue pending installation of a beam deflector and external target. Serious problems remain in precisely characterizing the neutron spectrum particularly at the very low energy or epithermal range. Owing to the very high values of the RBE for chromosomal breakage in the material used, the unavoidable presence of the contaminating gamma rays is not as serious an objection in the present work. The dose figures which have been given in all cases in rep are probably accurate to better than ±15–20 per cent. A more detailed discussion of the fast neutron dosimetry in the cyclotron facility is scheduled for future publication.

REFERENCES

[1] Kirby-Smith, J. S. and Daniels, D. S. *Genetics*, 1953, **38** 375.
[2] Bishop, C. J. *Stain Technol.* 1949, **24** 9.
[3] Conger, A. D. *ibid*, 1953, **28** 289.

CHROMOSOME BREAKAGE BY DIEPOXIDE AND BY X-RAYS

G. Roy Lane

Botany Department, University of Southampton

The effects of X-rays and other ionizing radiations on the meristematic cells of the root of *Vicia faba* have been studied by a number of workers, *e.g.* Darlington and La Cour[1], Ford[2], Thoday[3, 4], Gray and Scholes[5], and Revell[6, 7]. The latter author has made a special study of the effects of di(2 : 3-epoxypropyl) ether and some other radiomimetic substances on *Vicia* root cells whilst McLeish[8] has studied the effects of maleic hydrazide on the same material.

This paper deals with experiments now being carried out with X-rays and radiomimetic substances on the root cells of *Tradescantia*. This material was chosen for the following reasons :

(*1*) The effects of radiation on the haploid pollen cells of this plant have been extensively studied by many workers and of certain radiomimetic substances by Darlington and Koller[9] and recently by Smith and Lofty[10]. Until now, no study has been made of the effects on the diploid root meristem cells of this plant, although it forms adventitious roots freely and the roots can be grown readily in culture under controlled conditions.

(*2*) The chromosome breaks induced by diepoxide in the root cells of *Vicia* occur largely in the demonstrable heterochromatin (Revell[6, 7]). So also do those induced by maleic hydrazide (McLeish[8]). The effects on *Tradescantia* chromosomes, which possess no demonstrable heterochromatin should make an interesting comparison.

(*3*) Chromosome reunion (R″) to form polycentrics and rings is relatively rare in *Vicia* following radiation treatment. This has been related to the presence of heterochromatin by Darlington[11]. Chromosome reunion (R″) probably does not occur at all in the first mitosis after treatment with diepoxide or maleic hydrazide, reunion being entirely between chromatids, *i.e.* of the SR and R′ types. In *Tradescantia* pollen cells, chromosome reunion is extremely common and predominates if treatment is carried out during the first part of the resting phase. Here again the effects of diepoxide and maleic hydrazide on the root cells of *Tradescantia* should make an interesting comparison.

(*4*) Revell[12, 13] has postulated a new interpretation of the mode of origin of X-ray and radiomimetic chromosome structural changes, based on his work on *Vicia*. Results from *Tradescantia* in which the chromosomes differ in their chromatic make up and in their properties of reunion may be expected to assist in assessing the alternative interpretations.

EXPERIMENTAL

Details of the culture and technique used in this work will be published elsewhere. The plants used are of a single clone of *Tradescantia paludosa*.

Roots are induced by appropriate pruning and grown in culture at controlled temperature (22° C.) and illumination (16 hr/day) in aerated culture vessels.

Treatment with diepoxide solution is carried out at the controlled temperature and for the last 5 hours before fixation the roots are grown in 0·05 per cent colchicine, also at the controlled temperature. Treatment with X-rays is carried out as nearly as possible at the temperature of culture.

This material does not respond to the usual maceration methods used in making Feulgen squash preparations after aqueous fixation. New methods of maceration had to be worked out. The root tips are fixed in La Cour's 2 BD osmic fixative, bleached in hydrogen peroxide and placed in an extract of *Bacterium aroideae* for 3 to 5 days, a method suggested by the work of Wood[14]. This treatment greatly assists separation of the cells during squashing and does not interfere with subsequent staining. The root tips are stained by the Feulgen method, hydrolysis being carried on for 20 minutes and staining for one hour.

The chromosomes of *Tradescantia paludosa* are so well known that no detailed description is necessary. There are six pairs of chromosomes in the somatic cells all with approximately median centromeres, consequently the individual chromosomes are not easy to distinguish from one another. One pair has arms of approximately equal lengths and is fairly easily picked out. In the other five pairs the length of the shorter arm is from about one-half to four-fifths of that of the longer arm (*Figure 2*). There is no demonstrable heterochromatin and no distinguishable nucleolar constriction.

The material was given X-ray doses of 50 r–100 r (intensity 27 r/min. approx.) and fixed at various intervals from 6 hours to 4 days after treatment.

Diepoxide treatment was given with an M/2,000 solution for 10, 20, 30 and 40 minutes. Material was fixed at intervals from 12 to 78 hours after treatment.

RESULTS

X-rays—The effects of X-rays are similar to those shown in the pollen cells. There is, as is to be expected, a complete overlap of the different effects obtained by earlier or later treatment of the pollen grains. This is due to the lack of any synchronization in the development of the cells and to the varying rates of development.

At 36 hours after 200 r (22° C.) most of the reunion is of the R′ or SR type but dicentrics and centric rings (R″) and minutes (m) also appear in considerable numbers together with X_2 cells showing breaks and micronuclei. Dicentrics and rings survive in X_2 mitoses and at 3 days paired cells showing equal-sized dicentrics or rings are quite frequent.

Diepoxide—After a treatment of 20 minutes with M/2,000 diepoxide the breakage is quantitatively approximately the same as that obtained by Revell[6, 7] with *Vicia* using the same dose (*Table 1* and *Figure 1*). Reunion is entirely between chromatids, either chromatid reunion (B′) or sister reunion (SR), at all the time intervals (*Figure 2*). No dicentrics or rings (R″) like those observed after X-ray treatment were observed even at the longest time interval of 60 hours.

Approximately half of the changes are simple chromosome breaks, the majority of which show sister reunion in both centric and acentric fragments.

Table I.—Frequency of breaks and interchanges after treatment with di-(2 : 3-epoxypropyl) ether, M/2,000 for 20 minutes, and M/2,000 for 30 minutes

Treatment	Time interval in hours	Total cells	Damaged cells	B″	Single B	Chromatid interchanges			Total interchanges	Total B	Total B	Ratio of interchanges to B″
						Between chromosomes	Within chromosomes	Tri-radials				
*M/2,000 20 min	18	100	34	20	5	3	4	0	7	19	39	0·35
M/2,000, 20 min	22	100	40	28	9	9	5	0	14	37	71	0·50
M/2,000, 20 min	36	100	54	34	12	18	5	1	24	60	94	0·71
M/2,000 20 min	48	100	65	68	7	14	7	2	23	53	121	0·34
	Totals	400	193	150	33	44	21	3	68	169	325	0·47
M/2,000, 30 min	31	100	52	30	11	27	13	4	44	99	128	1·47
M/2,000, 30 min	52	100	85	74	20	65	33	6	104	228	302	1·41
	Totals	200	137	104	31	92	46	10	148	327	430	1·42

* 50 cells from each of 2 slides

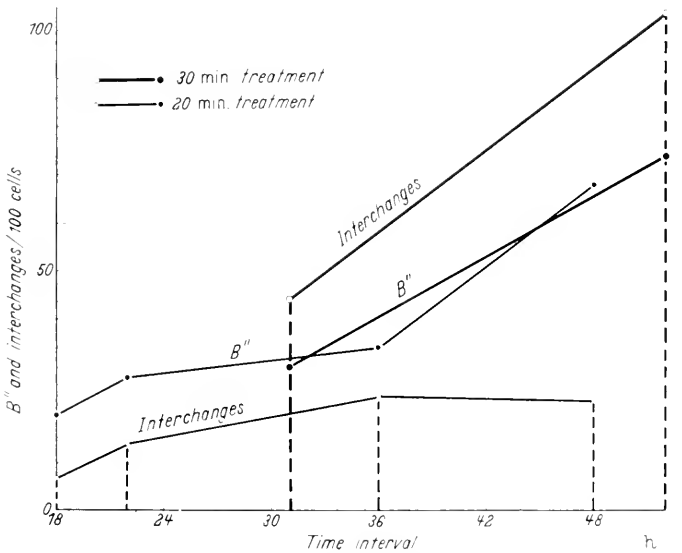


Figure 1. Frequency of breaks and interchanges after treatment with di(2 : 3-epoxypropyl) ether M/2,000 for 20 minutes and M/2,000 for 30 minutes

A few single chromatid breaks occur, but most of the remaining structural changes are chromatid interchanges, either symmetrical or asymmetrical (in about equal quantity). A few triradial figures appear with accompanying centric or acentric fragments which may or may not show sister reunion (*Figure 3*).

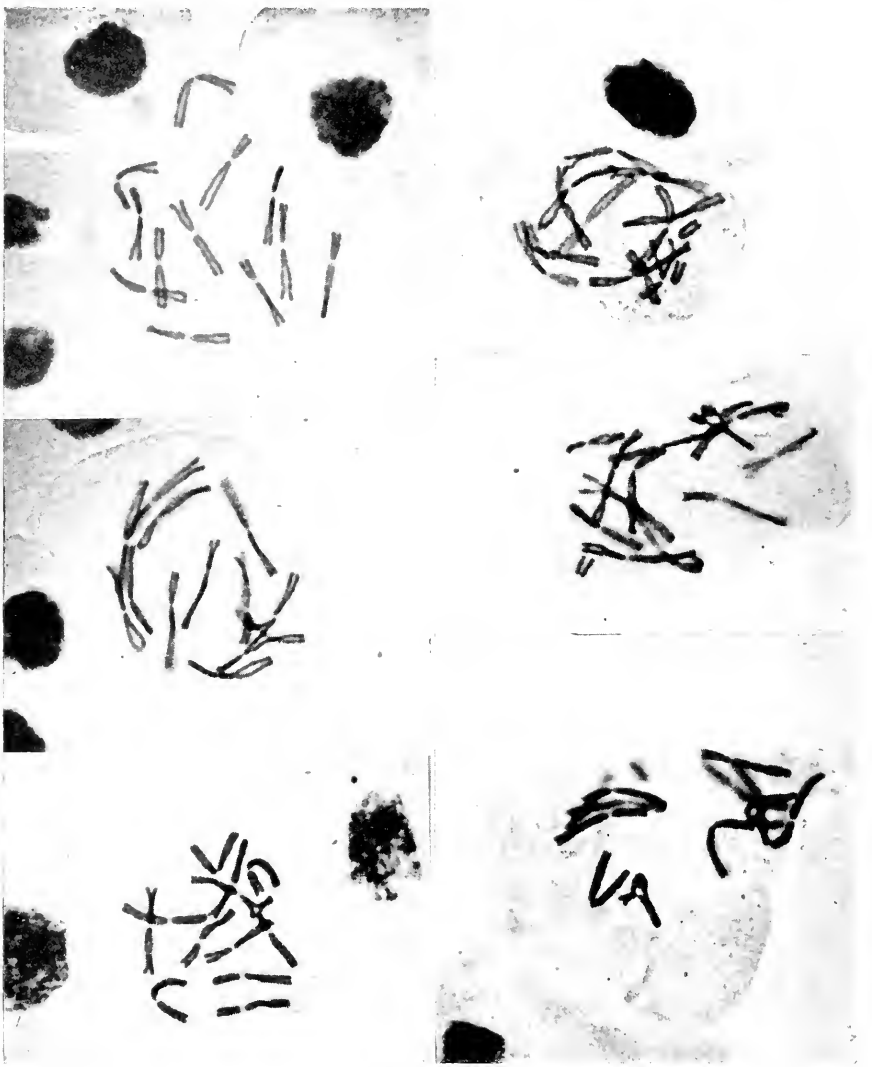


Figure 2

Approximately one-third of the interchanges are within the same chromosome, frequently between the two arms (*Figure 2*).

With treatment of 30 minutes the proportion of interchanges appears to be considerably higher, the ratio of chromatid interchanges to chromosome breaks rises to 1·4 as compared with 0·47 for the lower dose (*Table 1* and *Figure 1*). This is at variance with the results with *Vicia* where Revell[6, 7]

found that the ratio of interchanges to B″ remained at between 0·41 and 0·52 at all doses tried. This high ratio of 1·4 is consistent in the two time intervals analysed (31 and 52 hours), but in view of the relatively small

Figure 3

sample (200 cells) needs confirmation by further experiment and analysis.

Another difference in the interchanges induced by diepoxide in *Tradescantia* roots is that there appears to be no tendency towards interchange

between homologous chromosomes at apparently homologous points, as found by Revell[6, 7] in *Vicia*. As regards localization of breaks in general, this is difficult to estimate in *Tradescantia* owing to the close similarity in form between the different chromosomes. Further analysis should enable me to make a decision on this important point.

A few minute chromosome fragments (m) are found after all treatments, often associated with chromatids of unequal length or chromatid breaks. These are probably mainly minute interchanges of the type figured by

Table II.—Frequency of mitoses and of micronuclei after treatment with M/2,000 di-(2 : 3-epoxypropyl) ether. Roots grown in 0·05 per cent colchicine for 5 hours before fixation. Two slides for each treatment

Treatment	*Time interval in hours*	*Total no. of cells*	*Percentage mitoses*	*Percentage resting cells with micronuclei*	*Micronuclei per 100 resting cells*
None	—	1,298	7·30	0·25	0·25
M/2,000, 10 min	48	1,412	10·20	5·37	5·92
M/2,000, 20 min	12	1,282	5·85	0·58	0·75
,,	18	1,240	2·58	0·66	0·66
,,	24	1,249	1·68	2·28	2·62
,,	30	1,306	4·90	3·38	3·90
,,	36	1,398	6·93	7·75	8·55
,,	42	1,402	7·45	7·55	9·10
,,	48	1,417	7·35	8·65	9·55
,,	54	1,044	7·27	13·80	15·60
,,	60	1,647	11·20	18·00	20·50
M/2,000, 30 min	22	1,221	0·49	1·24	1·24
,,	31	1,250	2·16	1·88	2·13
,,	52	1,735	4·85	16·70	25·40
,,	78	1,411	5·38	10·10	11·60
M/2,000, 40 min	22	1,214	0·33	0·83	1·08

Revell[6] in relation to his explanation of the mode of origin of the chromosome breaks with sister reunion.

An occasional dicentric chromosome appears in the 48-hr sample from 10-min treatment. At this time-interval after the smaller dose no primary breakage is seen. Most of the mitoses are evidently of the second cell generation after treatment (T_2). These dicentrics are accompanied by a micronucleus in the same or neighbouring mitosis, and clearly have their origin in asymmetric interchanges, the micronucleus representing the acentric fragment. Similarly, neighbouring cells in this sample are found with short chromosomes of equal size, the products of separation of centric fragments which have not undergone sister reunion. Sister reunion is frequently absent in the centric fragment when the break is close to the centromere.

An analysis was made of the frequency of mitosis (after the usual colchicine treatment) and of micronuclei in the resting cells at different time intervals after doses of diepoxide (*Table II* and *Figure 4*). This shows that mitosis is suppressed by the diepoxide, the duration of the effect being dependent upon the dose. It can be seen that the frequency of mitosis is reduced from that of untreated samples. Mitosis drops to a minimum at about 24 hours after 20 minutes' treatment and recovers by about 36 hours. It rises still higher right up to 60 hours. After 30 minutes' treatment the recovery is slower, whereas after 10 minutes' treatment the mitotic frequency has already climbed to a high level and the cells in mitosis appear to be already of the

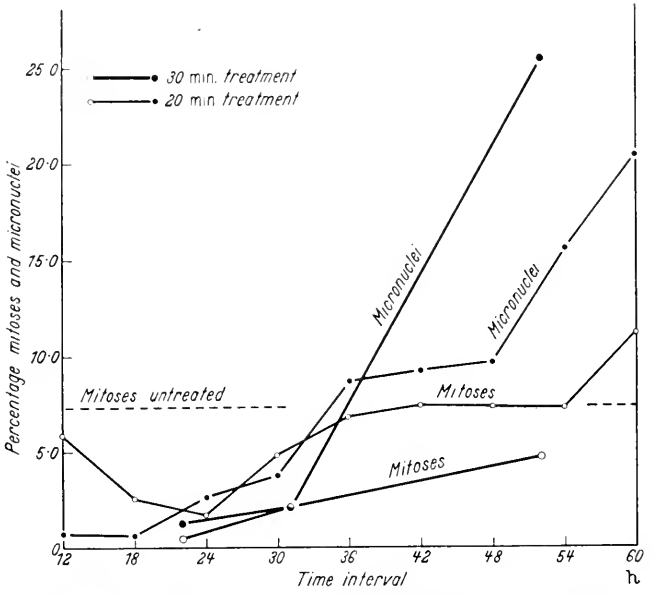


Figure 4. Frequency of mitoses and of micronuclei after treatment with M/2,000 di(2 : 3-epoxypropyl) ether for 20 minutes and 30 minutes

T_2 cell generation. This indicates that after this dose, suppression is of still shorter duration.

The different degree of mitotic suppression with different doses means that it is dangerous as with X-ray effects to draw comparisons between the effects of different doses at one given time period after treatment.

The data obtained on the frequency of micronuclei after treatment also show the effect of dose in retarding mitosis. At 30 hours the frequency is less than after the larger dose.

SURVEY

The data so far obtained on the effects of diepoxide on the meristematic root cells of *Tradescantia* raise many questions to which there is as yet no certain answer. It is useful, however, to consider these questions in order to clarify the direction which future investigation should take.

One thing is clear, that in spite of the lack of demonstrable heterochromatin, di(2 : 3-epoxypropyl) ether is as effective in causing structural change in the chromosomes of *Tradescantia* as it is in *Vicia*. The changes in *Vicia*, though not confined to the demonstrable heterochromatic segments, occur largely in them. It may be that changes outside these segments occur in small undetectable segments of heterochromatin or at points which have certain properties in common with heterochromatin. This of course raises the whole question of the definition of heterochromatin.

The chromosomes of *Tradescantia* are broken at many scattered locations. Acentrics of certain lengths seem to predominate, but it has not yet been possible to determine with certainty whether any particular points on the chromosomes are sensitive to diepoxide.

As Revell[6] has pointed out, there is considerable evidence that the heterochromatic regions of *Vicia* associate with one another in the chromocentres during the resting phase. These are just the regions in which, in the main, breakage by diepoxide occurs. It is probable that no such association between special regions sensitive to diepoxide occurs in *Tradescantia* where there is no demonstrable heterochromatin and no obvious chromocentres. Consequently it might be expected that interchange between chromosomes (reunion) would occur in a more random manner. My observations suggest that this is indeed the case. There is no such tendency towards homologous chromatid interchange.

Revell[6, 7] lays stress on the tendency for apparently homologous interchange to occur in *Vicia* and compares this phenomenon with chiasma formation. Clearly, reunion or interchange can take place only at points of association. In meiosis, and to a certain extent in mitosis, in *Vicia* these points of association are not random. Consequently, interchange will also tend to occur between certain restricted regions of the chromosomes which are normally associated rather than between points of chance association.

This might be held to account for the lack of an exponential relation between the frequency of interchanges and the dose. There are indications in my data that there may be such an exponential relationship in *Tradescantia*. If both chromosome breaks (B″) and interchanges show a linear relationship with dose, their ratio should remain constant at all doses provided that the threshold dose, the origin of the curves, is the same for both (and there is no reason to believe that it is not). This is the case in *Vicia* according to Revell[6, 7]. In *Tradescantia* the ratio rises from 0·5 at 20 minutes' exposure to approximately 1·4 at 30 minutes' exposure.

The apparent great difference with a relatively small change of dose may not truly reflect the relationship. Firstly, the samples are relatively small and secondly, the actual dose may change to a much greater degree than is suggested by the time values of the treatment. I have now reason to believe that penetration into the root in *Tradescantia* may be much slower than in *Vicia*. Certainly fixatives penetrate more slowly. It is therefore planned to carry out experiments with longer treatment at lower concentrations.

Revell[12] has interpreted all types of aberration including all the chromosome breaks with sister reunion (and presumably those without) as chromatid interchanges. Certainly there is considerable evidence that some of

the chromosome breaks have their origin in interchange of the type he describes. This may well account for the greater than linear relation between chromosome breaks with sister reunion (so-called isochromatid breaks) and the dose, reported by some workers in irradiated material. I have observed an apparent chromosome break with sister reunion of both centric and acentric fragments with the two fragments interlocked by the reunion. This clearly cannot be the result of failure of reproduction of broken chromosome ends.

However, other evidence suggests that only a fraction of the sister reunions are the result of minute interchange at loops. Firstly, if all B″ are the result of interchange at loops, one would expect the frequency of the three alternative types of detectable interchange to be approximately equal. In fact, the B″ with sister reunion far outnumber the other types (*i.e.* minutes (m) with or without B′ and unequal or looped chromatids). Secondly, after treatment with diepoxide, breaks cutting straight across both chromatids without sister reunion in the centric or acentric fragment occur fairly frequently. In addition to these clear breaks, constrictions or lesions occur either on both chromatids at apparently the same locus or on one chromatid opposite a chromatid break. These constrictions have been observed by McLeish[8] in *Vicia* after treatment with maleic hydrazide and by many workers in irradiated material. They are usually interpreted as restitution and may be considered as evidence supporting the view that diepoxide and other radiomimetic substances produce direct chromosome breakage by local inhibition of chromosome synthesis, and that the aberration is not always an exchange as Revell[12] has suggested.

Finally, Revell's suggestion that the diepoxide effects are to be considered as a kind of artificially induced chiasma formation is a tempting one to follow, at least in regard to the clear cases of interchange. The similarities between certain types of interchange and the chiasma are obvious, and they both occur at a restricted period in the cell cycle, probably at or near the time of chromosome reduplication. However, it should not be forgotten that the differences are many, a most significant one being that the chiasma is never an asymmetric interchange. This suggests a different mechanism for the two types of chromatid exchange, the chiasma and the artificially induced interchange. On the other hand, the complete absence of chromosome reunion (R″), even with treatment during early resting phase, suggests that breakage occurs near to the time of reduplication at whatever part of the resting phase the treatment is given.

SUMMARY

(*1*) Di(2 : 3-epoxypropyl) ether causes chromosome structural changes in the meristematic cells of the roots of *Tradescantia paludosa*. These changes are similar to those reported in *Vicia faba* root after similar treatment. This substance also has a delaying effect on mitosis, the effect increasing with dose.

(*2*) The chromosome breaks and reunions do not appear to be localized as in *Vicia* nor is there the same tendency towards apparently homologous chromatid interchange between homologous chromosomes. From the limited data so far obtained, interchange appears to be favoured by higher dose.

(3) Some of the apparent chromosome breaks have their origin in chromatid interchange.

(4) Dicentrics and centric rings are frequent after X-ray treatment, but no evidence was found of chromosome reunion (R″) after treatment with diepoxide even at the long time-intervals.

ACKNOWLEDGEMENTS

I am indebted to Professor P. C. Koller and to Dr. S. H. Revell for supplying me with samples of diepoxide, to Dr. J. R. Clarkson for irradiating material and to Mrs. J. Paton for invaluable assistance. This investigation is supported by grants from the British Empire Cancer Campaign.

REFERENCES

[1] Darlington, C. D. and La Cour, L. F. *J. Genet.* 1945, **46** 180.
[2] Ford, C. E. Proc. 8th Intern. Cong. Genet., *Heriditas* Suppl. 1949, 570.
[3] Thoday, J. M. Proc. 8th Intern. Cong. Genet., *ibid*, 1949, 672.
[4] Thoday, J. M. *Brit. J. Radiol.* 1951, **24** 572 and 622.
[5] Gray, L. H. and Scholes, M. E. *ibid*, 1951, **24** 82.
[6] Revell, S. H. *Brit. Emp. Cancer Camp. Ann. Rep.* 1953, **30** 42.
[7] Revell, S. H. Symposium on Chromosome Breakage, *Heredity* Suppl. 1953, 107.
[8] McLeish, J. Symposium on Chromosome Breakage, *ibid*, 1953, 125.
[9] Darlington, C. D. and Koller, P. C. *Heredity*, 1947, **1** 187.
[10] Smith, H. H. and Lofty, T. A. *Amer. J. Bot.* 1954, **41** 589.
[11] Darlington, C. D. *John Innes Hort. Inst. Ann. Rep.* 1952, **42** 26.
[12] Revell, S. H. *Brit. Emp. Cancer Camp. Ann. Rep.* 1954, **31** 33.
[13] Revell, S. H. This Symposium.
[14] Wood, R. K. S. *Nature, Lond.* 1951, **167** 771.

THE RELATIONSHIP BETWEEN CHROMOSOME FRAGMENTATION AND REJOINING IN *TRILLIUM ERECTUM* FOLLOWING THERMAL NEUTRON AND X-IRRADIATION

ELEANOR E. DESCHNER*

Brookhaven National Laboratory, Biology Dept., Upton, Long Island, New York

AN investigation into one of the factors affecting the degree of radio-sensitivity of the nuclear cycle in *Trillium erectum* was reported by SPARROW and MALDAWER[1] in 1950. Evidence was presented by them indicating that greater rejoining occurred at a relatively radio-resistant stage, interphase, accounting for a part of the difference in the total visible chromosome fragmentation while a small amount of rejoining occurred after X-irradiation at first meiotic metaphase, a highly sensitive stage. However, the increased number of reunions at the apparently resistant interphase stage was not sufficient to explain the decrease in total visible fragmentation.

A further study along these lines has been carried out in an effort to determine if this inverse relationship between breakage and rejoining would hold true at other stages of meiosis and if, in general, the frequency of reunions be closely associated with the degree of sensitivity. In addition, a similar study was undertaken using thermal neutron irradiation in an effort to learn how the two radiations differ, if at all, in their effect on the processes of breakage and reunion[2].

Propiono-carmine smears of anthers irradiated at known stages of microsporogenesis were examined at first anaphase and microspore anaphase in order to pick up both immediately visible and delayed breakage. Fragments were scored as an index of the breakage sensitivity and dicentric and ring chromosomes were used as an index of the degree of reunion.

Data collected at first anaphase as a result of 50 r X-irradiation at zygotene, pachytene, and diplotene indicates that while no statistically significant difference in breakage sensitivity existed, the amount of recombination of broken ends was significantly higher at diplotene. Cells irradiated at diplotene displayed a higher breakage sensitivity at microspore anaphase than at first anaphase although the same degree of rejoining was found to occur at both stages. The combined data at first anaphase and microspore anaphase revealed that a significantly greater degree of fragmentation and reunion occurred after diplotene irradiation than after pachytene or zygotene irradiation.

In comparing the relative sensitivity of diplotene and first metaphase, an analysis of variance showed that there was no significant difference in

* Present address : Radiobiological Research Dept., Mount Vernon Hospital, Northwood, Middlesex, England.

fragmentation occurring at microspore anaphase between stages nor was there a difference in the number of reunions taking place as a result of diplotene and first metaphase irradiation. However, when the total amount of reunion occurring as a result of diplotene irradiation was calculated, that is, the combined rejoining at first anaphase and microspore anaphase, it was found that rejoining was significantly higher in cells irradiated at diplotene than in cells irradiated at first metaphase, although both stages were equally sensitive to breakage. The combined data of first anaphase and microspore anaphase also indicates that rejoining calculated as a function of fragmentation occurs 2·3 times more frequently after diplotene and interphase than after first metaphase X-irradiation.

Ratios formed between the number of fragments and rejoins observed for each stage irradiated with thermal neutrons and X-rays indicate an over-all similarity in response to both types of radiation. Diplotene chromosomes are comparably sensitive and appear to have a greater ability to rejoin than chromosomes at other stages while first metaphase irradiated material consistently shows high sensitivity and low rejoining capacity.

In conclusion, no consistent relationship has been found to exist between the rejoining capacity and degrees of sensitivity exhibited by chromosomes of *Trillium erectum* exposed to irradiation at various stages of microsporogenesis. Consideration of the data seems to indicate that the processes of breakage and reunion may be controlled by two independent mechanisms or alternately that they are two processes whose relationship in time is obscured because while they are closely associated, both phenomena do not proceed at the same rate. In addition, it would seem that not only are there differences in radio-sensitivity of various stages to chromosome breakage, as for example, first metaphase and interphase, but also, stages similar in sensitivity to fragmentation, like first metaphase and diplotene, differ with respect to the degree of rejoining which follows.

REFERENCES

[1] Sparrow, A. H. and Maldawer, M. *Proc. Nat. Acad. Sci.* 1950, **36** 636–643.
[2] Deschner, Eleanor E. and Sparrow, A. H. *Genetics*, in press.

DISCUSSION ON PAPERS BY REVELL, SWANSON, KIRBY-SMITH, LANE AND DESCHNER

L. H. Gray : '*Contact first' hypothesis*—I think it is extremely interesting that Revell has felt compelled to return to the view that exchanges only occur between chromatids at points at which they lie in contact, in order to account for his observations on the aberrations induced in *Vicia* chromosomes by diepoxide. Over twenty years ago this view was advanced in connection with the production of exchanges by ionizing radiation, and so far as I know, the alternative hypothesis that exchanges arise by union between chromosome ends which result from independently produced breaks was adopted in preference to the 'contact first' hypothesis when it was shown that exchanges resulting from the irradiation of *Drosophila* sperm increase as the 3/2 power of the dose. Subsequent studies with *Tradescantia*, *Vicia* and many other materials have, so far as I know, always yielded the result that exchanges induced by X- or gamma rays increase more rapidly than the first power of the dose, and often as the square of the dose.

In one respect the two hypotheses do not differ very greatly, for it was found necessary by Lea and Catcheside, in developing quantitatively the generally accepted view of interchange formation, to assume that in *Tradescantia* exchanges rarely take place between breaks which are more than 1 micron apart at the time of their formation, and it might become a rather fine point to distinguish between this and actual contact. There is, however, one big and crucial difference, as Revell has pointed out, namely, that while the usually accepted hypothesis supposes that a chromosome may be broken anywhere along its length, Revell supposes that breakage is secondary to exchange and can only occur at places at which chromatids are already in contact. These are two hypotheses between which it should not be too difficult to decide by experiment.

It is clear, in the case of both chemically induced breaks and those induced by ionizing agents, that many steps intervene between the acts of initiation and the definitive formation of the aberrations, and in my view we already know of a number of differences between aberrations induced by diepoxide and those induced by ionizing radiation which make it clear that the steps cannot be identically the same in the two cases. This is shown (*a*) by the selective localization of aberrations induced by diepoxide (as well as by some other chemical agents) within restricted regions of the chromosome, by contrast with the more or less random distribution of radiation-induced aberrations, and (*b*) by the fact that in the case of *Vicia* meristem cells studied by Revell, the chemical agent is most effective when given during the first half of interphase, whereas ionizing radiation is most effective in the second half of interphase. I would suggest, therefore, that the hypothesis which we adopt as to the mode of action of each agent should be that which best accommodates the facts known to us about the aberrations produced by that particular agent, without any attempt to arrive at a single hypothesis which would be applicable to all agents.

For myself, I find no compelling reason at the present time to abandon the accepted view of aberration production by ionizing radiations. In the case of diepoxide, Revell has been led by the high proportion of aberrations which involve the heterochromatic regions of the long M chromosome, and from the approximate linear relation between exchanges and dose, to adopt the 'contact first' hypothesis for the chemical agent. By the same token, the random distribution of breaks and the square dose law for exchange production indicate a two-particle process for X- and gamma radiation. I agree with Revell that the two electrons might not initiate breaks in two different chromosomes ; it might be that even when two chromatids are lying in contact an amount of energy requiring the co-operative action of at

least two electrons is necessary to provide a disturbance adequate to initiate an exchange. I prefer the alternative view, which I think is supported by the dose relations observed with neutrons and α-particles. At not too high doses, exchanges which follow a square-law dose with X-rays are linearly related to dose when produced by neutrons, but even so show a small square-law component at high neutron doses. Again, they are linearly related to dose with all doses of α-radiation so far studied. Having regard to the number of electrons, protons and α-particles by which a cell is traversed when exposed to a given dose of X-, neutron- or α-radiation, the dose relations observed with these three radiations are just those to be expected if exchanges result from two independently produced breaks. Moreover, in the case of α-radiation, such low doses have been used that most of the aberrations observed have been produced in cells whose nuclei have been traversed by only one α-particle. It would seem difficult to account for the high efficiency with which α-particles produce aberrations if the induction of aberrations is restricted to occasions on which a particle passes through or in the immediate vicinity of a region in which two chromatid threads are lying in contact.

We all exercise some degree of selection, conforming our hypothesis to fit the facts which impress us most, and it may be that I am placing too much emphasis on the quantitative aspects of the dose relations observed with different types of radiation. The fact that Revell has adopted the 'contact first' hypothesis for chromosome breakage cannot but result in a critical re-examination of the ionizing radiation data, and will, we hope, lead to further experiments designed to decide between the alternative hypotheses.

R.B.E. FOR X-RAYS OF DIFFERENT TUBE VOLTAGES

In connection with the papers by Kirby-Smith and Daniels, and by Swanson, I would like to add a few remarks concerning the influence of kilovoltage and filtration on the yield of aberrations produced per unit dose. On a number of occasions (LEA[1], GRAY[2], SPIERS[3]) attention has been drawn to the fact that the mean energy of the secondary electrons generated in tissue remains almost constant over the entire range of photon energies from 25 kV to 100 kV, and that on this account it would be surprising if biological efficiency showed any appreciable dependence on X-ray tube kilovoltage over the range 50–200 kV. As is well known, the constancy of the mean electron energy arises from a fortuitous balance between the varying proportions of the more energetic photoelectrons and the low energy recoil electrons, and I thought it would be of interest to see how far this balance might be disturbed in the case of a phenomenon such as the production of chromosome structural damage for which we have reason to believe that the contribution of the very slow electrons should be heavily weighted. From a consideration of the relative effectiveness with which X-rays, neutrons and α-particles produce chromosome aberrations in *Tradescantia* microspores, Lea concluded that in order to break a chromatid thread an electron must (*a*) have sufficient residual range (0·1 μ) to traverse the thread and (*b*) dissipate at least 0·5 keV of energy in crossing the thread. (Lea[1], p. 276). In terms of the energy of the electron as it enters the chromatid thread, this limits chromatid breakage to electrons having energies between about 1·6 keV and 2·8 keV. Having applications to radiation chemistry as well as to chromosome breakage in mind at the time I made my calculations, I have assigned unit efficiency either to

(*a*) electrons having energy of 0·5 keV (Column I of the Table); or
(*b*) electrons having energy between 0·5 keV and 3 keV. (Column II of the Table)

and zero efficiency to all contributions to the total dose from electrons whose energies lie outside the assigned limits.

It will be seen that the second postulate resembles that of Lea but is not identical with it.

Some typical results are given in the table. Allowance has been made for :

(*1*) The low energy electrons (δ-rays) produced by the more energetic electrons.

(*2*) The relative contributions of photoelectric absorption and Compton scattering to the total tissue dose, and the distribution of energy among the recoil electrons.

(*3*) The spectral distribution of photon energy from tubes operated at particular kilovoltages and filtrations, where such data were available. Unfortunately no such data are available at present for the radiations used by Kirby-Smith and Daniels. The figures given in columns I and II for lightly and heavily filtered 250 kvp X-radiation therefore refer to the equivalent monochromatic X-radiation.

On the basis of either postulate, a significant variation of biological efficiency with X-ray quality in the range 50–200 kV is theoretically to be expected. In the case of postulate (*a*) the biological efficiency falls steeply from unity at 0·5 keV to a local minimum value of 0·135 at 29 keV, rises to a local maximum of 0·183 at

Table I.—Relative Effectiveness of X- and Gamma Radiation

Calculated values of τ_i compared with experimental data of Kirby-Smith and Daniels. (Chromosome Structural Damage)
Column I values of τ_i for $Q=0{\cdot}5$ keV
Column II values of τ_i for $0{\cdot}5<Q<3{\cdot}0$ keV

Radiation		*Relative Efficiency*		*Experiment*
Description	*Specification*	*I*	*II*	*K-S and D*
Cu_K characteristic	8 keV	1·30	2·04	
Mo_K characteristic	17·3 keV	1·05	1·32	
90 kvp	Spectrum	1·06	1·18	
200 kvp				
0·5 mm Cu filter	Spectrum	1·24	1·52	
250 kvp	≡62 keV			
3 mm Al filter	(H.V.L.=0·5 mm Cu)	1·23	1·55	1·33
220 kvp				
Thoraeus filter	Spectrum	1·15	1·31	
250 kvp	=206 keV			
4 mm Cu filter	≡(H.V.L.=4 mm Cu)	1·00	1·00	1·00
^{60}Co gamma rays	Spectrum	0·57	0·49	0·67

85 keV, and falls to 0·077 for ^{60}Co gamma rays. In the case of postulate (*b*) the minimum value is 0·242 at 34 keV, the maximum is 0·40 at 76 keV, and the ^{60}Co gamma ray value is 0·115. Both postulates give a computed ratio of X-ray to gamma ray efficiency somewhat greater than is observed. It is interesting to note, however, that the observed difference of 33 per cent in biological efficiency as between 250 kvp filtered by 3 mm Al and the same kilovoltage filtered by 4 mm Cu is to be anticipated in terms of postulate (*b*). Column II of the table in fact shows a computed difference in efficiency distinctly greater than that which was observed, but the computed ratio would probably be somewhat reduced if allowance were made for the spectral distribution of the two X-ray beams.

S. H. Revell : I quite agree with Gray that the exchange hypothesis which I have suggested is in one respect very like the version of the breakage-and-reunion hypothesis evolved by Catcheside and Lea. I also think that there is probably no point in spending too much time considering whether or not this exchange hypothesis

conforms exactly with the 'contact' hypothesis visualized by earlier workers. It is much more important, as well as more interesting, to try to find out what actually happens.

Again, it is certainly essential, as Gray says, to discover whether the independently induced events, which are usually involved in each X-ray *inter*change, can occur anywhere on the 'chromosome' or only at loci in the defined associations. I think, however, that it is also very important to recognize that this need not be just a question of whether breakage is primary or secondary to exchange initiation, because these events might not be breaks at all. The conclusion that breakage has to happen at some stage in exchange induction only follows from the assumption that the undoubtedly permanent genetic *pattern* is actually maintained as a permanent *structure*.

I also agree that the earlier stages in the actions of ionizing radiations and, say, di-(2 : 3-epoxypropyl) ether must be different ; but the 'chromatid' aberrations which finally result from these two types of treatment are qualitatively indistinguishable, at least so far as I can see ; and therefore I prefer to think that the last stage of both actions is probably the same. It is, of course, quite true that 'chromatid' changes produced in these two different ways can be distinguished statistically ; the radiation changes are more nearly random in distribution and they show a much higher proportion of 'open ends' (or 'imperfect exchanges') than do the diepoxide changes ; but I do not think that these quantitative differences are by themselves sufficient reason for supposing that the two agencies are inducing exactly the same types of aberrations by two entirely different mechanisms.

G. Östergren : Our studies on chromosome breakage in the onion by means of coumarin suggest strongly that the figures which look like half-chromatid or part-chromatid changes may in reality be ordinary full chromatid changes that are masked by the presence of matrix. The reason for this opinion is the fact that in the next division we get practically exclusively chromosome type changes. Furthermore, the presence in the X_2 cells of induced constrictions or attached fragments, occurring in both sister chromatids at the same locus, suggests to us that the treatment has induced mutation to a labile state of some loci. These have reproduced to give two labile sister loci which break during a critical period in the prophase of the X_2 division (Östergren and Wakonig[4]). It seems to me that similar effects may occur in Revell's diepoxide treatments. The main effect of his treatment may be mutations to a labile state occurring in early resting stage. After that follows reproduction to give pairs of labile loci, one in each chromatid. Later on, during a critical period in early prophase, these labile loci break and the breaks are recombined. As breakage and reunion often occur in chromosomes having a matrix, many of the changes are masked to subchromatid changes, although in reality they may be of full chromatid type. It is conceivable that besides false sub-chromatid changes there may also exist some true changes of this kind. Some of the labile loci suggested by us may survive one or more mitoses and cause an effect of delayed breakage (our observations on coumarin indicate that this may be the case).

J. A. V. Butler : It would appear that the time for the explanation of these chromosome breakage effects in terms of molecular structure has not yet arrived. However, there are some simple facts which have to be allowed for. The chromosome is a large structure, *e.g.* the rat chromosome contains 10^5 particles of DNA. According to the genetical results these must be united in a linear order (or multiples of a linear order, as there may be multiple strands). How are they joined together ? The chromosomes also contain proteins (histone) and the simplest possibility is that the junctions between DNA particles are made of histone. This would perhaps give a point of weakness at the junction of DNA and histone at which breaks would most easily occur. The junction would presumably be a salt bond between NH_3^+ and PO_4^-. If by the action of radiation either of these terminal groups were removed

the link would be irretrievably broken ; but if the salt bond is merely dissociated it can be remade and in the case of newly synthesized twin chromatids it could be remade in more than one way. This gives a possible explanation of chromosome breaks and rearrangements.

REFERENCES

[1] Lea, D. E. *Actions of Radiations on Living Cells*. London, Cambridge University Press, 1946.
[2] Gray, L. H. *Brit. J. Radiol.* 1947, Suppl. **1** 7.
[3] Spiers, F. W. *Discussions of the Faraday Society*, 1952, **12** 13.
[4] Östergren, G. and Wakonig, G. *Proc. 9th Int. Cong. Genetics, Bellagio*, 1953. Botaniska Notiser, 1954.

STUDIES ON THE EXPERIMENTAL CONTROL OF THE MUTATION PROCESS

Åke Gustafsson

Stockholm 51, Sweden

The work carried out by a Swedish group for theoretical and applied mutation research in plants combines radiobiological aspects, in a broad sense, with radiation chemistry and genetics. A series of organisms, mostly phanerogam species of agricultural character, are included in this research work. The radiations range from the sparsely ionizing gamma rays of a cobalt 60 source, electrons from a synchrotron or from disintegrating phosphorus 32, to X-rays of varying hardness, fast and thermal neutrons, as well as alpha rays and fission fragments. Recently also the effects of ultraviolet, radioactive substances of different kinds and various chemical compounds have been studied. The material treated consists either of dormant or germinating seeds and pollen grains given one-time doses, or living plants irradiated over the entire or part of the vegetation period. Some results of this group work have recently been summarized[1].

The experiments to be reported here concern the course of the mutation process, its control and intentional experimental direction. Generally said mutations, whether spontaneous or induced, are detrimental in character, *i.e.*, in the homozygous condition and in an environment optimal to the mother variety they decrease viability (which is here measured in an exact way). In the single or heterozygous condition some mutations, however, although detrimental when homozygous, increase viability. A very few, say one or two of a thousand genotypical changes, are directly beneficial to the organism, *i.e.* they augment viability both as regards vegetative matter and seed production, also under conditions optimal to the mother strain.

Every organism, variety or species, has its own mode of mutating, depending on its genotypical constitution, chromosome number or population structure. Some mutations are very rare, others more frequent ; some genes are highly stable, others unstable, etc. The statistical or random character of the mutation process argues against a Lamarckian interpretation of evolution, *i.e.* genes do not change owing to environmental influences in a direction parallel to the type of environment. This does not exclude the possibility that we are able of experimentally controlling or even directing the mutation process. Such a state of things was pointed out by Gustafsson[2].

The first task undertaken involved a change of the course of mutation, so that its random character is obliterated. A series of papers were published on this matter by Gustafsson[3] and co-workers. In 1948 Gustafsson and Mac key[4] obtained some data indicating that mutations destroying chlorophyll formation are not the same, statistically seen, after treatments with mustard gas substances as with X-irradiation or spontaneously. This was

further worked out and definitely proved by MAC KEY[5]. He stated the following values of significance for a difference in mutating

between mustards and	X-rays	P = 0·04
,,	,,	,,	neutrons	P = 0·001
,,	,,	,,	^{32}P	P = 0·002

Similarly D'AMATO[6] found that acridin derivatives altered the mutation process, so that albina mutants are not formed, although xantha and viridis mutants are frequent. We are safe in concluding that we now possess the means of changing the spectrum of the mutation process (KAPLAN[7], MULLER[16]). In the cases mentioned this is the more remarkable since chlorophyll mutations arise after changes in a great many gene loci, say roughly 250–300. Of these about one half presumably cause albinas (125–150) ; somewhat less than one half cause viridis types (100–125) ; less than a tenth, xanthas and albovirides (10–15 respectively) ; the remaining very rare types of mutants comprise according to this method of computation approximately 15–20 loci.

Table I

	Radiation source				
	Cobalt 60 gamma rays	160 MeV protons	180 kV X-rays	Pile neutrons	Radon alpha rays
Per cent rare chlorophyll mutants	6·6	10·3	8·6	12·3	23·1
Sum of mutants	319	639	248	399	65
Ion pairs per μ tissue ..	8	16	100	1,000–3,000 400	3,700

An indication that individual loci may be changed by different physiological treatments before irradiation was afforded in 1940 and 1947 by the induction of the rare so-called alboxantha mutants. These preferably arise when germinating seeds are irradiated. A similar experiment was then made by ANDERSON[8] (cf. EHRENBERG *et al*[9]). He found that in *Escherichia coli* a special mutation was depending for its origin on the oxygen pressure at the time of irradiation.

In recent studies EHRENBERG and ANDERSSON[10] as well as EHRENBERG and SAELAND[11] detected that these rare types of chlorophyll mutations to a certain extent depend on the character of the radiation employed, as visualized in *Table I.*

In the case of these chlorophyll mutants we deal with lethal or semilethal changes of the genotype. The ultimate aim of our experimental work, however, is to induce specific mutations leading to changes of viable or even high-productive character. In fact, we now know in barley how to increase its stiffness of straw—a property of supreme agricultural value—by means of mutation. All so-called erectoides mutations so far studied possess an extremely stiff straw. The anatomical and physiological background of this peculiar characteristic has been worked out by WETTSTEIN[12]. The fifteen or twenty loci, giving erectoid mutations, behave in a similar fashion. Recently EHRENBERG and NYBOM[13] discovered that there is an

increase of the relative frequency of these erectoid mutants with the increase in ionization density of the radiation. In fact, with thermal neutrons and dry seeds they distinctly predominate, although with X-rays they comprise no more than twenty per cent of all viable mutations found in the second generation. If this important finding holds true also in repeated experimentation, we may state that the problem of directed mutation is solved and that man is now, in principle, capable of producing new hereditary factors (genes) at will, beneficial to himself and his interests.

Here it ought to be added that DEMEREC[14] and KAPLAN[15] have presented evidence from microbial genetics and GILES[17] from studies on *neurospora* that such a planned direction of the mutation process is no longer a utopian idea but, in fact, a reality.

REFERENCES

[1] GUSTAFSSON, Å. *et al.* *Acta Agric. Scand.* 1954, **IV** 359.
[2] GUSTAFSSON, Å. *Lunds Univ. Årssler. N. 7, Avd. 2*, 1940, Bd. 36, Nr. 11, 1.
[3] GUSTAFSSON, Å. *Hereditas*, 1947 **33** 1.
[4] GUSTAFSSON, Å. and MAC KEY, J. *Hereditas*, 1948, **34** 371.
[5] MAC KEY, J. *Acta Agric. Scand.* 1954, **IV** 419.
[6] D'AMATO, F. *Caryolgia*, 1950, **3** 211.
[7] KAPLAN, R. W. *Z. Pflanzenzücht*, 1953, **32** 121.
[8] ANDERSON, E. H. *Proc. Nat. Acad. Sci.* 1951, **37** 340.
[9] EHRENBERG, L. *et al.* *Hereditas*, 1953 **39** 493.
[10] EHRENBERG, L. and ANDERSSON, G. *Nature, Lond.* 1954, **173** 1086.
[11] EHRENBERG, L. and SAELAND, E. *J. Nucl. Energy*, 1954, **I** 150.
[12] WETTSTEIN, D. von. *Acta Agric. Scand.* 1954, **IV** 491.
[13] EHRENBERG, L. and NYBOM, N. *Acta Agric. Scand.* 1954, **IV** 396.
[14] DEMEREC, M. *Evolution*, 1953, **7** 43.
[15] KAPLAN, R. W. *Ann. Rev. Microbiol.* 1952, **6** 49.
[16] MULLER, H. J. *Radiation Biology*, 1954, **I** 375–626.
[17] GILES, N. H. *Cold Spring Harbor Symp. Quant. Biol.* 1952, **16** 283.

DISCUSSION

F. H. SOBELS : I would be very much interested to hear Gustafsson's interpretation of this directed mutagenesis. How does he visualize the specific response of certain loci to a particular type of treatment ?

Å. GUSTAFSSON : With regard to what we call the group mutability or the changed spectrum of mutation, described first in barley and *Antirrhinum*, we have considered that certain types of genes giving a parallel pattern of mutation have a characteristic in common, some distinct fine-structure (for instance specific dissociation constants, KAPLAN). The change of cell environment (e.g. by increasing hydration) should lead to an activation of certain types of genes irrespective of their position in the genome.

Some types of mutations, like the viridis chlorophyll mutations in barley, seem to increase in frequency when chromosome disturbances accumulate. Then the viridis type of mutation may, for its mode of origin, be less dependent on changes in individual loci (gene mutations) than on simultaneous changes in several loci (deficiencies or duplications) or on chromosomal rearrangements (weak position effects).

Here, the parallel or directed formation of distinct types of mutation, under the influence of specific chemical or physical mutagens, may be explained by postulating a specific fine-structure common to certain types of genes. The fine-structure is then rearranged or broken down in a parallel manner, for instance by the presence of oxygen at the time of γ- or X-irradiation, by indirect rather than by direct effects, by increased hydration of the cell, or—as the case may be—by densely rather than by sparsely ionizing agents.

THE INFLUENCE OF POST-RADIATION FACTORS ON EFFECTS PRODUCED IN BARLEY

L. Ehrenberg

Institute of Organic Chemistry and Biochemistry, University, Stockholm

As a background for the data to be given, attention is first called to the properties of the barley plant as experimental object. In *Figure 1* the fate of a barley seed is summarized. The dry, dormant seed, which can be stored for a definite time, germinates after sowing, giving rise to a seedling

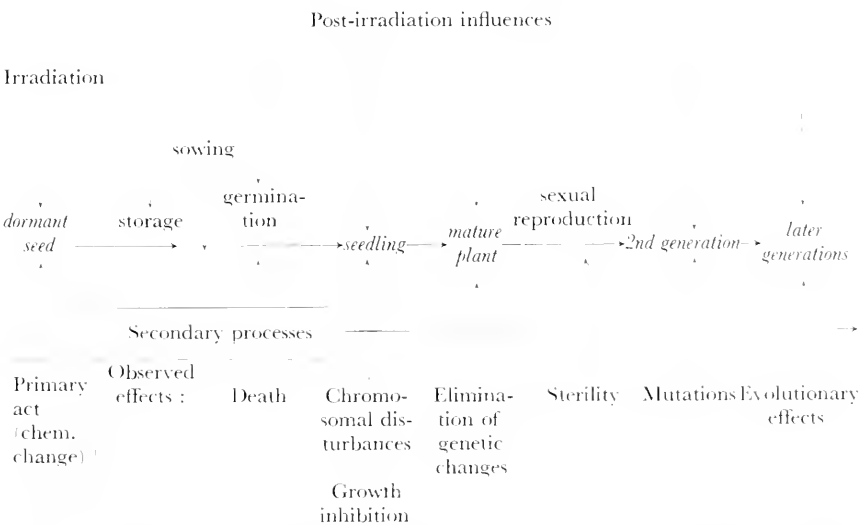


Figure 1. Development of a barley seed, irradiated in the dormant state. Observed effects and possible post-irradiation influences

and, after further growth, to a mature plant. By sexual reproduction the latter gives rise to second generation seeds, which pass through the same ontogenic cycle, and an unlimited number of later generations then follow. In irradiation experiments designed to produce mutations[1], any of these developmental stages can be irradiated. In the present study dormant seeds or seeds at a time just after the start of germination were irradiated.

The (biological) observed effects registered at any moment after the irradiation are due to the chemical changes produced at the moment of irradiation (primary act) and to a series of secondary processes following the primary act. These may be studied (*a*) by a variation of the conditions under which the radiation is performed, and (*b*) by post-irradiation treatment at different times.

When the irradiation conditions are changed (*e.g.* temperature, oxygen tension, presence of protectors), it is often difficult to prove if there is an influence at the moment of energy absorption or if the treatment affects the secondary reactions. To a certain extent the absence of an effect of such a change, when applied immediately after the irradiation, can prove that the factor has an influence on the primary act. The post-irradiation influences provide information about the secondary processes, which may be chemical, biochemical or biological and are referred to here as *post-irradiation effects*, or *after-effects*.

The water content of the seeds at the moment of irradiation influences the radio-sensitivity markedly. For all observed effects[2, 3] of sparsely ionizing radiations (*e.g.* X-rays) the damage (or change) is greater when the water content of the seeds is reduced, and smaller when the water content is increased. In the case of such a densely ionizing radiation as fast neutrons

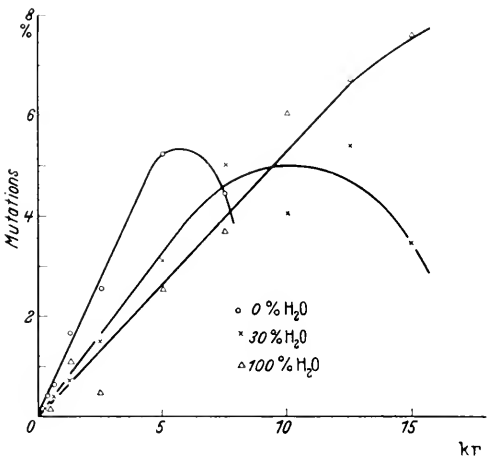


Figure 2. Mutation frequencies in per cent per spike progeny. Dormant seeds equilibrated with air of 0, 30 and 100 per cent relative humidity irradiated with different X-ray doses. Water content of the seeds 8·6, 10·5 and 18·1 per cent, respectively.

(around 8 MeV), the water content of the seeds has no effect on the radiation sensitivity. The growth of seeds equilibrated with dry air is four times more sensitive to X-rays than for seeds equilibrated with air of 100 per cent relative humidity. *Figure 2* illustrates the influence of the water content on the frequencies of chlorophyll-deficient mutations obtained after irradiation with X-rays (180 kV, unfiltered). At low doses the mutation frequencies are linear functions of dose, the greatest deviation being obtained for the lowest water content. For the production of mutations an increased water content protects. At higher doses the curves bend, because of the elimination of genetically changed cells which shows a different dependence on the water content of the seeds than does the mutation frequency. The influence of the water content on the radiation sensitivity of the seeds seems to be restricted to the primary reaction, since changes of the water content, immediately after the irradiation but still before the starting of the germination, have no influence on the observed effect (measurements of seedling growth).

Effect of germination temperature—Figure 3 shows the relative growth of seedlings, after irradiation of seeds of two different water contents with

different doses of X-rays. The seeds were sown at two different temperatures, +25° and +12° C. The damage developed is greater at the lower than at the higher temperature. This is in agreement with GELIN's[4] findings, that the frequency of chromosomal rearrangements in the root tips is about twice as high in the cold as in the warmth. *Figure 3* demonstrates

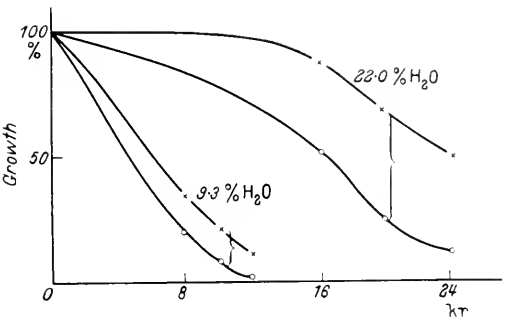


Figure 3. Relative growth of seedlings from seeds with 9·3 and 22·0 per cent water, respectively, treated with X-rays and grown at 25° C. (×) and 12° C. (o).

further what seems to be a general trend in experiments of this type, *viz.* that the temperature influence is smaller when seeds of a low water content are irradiated. In *Figure 4* a similar experiment, comparing temperature

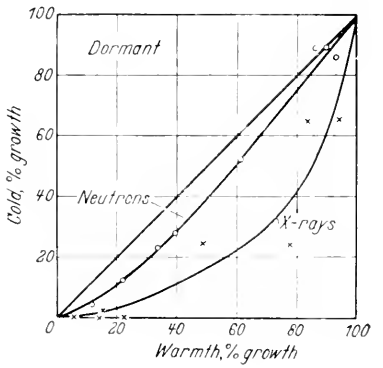


Figure 4. Dormant seeds irradiated with X-rays and neutrons (~ 8 MeV). Relative growth at 12° C. given as function of relative growth at 25° C.

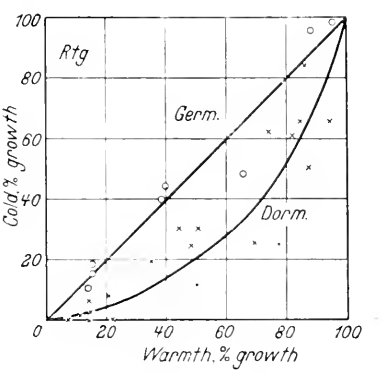


Figure 5. Germinating seeds (pre-soaked for 24 hours in water) and dormant seeds irradiated with different X-ray doses. Relative growth at 12° C. given as function of relative growth at 25° C.

effects on seeds irradiated with X-rays and neutrons, is shown diagrammatically : the relative growth at the lower temperature (+12° C.) is given as a function of the relative growth at the higher temperature (+25° C.). In the case of X-rays, a 25 per cent decrease of the growth in the warmth is found to correspond to as much as 70 per cent decrease in the cold (*i.e.* in accordance with the result given in *Figure 3*). For neutron irradiation, the influence of the germination temperature is appreciably smaller.

In *Figure 5* the influence of the germination temperature has been studied for the case of seeds irradiated with X-rays after the start of the germination

process and there is no influence of the temperature, contrary to the effect obtained with dormant seeds irradiated simultaneously (see *Figure 4*).

Effect of storage after irradiation—In *Figure 6* the relative growth of seeds sown after storage for two weeks at room temperature is given as a function

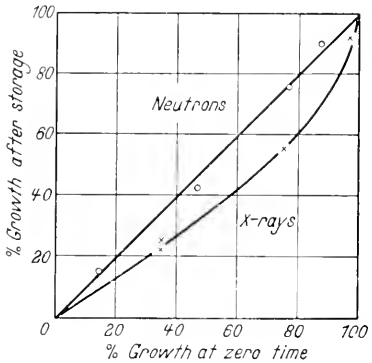


Figure 6. Dormant seeds irradiated with neutrons and X-rays. Relative growth after storage for 6 weeks at 20° C given as function of relative growth obtained after immediate sowing.

of the relative growth of parts of the same irradiated samples sown immediately after irradiation. The storage is found to lead to an increase in damage with X-rays, but not with fast neutrons. The storage effect is relatively greater when seeds of a high water content (*i.e.* equilibrated with moist air) irradiated and when the seeds are stored at about 25° C. than at about 12° C. When germinating seeds are irradiated and afterwards dried again and then stored, no change of the degree of radiation damage is observed.

CONCLUSION

Although the data presented (and summarized in *Table I*) do not suffice to explain the mechanism of radiational action, they provide a foundation for

Table I

State of barley seeds		*Effect on radiation damage (growth inhibition) of:*			
		lowered growth temperature		*storage (esp. high temp.)*	
		X-rays	*neutrons*	*X-rays*	*neutrons*
Dormant, equilibrated to	dry air	small increase	small or none	small increase	small or none
	moist air	large increase		large increase	
Germinating		none	—	none	—

further work on the biochemical level. The relative absence of post-irradiation effects in the case of neutron irradiation indicates that the damage is fixed at the moment of energy absorption, and is in agreement with the finding[2] that the neutron damage is confined chiefly to nuclear material.

In the case of X-irradiation of dormant seeds, especially those irradiated at high humidities, the development of the lesion can be interfered with. The effects of germination temperature and storage are additive. The relative protection obtained at the higher germination temperature, and which can be measured as a lower frequency of chromosomal rearrangements[4] as well as a reduced growth inhibition, cannot be explained only as a repair of chromosome breaks. Earlier results[2] had already indicated cytoplasmic damage by X-rays, as a cause of growth inhibition. The protective effect of an increase in water content seems to be related to the increased respiration rate.

When germinating seeds are irradiated, the damage seems to be caused by quite a different mechanism, and the observed effect cannot be influenced by post-irradiation factors. Compared to the dormant seeds, with a relatively low water content (10–20 per cent) and a low metabolic rate, the germinating seeds contain more water (about 40 per cent) and show a much higher metabolic rate, different enzyme systems being activated.

The difference in the action of neutrons and X-rays is well illustrated by barley seedlings ; these show a higher dry-weight after their growth has been inhibited by neutrons than by X-rays[5]. One cause for this difference might be that neutron damage is primarily located in the roots of the seedling and interferes with water uptake, while the X-rays cause a relatively greater inhibition of the shoot part of the plant.

REFERENCES

[1] Gustafsson, A. This Symposium.
[2] Ehrenberg, L. and Nybom, N. *Acta Agr. Scand.* 1954, **4** 396.
[3] Ehrenberg, L. and Andersson, G. *Nature, Lond.* 1954, **173** 1086.
[4] Gelin, O. *Agri. Hort. Genet.* 1953, **11** 66.
[5] Unpubl. investigation in co-operation with D. v. Wettstein.

PATHOLOGY OF MICE IRRADIATED AFTER INJECTION OF CYSTEAMINE (β MERCAPTOETHYLAMINE)

M. A. Gerebtzoff and Z. M. Bacq

Laboratory of Anatomy and Laboratory of Pathology of Liège University

There is no doubt that cysteamine injected into mice before a lethal irradiation, confers protection. But the site of this action is unknown. In what organs does the protection appear? And does it protect the cells themselves or a factor necessary to their regeneration? In an attempt to obtain an answer to these questions, we have studied the lesions in three radio-sensitive organs (spleen, thymus and intestinal epithelium) and in the liver; the importance of this organ in regeneration has been stressed by Maisin and his co-workers. We have compared C57 mice subjected to 700r with or without an injection of 3mg of cysteamine just before the irradiation. The detailed results are published elsewhere[1]. Only the main observations will be described here. For every organ listed above, we have measured the degeneration due to the primary action of X-rays, as seen 6 hours after irradiation, and the regeneration observed 3 to 6 days later.

(1) Spleen

(a) Degeneration—In the lymph nodes of the spleen, the spread of nuclear pycnosis is smaller in mice treated with cysteamine than in control animals. The relation between the intact surface and the total surface of the nodes is, in the mean, 0·151 for untreated mice and 0·371 for treated mice. The difference is quite significative. *(b) Regeneration*—Four days after irradiation, pycnotic nuclei are very rare in treated animals, but still numerous in some nodes of controls. In these, elimination of degenerated cells and regeneration are slower.

(2) Thymus

(a) Degeneration—The difference in pycnotic areas is not significative. Pycnosis is massive in both groups of mice. *(b) Regeneration*—Count of mitosis for 10 microscopic fields gives 48 mitosis for controls and 60 for treated animals. But a statistic study of the results shows that this difference is not quite significant.

We attribute the uncertain action of cysteamine on thymus to the strong radio-sensitivity of this organ.

(3) Intestine

(a) Degeneration—There is no difference between treated and untreated mice. *(b) Regeneration*—The number of mitotic nuclei is 61 for controls, 83 for treated animals. The difference is significant and regeneration is more intense after an injection of cysteamine.

(4) Liver

In this organ, the lesions are predominantly cytoplasmic. Their intensity seems to be related to the mode of fixation of the tissue. When the liver is fixed in formalin, there is no difference between treated and untreated mice : 6 hours after irradiation, the cytoplasm of hepatic cells shows a few small vacuoles ; 4 days later, the vacuolar state is very pronounced. When it is fixed in formalin and picric acid, there are marked differences between treated animals and controls : in these, the vacuolar state is evident 6 hours after irradiation and very pronounced 4 days later ; in cysteamine injected mice, the hepatic cells seem to remain normal.

The vacuolar state of the cytoplasm suggested lipidic degeneration. But a histochemical study showed that, if some lipids were present at the beginning of the degeneration, they disappeared later on. The lack of vacuolar degeneration in cysteamine-injected animals when the liver is fixed in a liquid containing picric acid may be related to the fact that this acid is an excellent fixation medium for glycogen. It is possible that the linkage between glycogen (and other polysaccharids) and proteins is fragile in the liver of irradiated animals. But this fragility is greater in controls than in cysteamine-treated mice : in these, picric fixation is sufficient to maintain the polysaccharids in the cytoplasm. This observation connects our study with some biochemical researches, particularly those of Fischer.

CONCLUSION

The injection of cysteamine before irradiation effects a direct protection of liver and spleen. It has a strong accelerating action on regeneration in spleen, intestine and probably thymus. Our observations favour the hypothesis that protection by cysteamine is effective at the level of some factors concerned with glycogen metabolism.

REFERENCE

[1] GEREBTZOFF, M. A. and BACQ, Z. M. *Experientia*, 1954, **10** 341.

SOME FACTORS CONTROLLING THE HAEMATOPOIETIC REGENERATION IN WHOLE BODY IRRADIATED RATS*

E. H. Betz

Department of Pathology, University of Liège, Belgium, National Foundation for Scientific Research

Many papers in the field of radiobiology have shown that there is a close relationship between the survival rate of whole body irradiated animals and their ability to regenerate the destroyed haematopoietic tissues. During the last years, it has been shown that different chemicals are able to protect animals against a lethal dose of X-rays. Whereas animals submitted to a lethal whole body irradiation do not show any regeneration of their haematopoietic tissues, there is an extensive regeneration of bone marrow, spleen, lymph nodes and thymus in the animals receiving a protective agent before the lethal irradiation. Quite similar results have been described in animals protected with potassium cyanide (Betz[1] ; Betz and Fruhling[2]), glutathione (Cronkite *et al*[3]), thiourea (Mole[4]), cysteine (Lorenz[5]). An identical stimulation of haematopoiesis is observed in animals protected by spleen or bone marrow homogenates injected after X-irradiation (Jacobson *et al*[6], Lorenz *et al*[7]).

In earlier experiments, we have shown that, in animals submitted to a lethal dose of X-rays, there is an inhibition of haematopoiesis which is independent of the tissular lesions themselves. It is possible, indeed, by grafting the spleen of an irradiated mouse to a normal one to induce an extensive regeneration of myeloid and lymphoid tissues within the graft. Such a regeneration would never have taken place if the splenic tissue had remained within the radiated body till death. From these observations, we may conclude that the regeneration of the haematopoietic tissues depends not only on tissular lesions, but also on the humoral conditions within the body where such tissues are living. These observations led us to investigate different factors which could possibly control the haemopoietic regeneration of irradiated rats (Betz[8]).

The first factor we started to investigate was the influence of the adrenal cortex. It is well known that whole body irradiation stimulates the activity of the adrenal cortex (Patt *et al*[9]). The increased production of adrenocortical hormones could influence the haematopoietic regeneration ; Baker and Ingle[10] have observed indeed an atrophy of bone marrow in rats treated with large doses of cortisone and ACTH. Therefore, the hypercorticism existing in whole body irradiated rats, could possibly explain the inhibition of the haematopoiesis observed in such animals.

* A more detailed paper on this subject has been published in *Revue d'Hématologie*, 1953, **8** 489.

(1) Influence of cortisone and desoxycorticosterone acetate (D.C.A.) on haematopoietic regeneration after a sub-lethal dose of X-rays

The experiments are made in order to see whether adrenocortical hormones are able to inhibit the haematopoietic regeneration after a sublethal irradiation (500 r). Former experiments have shown that rats submitted to such a dose of X-rays regenerate very quickly the haematopoietic tissues destroyed by the irradiation.

Methods : Six groups of 12 albino rats each were used. The animals of the first group are controls. They are irradiated with 500 r (Picker app., 250 kV, 18 mA, filter 0·25 mm Cu, F.D. 50 cm output 90 r/min.) The rats of the three following groups are irradiated in the same way. After the irradiation, the animals are injected daily with cortisone. The doses used were respectively 2·5, 5 and 10 mg daily. The rats of the two last groups are irradiated and injected daily with 3 or 5 mg of

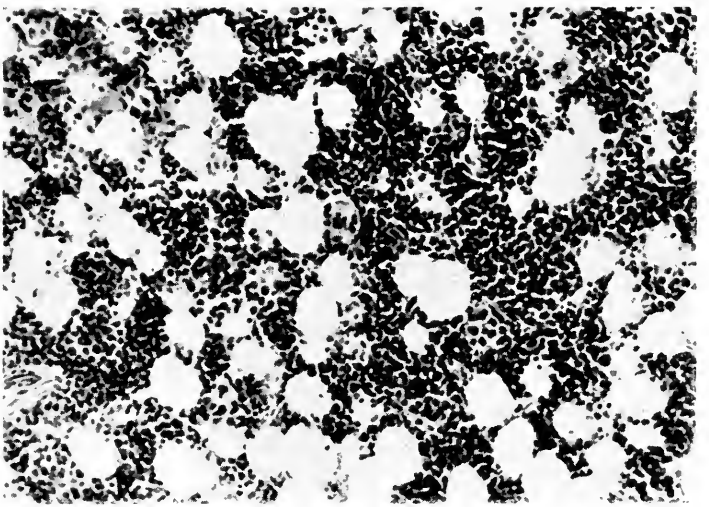


Figure 1. Femoral bone marrow of the rat, 15 days after 500 r ; *extensive regeneration of the myeloid tissues (200 ×)*

desoxycorticosterone acetate (D.C.A.). In each group, two animals are killed on the sixth, eighth, tenth, twelfth and fifteenth days. Cervical lymph nodes, spleen, thymus, femoral bone marrow are fixed in Helly's fluid, and studied histologically.

The control rats, which have been irradiated only, have an extensive and early regeneration of thymus, spleen, lymph nodes and bone marrow. The repair of the destroyed tissues starts on the fourth day after irradiation. The daily injection of 2·5 mg of cortisone does not modify in any way the histological picture of the haematopoietic tissues.

Daily injection of 5 mg of cortisone acetate to rats irradiated with 500 r does not influence the regeneration of spleen, lymph nodes, or bone marrow ; the regeneration of the thymus only is inhibited by such a dose of cortisone. On the contrary, the injection of 10 mg of cortisone daily does block the regeneration of spleen, lymph nodes and bone marrow as well as the repair of thymus. The histological picture of the haematopoietic

tissues of these rats is quite similar to that observed in rats receiving a lethal irradiation of 800 r. The daily injection of 3 or 5 mg of D.C.A. does not modify the regeneration of haematopoietic tissues.

(2) Influence of adrenalectomy on the haematopoietic regeneration

Methods: four groups of rats were used. The animals of the first group are irradiated with a lethal dose of X-rays (800 r). The rats of the second group are adrenalectomized and 4 days later submitted to 800 r. The animals of the third group and the fourth group are adrenalectomized and irradiated. In addition, they were supplemented with a dose of D.C.A. (3 mg daily) or cortisone (2·5 mg daily) too small to influence the haematopoietic activity. The haematopoietic tissues are taken and studied histologically between the fourth day and the moment of death.

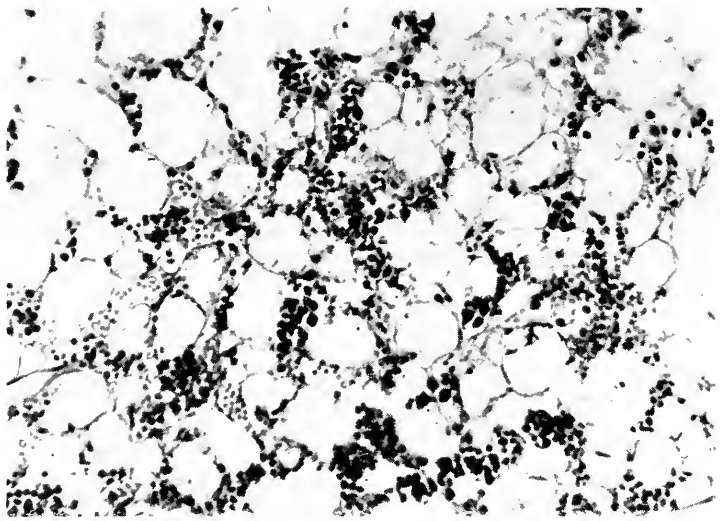


Figure 2. Femoral bone marrow of the rat, 15 days after 500 r *and injection of cortisone acetate* (10 mg *daily*) *: inhibition of the myelopoiesis (200 ×)*

The control rats receiving 800 r died between the sixth and the tenth day. They never showed any repair of the haematopoietic tissues destroyed by the irradiation. The adrenalectomized rats were very sensitive to a lethal dose of X-rays (800 r) and died before any regeneration could possibly take place (fourth day). In the adrenalectomized group of rats supplemented with a dose of D.C.A. (3 mg daily) or cortisone (2·5 mg) the resistance appeared to be normal. Some of them survived for 10 days. Although any possibility of hypercorticism was excluded, no haematopoietic regeneration has been observed in these rats.

The results of these experiments are not conclusive. Should an increased adrenocortical secretion be able to inhibit the haematopoietic regeneration, there is no doubt that the hypercorticism following a lethal whole body irradiation is not the only factor involved in the inhibition of haematopoiesis. The adrenalectomy does not succeed, indeed, in stimulating the haematopoietic regeneration of irradiated rats. Some other factors must be involved in the regulation of this phenomenon.

Recently SELYE[11] has shown that somatotrophic hormone is able to counteract the catabolic effect of protein which normally occurs after a stress or an injection of cortisone. On the other hand, it is known that the production and differentiation of blood cells is closely related with the protein metabolism. Therefore, we studied the effect of somatotrophic hormone on the haematopoietic regeneration of irradiated rats. We compared the results with those obtained by using other substances such as testosterone propionate and vitamin B_{12} whose effect on protein anabolism is also well known.

(3) Influence of somatotrophic hormone on the haematopoietic regeneration

Methods : Three groups of rats were studied, all irradiated with a lethal dose of X-rays (800r). The first group is used as control. The animals of the second group are irradiated and injected daily with 5I.U. of growth hormone. The rats of the

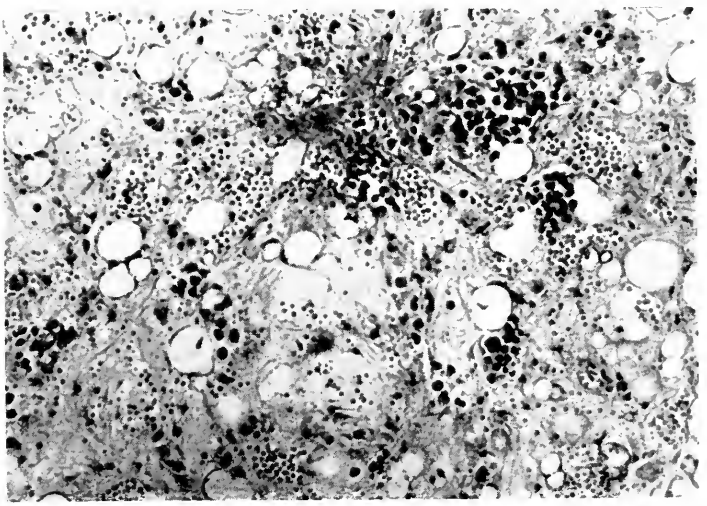


Figure 3. Femoral bone marrow of the rat, 10 days after 800r *: almost no regeneration of the marrow has taken place (200 ×)*

third group are adrenalectomized before the irradiation. Afterwards, they are injected daily with 2·5mg of cortisone and 5I.U. of growth hormone. The haematopoietic tissues are studied histologically.

In the control group, all the animals died within the eleven days following the irradiation, without showing any sign of haematopoietic regeneration. The rats irradiated and injected with growth hormone behaved like the controls ; they died between the fourth and the tenth day without regeneration of their haematopoietic tissues. The adrenalectomized rats injected with costerone and somatotrophic hormone proved more resistant to a lethal dose of X-rays ; about 25 per cent of the individuals of this group survived, showing an extensive regeneration of lymph nodes, spleen and bone marrow. In the bone marrow and spleen, the differences between control rats and treated rats are striking. In the control rats, the bone marrow is congested and oedematous; the myeloid cells have almost all disappeared ; the spleen is

small and lymphoid, or myeloid structures are no longer present. In the treated rats, the bone marrow is packed with myeloblasts and myelocytes and is even more cellular than the normal marrow. In the spleen the regeneration of lymphoid and myeloid cells is conspicuous ; numerous myeloid foci are to be seen in the red pulp.

Influence of testosterone propionate and vitamin B_{12} on the haematopoietic regeneration

The experiments were made on the same scheme as the one used for the assay of growth hormone. The two substances used in this experiment have the same action as growth hormone. They are quite inactive when injected to normal irradiated rats, while they do, on the contrary, stimulate the haematopoietic regeneration of adrenalectomized animals ; here again, about 20 per cent of the individuals survived a lethal whole body irradiation.

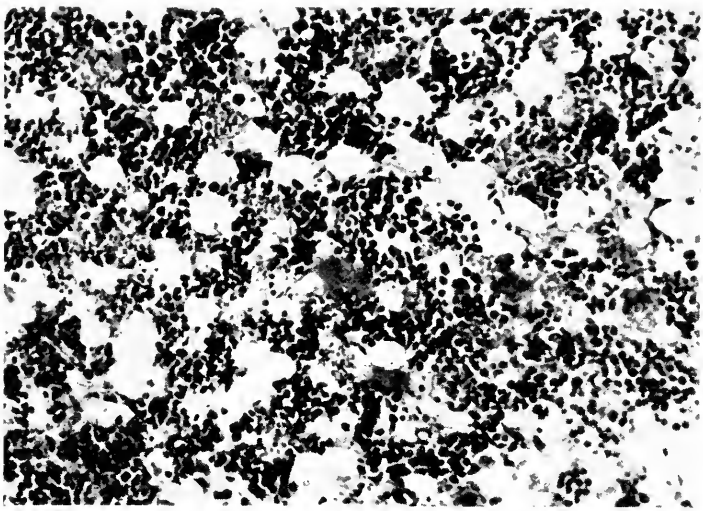


Figure 4. Femoral bone marrow of the rat 10 days after adrenalectomy, 800 r and injection of growth hormone and cortisone acetate (2·5 mg daily). Regeneration of myeloid tissue is conspicuous. (200 ×).

SURVEY

From these experiments, we may conclude that substances stimulating the anabolism of proteins are active in stimulating the haematopoietic regeneration of irradiated rats provided they are given to adrenalectomized animals. They are quite inactive in normal rats. The results indicate that, as far as haematopoiesis is concerned, there is an antagonistic effect of adrenal hormones and substances like growth hormone, testosterone propionate and vitamin B_{12}. It is likely that such an antagonism is related to the action of these substances on the metabolism of proteins.

In whole body irradiated rats, hypercorticism plays a role in the increased catabolism of proteins which is observed in animals. Betz and Jehotte[12] have shown that a lethal dose of X-rays produces in the rat a quick reduction of food intake, a reduction of nitrogen excretion, together with a negation

of the nitrogen balance. After five days, a secondary increase of the nitrogen excretion takes place with a still more marked negation of the nitrogen balance. Adrenalectomy prevents the secondary increase of nitrogen excretion although the nitrogen balance remains negative.

Our results confirm the relationship between the regeneration of blood cells and the metabolism of proteins. The disturbances of this metabolism which follows a whole body irradiation are poorly understood. Besides the hyperactivity of the adrenal cortex, there are other unknown factors interfering with the nitrogen metabolism. A better knowledge of these factors would probably be a great help in the discovery of the factors which control the haematopoiesis of the irradiated body.

REFERENCES

[1] Betz, E. H. *C.R. Soc. Biol.* 1950, **144** 1439.

[2] Betz, E. H. and Fruhling, L. *C.R. Soc. Biol.* 1940, **144** 1013.

[3] Cronkite, E. P., Brecher, G. and Chapman, W. H. *Proc. Soc. exp. biol.* 1951, **76** 396.

[4] Mole, R. H. *Colloquium centenaire de la découverte du Radium*, Paris, 1950.

[5] Lorenz, W. *Strahlentherapie*, 1952, **88** 190.

[6] Jacobson, L. O., Simmons, E. L., Marks, E. R., Robson, M. J., Bethard, W. F. and Gaston, E. O. *J. Lab. Clin. Med.* 1950, **35** 746.
Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J. and Eldredge, J. H. *J. Lab. Clin. Med.* 1951, **37** 683.

[7] Lorenz, E., Uphoff, D., Reid, T. R. and Shelton, E. *J. Nat. Cancer Inst.* 1951, **12** 197.

[8] Betz, E. H. *Ann. Endocr.* 1951, **12** 801.

[9] Patt, H. M., Swift, M. N., Tyree, E. B. and Johns, E. S. *Amer. J. Physiol.* 1947, **150** 480.

[10] Baker, B. L. and Ingle, D. J. *Endocrinology*, 1948, **43** 422.

[11] Selye, H. *Brit. med. J.* 1951, **10**, 263.

[12] Betz, E. H. and Jehotte, J. *C.R. Soc. Biol.*, in press.

ACTION DE LA CYSTÉAMINE SUR LES TUMEURS GREFFÉES, IRRADIÉES *IN LOCO*

S. Neukomm*, Mme L. Peguiron* et A. Herve†

* Service des Recherches Expérimentales du Centre Anticancéreux Romand, Lausanne.
† Service de Radiothérapie et Centre Anticancéreux, Université de Liège.

Depuis la découverte par Bacq et ses collaborateurs[1] de l'effet de radioprotection de la cystéamine (bétamercaptoéthylamine ou β-M) chez la souris, un grand nombre de travaux ont été consacrés à l'étude des amines soufrées d'où il ressort que la β-M et son dérivé oxydé sont à l'heure actuelle les protecteurs les plus efficaces puisqu'ils protègent à plus de 95 pour cent contre 700 r et entre 30 et 90 pour cent contre 1.000 r[2]. La grande efficacité du produit alliée à une faible toxicité (la dose mortelle 50 pour cent étant d'environ 0,35 mg par gramme de souris) a incité les chercheurs belges à l'utiliser en clinique pour le traitement du mal des rayons dont l'origine se trouve en partie liée à l'action indirecte des hautes doses d'irradiation[3]. Administré après la séance d'irradiation par l'injection intraveineuse lente, Herve[4] a montré qu'une seule injection de 200 mg était suffisante dans beaucoup de cas pour faire disparaître les symptômes majeurs du mal des rayons.

Nous avons recherché si la β-M injectée par voie intrapéritonéale était capable de protèger une tumeur irradiée localement. Il serait intéressant d'autre part de déterminer si la dose de β-M, utilisée cliniquement contre le mal des rayons est aussi susceptible de protèger la lésion irradiée.

C'est pour aborder ces problèmes que l'un de nous (A. H.) entreprit dès juillet 1952, au Centre anticancéreux de Lausanne, la première série d'expériences qui fait l'objet de la présente communication.

MATÉRIEL ET MÉTHODES

Nous avons utilisé pour ces recherches des souris de notre élevage (souche *E* et souche R_3) mâles ou femelles (*Tableau I*) greffées avec la tumeur de Caspari (adénocarcinome de la mamelle).

La greffe est faite par injection sous-cutanée, au moyen d'un trocart, de fragments de la grosseur d'une tête d'épingle. Le développement apparent de la greffe commence 10–15 jours après l'injection. Quelquefois, deux, voire trois, tumeurs se développent simultanément, ce qui explique que le nombre de tumeurs observables peut être plus grand que le nombre d'animaux greffés.

L'accroissement de chaque tumeur est mesuré en déterminant au moyen d'un pied-à-coulisse le petit (a) et la grand (b) diamètres. Le volume de ces tumeurs ovoïdes est directement proportionnel au produit a^2b. Pour la tumeur de Caspari, le facteur de proportionnalité est 1,06, donc négligeable. C'est-à-dire que la racine cubique du volume calculé $[(a^2b)^{1/3}]$ est très sensiblement égale au diamètre moyen de la tumeur. L'expérience a

Tableau I

Action protectrice de la β-M sur des tumeurs irradiées					*Témoins*		1500 r			3000 r		
					Sans	*+β-M*	*Sans*	*+β-M*	*+β-M*	*Sans*	*+β-M*	*+β-M*
Exp.	*Lots*	*Animaux*	*Tumeurs*	*Dose*	—	*Avant*	—	*Avant*	*Après*	—	*Avant*	*Après*
I	1	6 ♂ *E*	6	—	0,67 ± 0,12	—	—	—	—	—	—	—
	2	6	6	4 × 3 mg	—	0,70 ± 0,10	—	—	—	—	—	—
	3	6	4	—	—	—	0,70 ± 0,04	—	—	—	—	—
	4	6	3	4 · 3 mg	—	—	—	0,41 ± 0,02	—	—	—	—
II	1	6 ♀ R_3	7	—	0,72 ± 0,14	—	—	—	—	—	—	—
	2	6	7	4 · 3 mg	—	0,78 ± 0,07	—	—	—	—	—	—
	3	6	6	—	—	—	0,37 ± 0,09	—	—	—	—	—
	4	6	4	4 × 3 mg	—	—	—	0,40 ± 0,26	—	—	—	—
	5	7	3	—	—	—	—	—	—	0,25 ± 0,05	—	—
	6	7	2	4 × 3 mg	—	—	—	—	—	—	0,43 ± 0,10	—
III	1	11 ♀ *E*	11	—	0,64 ± 0,07	—	—	—	—	—	—	—
	2	11	19	—	—	—	0,50 ± 0,04	—	—	—	—	—
	3	11	14	1 · 4 mg	—	—	—	0,55 ± 0,04	—	—	—	—
	4	11	13	1 · 4 mg	—	—	—	—	0,48 ± 0,05	—	—	—
	5	11	8	—	—	—	—	—	—	0,42 ± 0,05	—	—
	6	11	10	1 · 4 mg	—	—	—	—	—	—	0,49 ± 0,04	—
	7	11	8	1 · 4 mg	—	—	—	—	—	—	—	0,47 ± 0,10
Moyenne pondérée					0,672	0,745	0,500	0,505	0,480	0,385	0,487	0,470
*Écart quadratique moyen**					0,035	0,045	0,035	0,035	0,050	0,047	0,055	0,100

* $[(\Sigma\epsilon^2)/\{n(n-1)\}]^{\frac{1}{2}}$

montré que l'accroissement du diamètre moyen suit une courbe linéaire en fonction du temps, tout au moins pendant la plus grande partie de l'évolution apparente des tumeurs (*Figure 1*). La vitesse de croissance est alors déterminée par la pente de la droite interpolée qui relie les divers points des courbes d'évolution du diamètre moyen en fonction du temps ; elle s'exprime en millimètres par jour.

Les irradiations sont faites avec un appareil de contact-thérapie en choisissant un localisateur approprié pour chaque tumeur*. Les tissus sains du voisinage sont protégés par des lames de plomb percées d'un trou de diamètre correspondant à celui des tumeurs. Pendant l'irradiation, l'animal

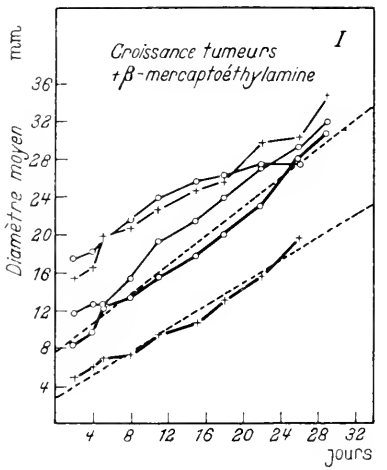


Figure 1

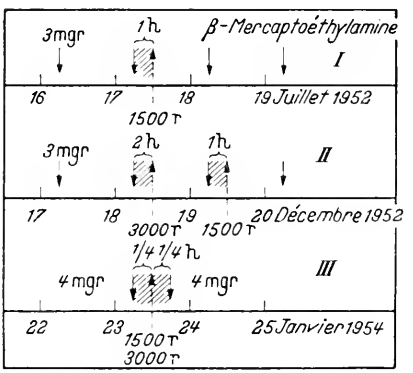


Figure 2

n'a pas besoin d'être tenu, car il a reçu préalablement (30–45′) une injection intrapéritonéale de Numal 'Roche' (0,2cm³ sol. à 1 pour cent) qui provoque une narcose d'une heure environ.

La β-M a été injectée 2h, 1h et $\frac{1}{4}$h avant, ou $\frac{1}{4}$h après l'irradiation par voie intrapéritonéale à la dose de 3 ou de 4mg par animal. Les doses de roentgen par tumeur ont été de 1.500 et 3.000.

Dans les séries *I* et *II*, les animaux ont reçu de la β-M pendant 4 jours consécutifs, alors que dans la série *III*, une seule injection a été faite avant ou après l'irradiation (*Figure 2*).

* Nous remercions M. le Prof. Babaiantz, chef du Service universitaire de radiologie, pour l'intérêt qu'il a bien voulu porter à ces travaux et les appareils qu'il a aimablement mis à notre disposition.

RÉSULTATS

Les résultats de nos trois séries de recherches, exprimés en accroissement du diamètre moyen (mm/jour), sont condensés dans le *Tableau I.* On constate que l'injection de β-M à des animaux porteurs de tumeurs non irradiées (témoins) ne provoque pas de modification significative de la vitesse de croissance des tumeurs ; le chiffre plus élevé de la moyenne pondérée comparativement aux animaux non traités est dû à l'absence d'un lot comparable dans la série *III* (*Figure 3*).

Pour les animaux dont les tumeurs ont reçu 1.500r, la β-M injectée avant ou après l'irradiation ne semble pas apporter de modifications au développement des tumeurs, tout au moins si l'on en juge sur la valeur des moyennes pondérées. Il est probable toutefois que ce résultat soit la conséquence d'un effet inhibiteur insuffisant de la dose de 1.500r qui n'est pas capable de 'révéler' la protection. Cette opinion est justifiée par le fait que l'application d'une dose de 3.000r chez des animaux traités de la même manière que

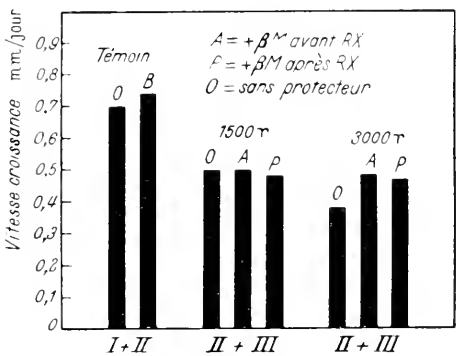


Figure 3

précédemment n'entraîne pas une diminution de la vitesse de croissance aussi importante que chez les animaux non traités par β-M. La différence entre les vitesses de croissance des tumeurs chez des animaux ayant reçu de la β-M avant ou après l'irradiation et chez des animaux n'ayant pas reçu de β-M est statistiquement significative. Tout se passe comme si, chez les animaux traités à la β-M, l'efficacité de la dose de 3.000r avait été diminuée de moitié, c'est-à-dire comme si l'on avait irradié avec une dose de 1.500r seulement.

Nos résultats ne permettent pas d'établir avec précision une relation entre le moment de l'administration de la β-M et l'importance de l'effet protecteur qu'elle exerce (*Figure 4*).

COMMENTAIRES

Chez la souris, l'injection intrapéritonéale de β-M protège les tumeurs irradiées localement par une forte dose de rayons X. Dans les expériences *I* et *II*, il faut noter que des injections ont aussi été faites le jour précédant l'irradiation et les jours suivant l'irradiation (*Figure 2*). A protection obtenue dans ces cas ne semble pas influencée par les injections faites à des moments éloignés de l'irradiation mais bien par la seule injection

précédant d'une à deux heures le moment de l'irradiation. Il apparaît comme certain que l'action de la β-M s'exerce dans un laps de temps très court (de l'ordre d'une heure). Ce fait peut être mis en corrélation avec le métabolisme et l'élimination rapides du produit[2-5]. Il faut noter que dans nos expériences, nous avons utilisé une dose considérable de β-M (150 mg/kg). Cette dose correspond à celle qui offre la meilleure protection contre le rayonnement X aux animaux irradiés *in toto* par une dose de 750 r (100 pour cent mortelle pour tous les témoins[2]).

La dose active en clinique contre le mal des rayons est de 3 mg/kg, c'est-à-dire 50 fois moindre que celle utilisée dans nos expériences. L'effet d'une

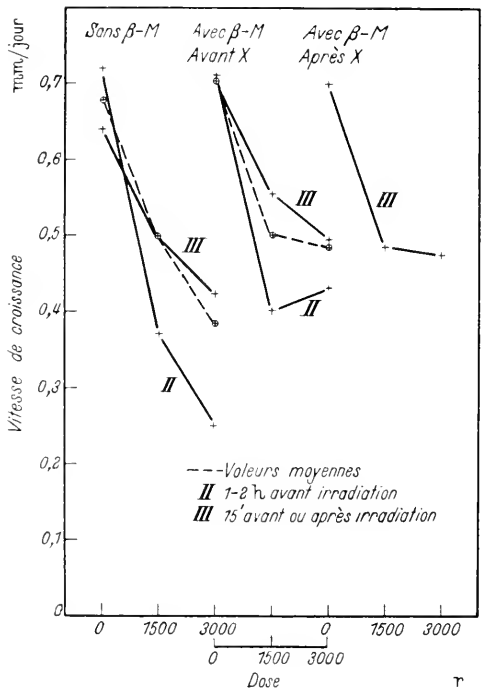


Figure 4. Effet protecteur de la β-M sur tumeurs irradiées

telle dose chez l'animal n'a pas été étudié. Il n'est, par conséquent, pas possible de transposer sur le plan clinique les conclusions de ce travail expérimental.

Les observations cliniques faites par l'un de nous et rapportées ailleurs[3, 4] n'ont jamais montré une résistance accrue de la lésion irradiée. Il semble certain que l'injection d'une petite dose de β-M effectuée plus d'une heure après l'irradiation soit incapable de modifier l'action des rayons X sur les cellules tumorales.

Dans le cadre de nos expériences il est difficile d'apprécier quantitativement la protection chimique. Nos observations nous conduisent à admettre qu'un test quantitatif dépend principalement :

(*a*) de la dose de rayons utilisée pour 'révéler' la protection, cette dose dépendant en partie du volume des tumeurs à irradier ;

(*b*) de la dose de protecteur administrée ;

(*c*) du moment de l'administration du protecteur par rapport au moment

de l'irradiation, ce facteur 'temps d'action du protecteur' étant étroitement lié au métabolisme du produit (destruction et élimination par l'organisme).

Le rôle du volume des tumeurs dans le choix de la dose de roentgen à donner est particulièrement évident dans l'expérience *I*, lot 3 (*Tableau I*). Dans ce cas, la vitesse de croissance des tumeurs irradiées par 1500r n'a pas subi de modification par rapport à celle des témoins non irradiés. Or les animaux de ce lot avaient tous des tumeurs très grosses ; il est donc très vraisemblable que la dose choisie n'a pas été suffisante pour provoquer un arrêt de croissance rigoureusement comparable à celui obtenu dans le lot 4.

CONCLUSIONS ET RÉSUMÉ

(*1*) La β-M seule ne semble pas capable de modifier la croissance des tumeurs non irradiées.

(*2*) La β-M injectée à forte dose par voie intrapéritonéale à des souris porteuses de tumeurs greffées protège les tumeurs contre l'irradiation locale. La protection est obtenue lorsque l'injection est relativement proche du moment de l'irradiation. La protection se manifeste d'autant mieux que la dose de rayons administrée est plus grande, tout au moins dans les limites choisies pour nos expériences.

(*3*) Les doses de β-M utilisées dans nos expériences préliminaires sont 50 fois supérieures à celles qui, en clinique, améliorent les symptômes du mal des rayons.

Il reste à voir si les doses cliniques de β-M et les conditions dans lesquelles elles sont administrées ont une incidence sur la régression des tumeurs irradiées.

ADDENDUM

This work was carried out in the Experimental Research Service of the Centre Anticancéreux Romand at Lausanne because we knew the details of growing Caspari tumour specially studied by Neukomm. The linear growth of tumour as a function of time seemed to be a good test of observation.

Nevertheless, in this work, we have neglected the concentration of cysteamine in the tumour itself.

Thanks to the use of labelled sulphur, Verly *et al* were able to follow the metabolism of the cysteamine and have showed that the concentration of this substance during the first twenty-four hours was larger in the liver and the intestine than in the other tissues.

A similar research programme would be very interesting for 'spontaneous' or 'experimental' tumours. It would be possible, in particular, to determine the final protecting effect upon certain types of lesions by concentrating a more important quantity from the injected cysteamine. We intend to study this problem.

REFERENCES

[1] Bacq, Z. M., Herve, A., Lecomte, J., Fischer, P., Blavier, J., Dechamp, G., Le Bihan, H. et Rayet, P. *Arch. Internat. Physiol.* 1951, **59** 442.

[2] Bacq, Z. M. et Herve, A. *Bull. Acad. Royale Méd. de Belgique*, 1952, **17** 13.

[3] Herve, A. *Revue médicale de Liège*, 1952, **7** 276.

[4] Herve, A. et Bacq, Z. M. *J. Radiol. et Electrol.* 1952, **33** 651.

[5] Verly, W., Bacq, Z. M., Rayet, P. et Urbain, M. F. *Biochimica et Physica Acta*, 1954, **13** 233.

ÉTUDE HISTOLOGIQUE DE LA RÉPARATION INTESTINALE CHEZ DES RATS IRRADIÉS SOUS DIVERSES CONDITIONS DE PROTECTION

H. Maisin et C. Fiévez

Laboratoires de Recherches pour le protection de la population civile annexés a l'Institut du Cancer de Louvain

Nous nous sommes surtout attardés à l'étude comparative des lésions et de la régénération de l'intestin grêle de rats irradiés d'une façon standard et protégés de diverses manières.

Nous avons choisi cet organe comme premier but d'étude, car c'est lui que nous rendons responsable des morts constatées chez les animaux les premiers jours qui suivent l'irradiation. En effet, ceux-ci présentent une diarrhée extrêmement abondante et les seuls animaux qui survivent 4 à 5 jours après l'irradiation sont ceux qui, pour une raison ou l'autre, protection mécanique[1, 2, 4] ou chimique par la bétamercaptoéthylamine (bécaptan)[3, 4] n'ont pas présenté cette diarrhée.

Notre matériel d'expérimentation est représenté par des rats blancs homozygotes. Tous nos animaux ont reçu 700 r sous 200 kV, $\frac{1}{4}$ mm Cu, 1 mm d'Al, à une distance F.P. de 55 cm. Notre appareil un Maximar de 250 kV de la General Electric Co. débite 34 r/minute mesurés dans l'air à l'intégrateur Victoreen. Certains n'ont pas été protégés (rats contrôles), d'autres ont été protégés par un écran de plomb de 5 mm d'épaisseur et de 2 cm de côté, placé soit sur leur région sous-ombilicale[1, 4] entraînant ainsi la protection d'une partie du gros intestin et des anses grêles, soit sur leur région sus-ombilicale[1, 4] entraînant alors la protection de la grosse partie des anses grêles, d'autres enfin ont reçu directement avant l'irradiation une injection intrapéritonéale de 7,5 mg de bécaptan par 100 g de poids sans aucune autre protection. Nous diluons 10 mg de bécaptan dans 1 cm^3 d'eau distillée. Nous avons également injecté du bécaptan à des animaux non irradiés, mais ces injections nous semblèrent sans effet.

Nous avons sacrifié dans chaque série au moins deux rats de 24 en 24 heures et nous nous limiterons aux résultats observés les 10 premiers jours qui suivent l'irradiation, c'est-à-dire pendant la période dite intestinale et les jours qui lui font immédiatement suite.

Les pièces ont été conservées 3 jours dans le Bouin et colorées à l'hématéine–éosine–safran.

Nous avons essayé de chiffrer les lésions et de juger de la régénération en comptant les mitoses des glandes de Lieberkuhn dans des conditions standardisées (grossissement 800), leur nombre chez les rats normaux est en moyenne de 5 par champ. Chez les rats non irradiés injectés de bécaptan le nombre de mitoses ne varie pas, que cette numération ait lieu 3 heures, 6 heures, 12 heures ou plusieurs jours après l'irradiation.

I. RATS CONTRÔLES

1 jour après l'irradiation—l'épithélium de surface est relativement peu lésé, la bordure ciliée est intacte dans la plus grande partie de sa longueur.

Les glandes de Lieberkuhn sont nettement abîmées. Elles paraissent tout d'abord raréfiées et la tunica propria plus lâche. Leurs noyaux présentent des lésions variées de dégénérescence. Les granulations des cellules de Paneth sont très visibles, mais souvent libérées dans la lumière glandulaire par effraction du pôle secréteur.

Le nombre de mitoses, au niveau des glandes de Lieberkuhn, est en moyenne de 1,25 par champ.

2 jours après l'irradiation—l'épithélium de surface présente des lésions plus sérieuses ; les noyaux sont moins nombreux beaucoup plus irréguliers, parfois très volumineux. En outre, leur situation dans la cellule n'est plus aussi strictement polaire. Certaines cellules, rares à vrai dire, en sont dépourvues. La bordure ciliée est respectée dans sa plus grande partie.

Les glandes de Lieberkuhn montrent une anisonucléose de plus en plus nette. Les limites cellulaires sont peu précises et le protoplasme en est plus granuleux. Les grains des cellules de Paneth sont déversés presqu'en totalité dans la lumière glandulaire et par conséquent de moins en moins nombreux dans le protoplasme cellulaire.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 0,6 par champ.

3 jours après l'irradiation—la hauteur des villosités est réduite et l'épithélium de surface est de plus en plus aplati. L'anisonucléose et les altérations chromatiniennes sont de plus en plus manifestes. Les protoplasmes sont vacuolisés. La bordure ciliée disparaît à beaucoup d'endroits.

Les glandes de Lieberkuhn montrent une lumière nettement plus grande par aplatissement de l'épithélium glandulaire. Certaines glandes présentent même un épithélium endothéliforme. Les cellules de Paneth ne sont plus reconnaissables.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 0,38 par champ.

4 jours après l'irradiation—les villosités sont de plus en plus aplaties et l'épithélium de surface est très altéré, la bordure ciliée a disparu. Bon nombre des cellules présentent des vacuoles très grosses, uniques ou multiples, refoulant le noyau ou ce qui en reste à un pôle. La plupart des glandes de Lieberkuhn sont dans le même état que le jour précédent, mais par endroits apparaissent de rares glandes de Lieberkuhn mieux formées, avec un épithélium plus régulier cylindrique semblant récupérer une aptitude fonctionnelle, des cellules de Paneth sont à nouveau bien reconnaissables.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 1,15 par champ. Celles-ci n'existent en fait qu'au sein des glandes qui prolifèrent.

5 jours après l'irradiation—l'épithélium de surface redevient cylindrique, toutefois, les villosités restent encore assez effacées. A certains endroits la bordure ciliée réapparaît.

Les glandes de Lieberkuhn en prolifération sont de plus en plus nombreuses à la lumière toujours plus élargie tandis que les glandes lésées se font de

plus en plus rares. Des cellules de Paneth existent dans la plupart des glandes néoformées.

Le nombre de mitoses au niveau des glandes de Lieberkuhn tend à redevenir normal, il est actuellement en moyenne de 3,7 par champ.

6 jours après l'irradiation—l'épithélium intestinal et la hauteur des villosités redeviennent normaux. La densité des glandes de Lieberkuhn et leurs dimensions paraissent normales. Toutefois elles restent plus allongées, leur lumière est presque fermée. Le nombre des cellules de Paneth et leurs granulations sont normalles.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 5,1 par champ.

Le dernier rat de notre série a été sacrifié le dixième jour ; la muqueuse intestinale y est redevenue entièrement normale. Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 6,5 par champ.

II. RATS DONT LA RÉGION SOUS-OMBILICALE A ÉTÉ PROTÉGÉE

Dans ce cas, la plus grande partie des anses grêles n'a pas été protégée et les lésions constatées à ce niveau sont superposables à celles observées chez les rats contrôles.

III. RATS DONT LA RÉGION SUS-OMBILICALE A ÉTÉ PROTÉGÉE

Dans ce cas la plus grande partie des anses grêles a été protégée mécaniquement et ce sont ces parties que nous allons décrire.

1 jour après l'irradiation—contrairement à ce qu'on serait en droit d'attendre, l'intestin grêle protégé présente après 24 heures, exactement les mêmes lésions que l'intestin grêle des rats contrôles, mêmes altérations nucléaires, même raréfaction des glandes de Lieberkuhn, même sidération des mitoses. Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 1,1 par champ.

2 jours après l'irradiation—les lésions constatées après 24 heures n'ont pas évolué comme chez les contrôles. Si l'aplatissement des villosités est assez manifeste, par contre au niveau de l'épithélium de surface, les noyaux restent polaires et les altérations nucléaires sont relativement peu prononcées.

Au niveau des glandes de Lieberkuhn, existent des altérations nucléaires certaines, mais l'épithélium glandulaire présente une activité mitotique considérable.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 6 par champ. Les cellules de Paneth sont intactes.

3 jours après l'irradiation—l'épithélium de surface est strictement normal à certains endroits, cependant sur de larges étendues, il présente des lésions comparables à celles décrites chez les animaux contrôles homologues, noyau plus central—anisonucléose—épithélium plus plat—perte de la bordure ciliée.

Les cellules des glandes de Lieberkuhn sont cylindriques, tassées les unes contre les autres, les lumières glandulaires sont plus élargies et leurs lésions peu visibles.

Les cellules de Paneth sont nombreuses et nettement visibles.

Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 7 par champ.

4 jours après l'irradiation—si l'épithélium de surface est redevenu normal dans sa plus grande partie, il reste encore des endroits cependant, à épithélium lésé. L'aspect de la sous-muqueuse de l'intestin grêle reprend une apparence normale. Seule l'activité mitotique y est plus grande. Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 8,4 par champ.

Dans les jours qui suivent, l'intestin redevenu complètement normal ne diffère de l'intestin non irradié que par une activité mitotique plus intense. Celle-ci ne se régularise que vers le dixième jour.

IV. RATS INJECTÉS IMMÉDIATEMENT AVANT L'IRRADIATION DE 7,5 MG DE BÉCAPTAN PAR 100 G DE POIDS

1 jour après l'irradiation—on peut dire que les lésions sont strictement semblables à celles observées chez les contrôles homologues.

Les deux préparations peuvent être superposées—même intégrité de l'épithélium de surface—mêmes lésions lieberkuhniennes. Le nombre de mitoses au niveau des glandes de Lieberkuhn est en moyenne de 1,4 par champ.

2 jours après l'irradiation—l'épithélium de surface présente les mêmes altérations que les homologues contrôles. Les glandes de Lieberkuhn sont nettement lésées. Leur épithélium est aplati et la lumière de ce fait très visible. Il montre de l'anisonucléose et des altérations nucléaires diverses.

Les cellules de Paneth sont très visibles. Le nombre de mitoses au niveau des glandes de Lieberkuhn est redevenu en moyenne de 1,7 par champ.

3 jours après l'irradiation—l'épithélium de surface peut toujours être comparé à celui des animaux homologues protégés mécaniquement. Quant aux glandes de Lieberkuhn, elles présentent une tendance nette à la régénération. Ceci peut être objectivé par le nombre de mitoses qui est en moyenne de 5,2 par champ.

4 jours après l'irradiation—l'épithélium de surface reprend un aspect normal. Il redevient plus cylindrique. Il peut être comparé à celui des animaux homologues protégés mécaniquement et à celui des animaux contrôles du sixième jour.

Les glandes de Lieberkuhn comme celles des animaux protégés mécaniquement présentent une activité régénératrice très intense. Le nombre de mitoses y est de 7,4 par champ.

5 jours après l'irradiation—comme pour les animaux homologues protégés mécaniquement l'intestin grêle reprend un aspect normal. L'activité mitotique des glandes de Lieberkuhn est toujours très intense. Nous avons dénombré 9,3 mitoses par champ.

Le sixième jour après l'irradiation l'activité mitotique au niveau des glandes de Lieberkuhn est toujours élevée. Nous avons compté en moyenne 8,4 mitoses par champ.

A partir du septième jour l'activité mitotique au niveau des glandes de Lieberkuhn redevient normale.

Nous avons cru bon synthétiser nos numérations de mitoses dans un diagramme (*Figure 1*) qui nous permettra d'établir des comparaisons et de tirer des conclusions. Rappelons que chez le rat normal le nombre de mitoses par champ microscopique au grossissement 800 est de cinq.

En résumé

Il a été montré dans ce laboratoire que les rats dont l'intestin grêle a été protégé mécaniquement[1, 2, 4] et que ceux qui ont reçu du bécaptan[3, 4] ne présentent pas les diarrhées, habituelles chez les animaux contrôles ou dont l'intestin n'a pas été protégé.

Actuellement, nous pouvons à la lumière de nos constatations histologiques supposer que l'absence de diarrhée est liée à une régénération plus rapide

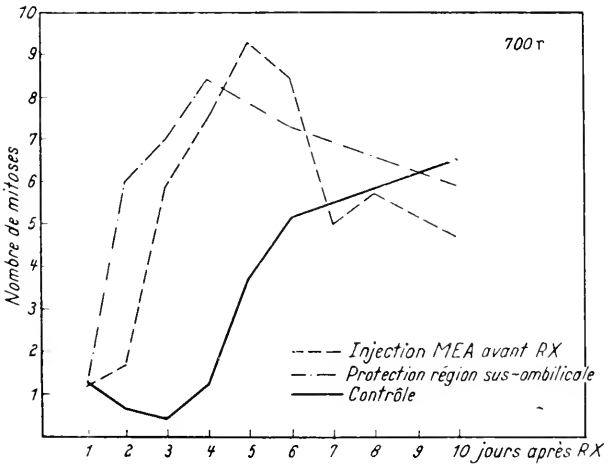


Figure 1. Étude histologique réparation intestinale chez rats irradiés sous diverses conditions protectrices

de l'intestin grêle, régénération obtenue tout aussi bien avec l'écran de plomb qu'avec le bécaptan. Rappelons que le premier jour après l'irradiation, les lésions sont semblables chez tous nos animaux.

Chez les animaux contrôles, la régénération intestinale ne se produit qu'à partir du cinquième jour. Il en est de même pour les parties d'intestin non protégées mécaniquement. La réparation presque complète ne s'observe qu'au sixième jour et les lésions sont maximales les troisième et quatrième jours.

Chez les animaux dont l'intestin a été protégé mécaniquement ou qui ont reçu une injection de bécaptan immédiatement avant l'irradiation, la régénération débute, chez les premiers dès le deuxième jour, chez les seconds dès le troisième jour et la réparation presque complète est réalisée dès le quatrième jour. Le maximum des lésions s'observe pour les rats protégés mécaniquement le premier jour, pour les rats injectés le deuxième jour.

Ceci est en contradiction avec les résultats obtenus par Van Lancker[5] dans notre laboratoire. Il croyait que l'intestin des animaux ayant reçu du bécaptan avant l'irradiation ne se comportait pas différemment de celui des animaux contrôles.

CONCLUSION

En faisant une revue de la littérature actuelle à ce sujet on peut voir que d'autres expériences avec d'autres substances donnent des résultats analogues.

Ainsi WILLIAMS et DE LONG[6] trouvent au niveau de l'intestin de rats irradiés par 700 r, une activité mitotique augmentée dès la 73ème heure suivant l'irradiation après leur avoir injecté du glutathion réduit ou du para-aminopropiophenone. CRONKITE, BRECHER et CHAPMAN[7] trouvent cette stimulation mitotique après injections de glutathion, au niveau de la rate, de la moëlle et du thymus de souris irradiées.

Des faits semblables sont décrits par ROSENTHAL, GOLDSCHMIDT et PICKERING[8] avec la cystéine, par BETZ et FRUHLING avec le cyanure de potassium[9] qui, tout en n'évitant pas les radiolésions des organes hématopoïétiques, en rendent cependant leur réparation beaucoup plus rapide.

Tous ces auteurs insistent donc sur cette régénération plus rapide.

Doit-on assimiler l'action protectrice du bécaptan (ou éventuellement d'autres substances) au niveau de l'intestin à celle réalisée par un écran de plomb ?

L'histologie élémentaire tend à nous faire répondre à cette question par l'affirmative. En effet, à part le léger retard de régénération observée chez les animaux injectés, les lésions et les réparations sont identiques. Les deux types de protection réalisent les mêmes aspects morphologiques, comme si tous les deux gardaient à un plus grand nombre de cellules leurs potentialités reproductrices avec comme conséquence une réparation plus rapide et plus complète.

Cependant, malgré cette identité morphologique il est peu probable et peu logique d'admettre que cette protection s'exerce au même niveau.

Mais ceci ne ressort plus des disciplines histologiques élémentaires.

REFERENCES

[1] MAISIN, J., DUNJIC, A. et MAISIN, H. *C.R. Soc. Biol. Paris*, 1954, **148** 611.

[2] MAISIN, J., MAISIN, H. et DUNJIC, A. *C.R. Soc. Biol. Paris*, 1954, **148** 743.

[3] MAISIN, J., MAISIN, H. et DUNJIC, A. *C.R. Soc. Biol. Paris*, 1954, **148.**

[4] MAISIN, J., MAISIN, H. et DUNJIC, A. *Deuxième Symposium de Radiobiologie, Liège, 1954.* Butterworths Scientific Publications, London, 1955.

[5] VAN LANCKER, J. *C.R. Soc. Biol. Paris*, 1953. **147** 2057.

[6] WILLIAMS, R. B., Jr. et DE LONG, R. P. *Fed. Proc.* 1953, **12** 406.

[7] CRONKITE, E. P., BRECHER, G. et CHAPMAN, W. H. *Proc. Soc. exp. Biol. N.Y.* 1951, **76** 396.

[8] ROSENTHAL, R. L., GOLDSCHMIDT, L. et PICKERING, B. I. *Amer. J. Physiol.* 1951, **166** 15.

[9] BETZ, H. et FRUHLING, L. *C.R. Soc. Biol. Paris*, 1950, **144** 1013.

DISCUSSION TO PAPERS BY GEREBTZOFF AND BACQ, BETZ, NEUKOMM, PEGUIRON AND HERVE, AND MAISIN AND FIÉVEZ

Z. M. Bacq : To my mind, the facts gathered by Jacobson and Cole, by Lamerton, Loutit and Maisin, and by those who worked as chemists or cytologists with radioprotectors, are just parts of one general story. We must put all these facts together and try to formulate some kind of temporary logical idea which might be useful for further work in these fields. This is what I have attempted in a book with Alexander, which is now in press.[1]

We think that the permeability of the nuclear membrane in haematopoietic tissues of mammals is much increased by ionizing radiations. The nuclear factor (Cole) escapes in large quantities in the cytoplasm, the activity of which is first very much increased (Altman, Richmond and Salomon ; Nizet, Herve and Bacq) ; this factor might escape in the blood ; it is rapidly destroyed and not resynthetized because the nuclei are heavily damaged, if the whole body has received a sufficient dose of radiation.

If part of the haematopoietic system (bone marrow or spleen) has been shielded or if a chemical protector has been injected, the primary damage to the haematopoietic system is decreased ; a substantial proportion of nuclei escape destruction ; in these nuclei, the growth factor is synthetized in larger quantities, and the regeneration of the haematopoietic system (about which everybody agrees) is activated.

In my mind, chemical protectors are comparable to an imperfect physical screen which decreases the primary radiation damage. During the first years after the discovery of the phenomenon of chemical protection, many papers, even by ourselves, were published showing that the behaviour of rodents protected by cysteine, glutathione or cysteamine was exactly the same as that of the irradiated controls during the first 2 to 4 days after irradiation. A closer analysis shows that this idea is wrong ; Gerebtzoff and myself have just given evidence that the *primary* cellular damage (at 6 hours) is less in the spleen of mice irradiated under protection of cysteamine ; Devik, who is here (I hope that he will give us first-hand evidence), has observed that very soon (1 hour) after irradiation, the number of mitotic abnormalities after X-irradiation is significantly reduced if cysteine or cysteamine has been injected before irradiation ; when looking with Gray through some of my published data[2], I agreed that small differences which I had considered to be within the limit of error, might show a significant lesser damage in cysteamine injected animals.

Thus our general idea seems to be consistent with the available observations.

We would also like to suggest that the factor responsible for the polycythemia in chronic anoxia is identical with the factor which, according to Jacobson and Cole is active on haematopoietic tissues when injected after irradiation[1].

The behaviour of the adrenals is apparently complicated. We[3] have confirmed previous evidence published by Patt. There is a very rapid drop (already marked 1 hour after 800 r, maximal at the second hour) in cholesterol values of the suprarenals of the rat ; the values are normal at 24 hours and drop again to a very low level at 4 days (*see Figure 1*). The main interest of the figure is that during the first 24 hours, the behaviour of the adrenals of rats protected by cysteamine (10 mg/100g) is the same as that of the controls.

Here again it looks at first sight that cysteamine has not protected against the *primary* action, but we think that further work may show that for a certain, lesser dose of X-rays, there will be a difference between chemically protected and control rats.

But, as I said in the discussion following Court-Brown's paper, the first hours after irradiation are not a favourable period to observe the action of chemical protectors.

The ascorbic acid of the suprarenals shows variations parallel to that of cholesterol both in controls and cysteamine-protected rats. More than 300 rats have been used in this study.

We are not at all prepared to say that the cysteamine-injected rats survive because their adrenals are in normal condition from the first to the fourth day. It might

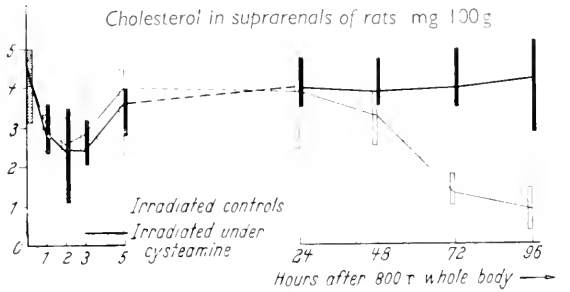


Figure 1

very well be that the adrenals of these animals are in better shape *because* the liver and other important organs have been less damaged in the presence of cysteamine. The adrenals are not at all necessary for the demonstration of cysteamine action ; recent observations of Fischer show that cysteamine protects adrenalectomized rats against X-radiation.

So far as I know, histological observation has not revealed changes in the suprarenals as early as 1 hour after irradiation.

Irradiation increases rapidly the amount of ACTH present in the hypophysis ; ACTH falls after 24 hours to subnormal levels[4]. In the pig, there is a marked increase of neutral steroid excretion in the urine during the first day only, after irradiation[5]. Thus the existence of a short hypophyso-adrenal reaction immediately after a heavy dose of X-rays seems undeniable. After 24 hours, the story is indeed much more complicated.

F. Devik[6,7] : In the discussion of morphological effects of ionizing radiations it may be of interest to point out that the mitoses in the bone marrow of mice may show

Table I

Time in hours after 200 r whole body irr.	1	2	3	4	6	9	18	29	51
Cysteamine 3 mg intraperit. 10 min before irradiation	55 (2)	69 (5)	63 (2)	63 (5)	70 (5)	64 (5)	41 (20)	18 (4)	12 4
Controls	85 (2)	78 (5)	83 2)	82 (5)	87 (5)	80 (5)	62 (18)	21 4)	8 (4)

pronounced radiation effects 1 hour or less after 200 r whole body X-irradiation. These mitotic abnormalities, which mainly disappear in the course of 1–2 days, were found to be significantly reduced in frequency by hypoxia treatment during irradiation, and by pretreatment with cysteine, cystamine, and cysteamine as shown in *Table I*. The figures indicate the mean percentage of abnormal anaphases. Usually

50 anaphases were analysed in each specimen. The figures in parentheses indicate the number of mice which were investigated.

Betz : Gerebtzoff a signalé des différences dans l'extension des dégénérescences cellulaires dans la rate de souris irradiées et de souris irradiées après protection par la cystéinamine. Je voudrais lui demander si ces différences atteignent au même titre les tissus myéloïdes et les tissus lymphoïdes de cet organe.

M. A. Gerebtzoff : I did not study, from the quantitative point of view, the difference in reaction between lymphoid and myeloid tissues in spleen, but it appears that the protection of myeloid tissue by cysteamine is stronger than the protection of lymphoid tissue.

Harvey M. Patt : Our studies[8, 9] of the adrenal response to X-irradiation would seem to support, at least in part, the interpretation advanced by Betz. The data presented by Bacq are essentially a confirmation of earlier work. Ionizing radiations, in common with other noxious stimuli, induce changes that are presumed to reflect an increased demand for the adrenal hormones. The functional response of the adrenals is mediated by the pituitary and closely resembles that seen following a host of injuries. While the early decrease in adrenal lipids is probably indicative of an increased requirement for cortical secretions, the rise in adrenal cholesterol in the rat several days after median lethal irradiation may represent over-stimulation in excess of cortical hormone demand. That the elevated cholesterol is not a result of adrenal exhaustion is suggested by its absence with higher dosages. Although adrenal lipids are usually depleted before death, it is not known whether this is a cause or an effect of the more terminal events. It is particularly noteworthy that Edwards and Sommers[10] were unable to detect any fundamental difference in radiation reactions as a consequence of adrenalectomy of shielded or irradiated parabionts and that Schneider *et al*[11] observed that the adrenals of irradiated rats were capable of sustaining non-irradiated adrenalectomized parabionts. Absence of the adrenals did not compromise protection of irradiated animals by parabiosis. These results strongly suggest that the adrenals of lethally irradiated rats suffer no basic impairment.

Turning to the mechanism of protection by β-mercaptoethylamine, there is, at present, no direct evidence and little reason to assume that the effect is a consequence of the protection of a specific restoration factor. Arguments for the latter fail to consider a very basic matter, namely the relative radio-sensitivities for different morphological and functional responses. We may note in particular (*1*) the difficulty of differentiating the extent of initial injury in the lethal range by ordinary histological criteria, (*2*) that many responses, *e.g.* lymphoid involution, are related more to the radiation dose than to the lethal or morbid effect, (*3*) that near maximal initial effects may occur at dosages considerably below those required for acute lethality. It is possible under appropriate experimental conditions to demonstrate prevention of injury in a variety of systems by agents such as β-mercaptoethylamine and cysteine. The sparing of comparatively few cells initially might exert a profound influence on the recovery pattern of the tissue as a whole. It should be remarked that it is necessary to consider another matter in interpretation of protective phenomena, namely, the temporal and spatial distribution of a given agent in the biological system. Localization of β-mercaptoethylamine to specific sites, as discussed by Eldjarn*, might preclude a more generalized protective action in the animal.

Bacq has alluded to the possibility that the effects of β-mercaptoethylamine on the one hand and of spleen shielding and homogenates on the other may be related. This is so to the extent that both modify the acute lethal action. In fact, cysteine has been shown by Simmons *et al* to synergize with spleen shielding. This, however, does not imply necessarily that the basic mechanisms underlying the protection are

* See page 116.

similar. The chemical agents under discussion are rather more general in their effects and are concerned apparently with the more immediate physico-chemical ramifications of energy transfer, probably because they serve as hydrogen donors. Tissue shielding and transplants may be thought of as procedures which do not modify the initial injury but rather encourage the recovery of specific physiological systems.

BETZ : Bacq has shown us his results on the depletion of the cortical cortex of irradiated rats and of those irradiated and protected by cysteamine. We made similar observations 5 years ago, when studying the behaviour of the adrenal cortex of mice protected by injection of KCN. We saw indeed that the animals protected by KNC had a much less marked depletion of the cortical lipids than the controls. Our observations were made on sections on the adrenal stained with Soudan III. I would like to add to Bacq's comment that it is perfectly possible to detect by histological means the depletion of the adrenal cortex. Even the first phase of depletion occurring after 2 or 3 hours and lasting a short time is to be seen histologically.

Bacq told us about a hypothesis on the mechanism of action of some chemical protectors. It is quite possible that some chemicals act by protecting a factor stimulating the haematopoietic regeneration, or by protecting some cells producing this factor. This is an interesting working hypothesis. So far as the mechanism of action of the spleen and bone marrow homogenates are concerned, I would like to suggest that they also act by the pathway of the nucleoprotein anabolism. Some results published by Kelly and Jones show that such preparations enhance the synthesis of DNA in irradiated animals.

I know very well Patt's work on the adrenal depletion in irradiated rats and I agree entirely with him. I repeated his chemical studies and found the same results. The terminal drop of the ascorbic acid and cholesterol in the adrenals of animals submitted to a lethal dose of X-rays seems to be a sign of hyperactivity rather than a sign of exhaustion of the gland.

I quite agree with Mole that the adrenals are necessary to the survival of irradiated animals. As I showed in my papers, our adrenalectomized rats were very sensitive to a whole body irradiation. Some amount of cortical hormones is necessary to maintain a normal resistance. But this does not rule out the possibility that a large excess of such hormone may be noxious to the haematopoietic tissues. We feel that the irradiated body produces an excess of cortical hormones : as the results shown here indicate, this excess plays a role in the inhibition of the haematopoiesis we observe after a lethal dose of X-rays.

J. S. MITCHELL : Although the methods of classical histology show no obvious changes shortly after irradiation, studies which I have carried out over a number of years with the use of ultraviolet photomicrography have shown changes in cellular nucleic acid metabolism in several types of cells within relatively short times after irradiation. In my early work [12, 13] on the accumulation of pentose nucleotides in cytoplasm after irradiation and inhibition of synthesis of deoxyribonucleic acid by irradiation, a substantial increase in cytoplasmic absorption at 2,537 Å was found in a number of human malignant tumours at 80 minutes after irradiation. The magnitude of the change was consistent with the presence in the irradiated cytoplasm of ribonucleotides in local concentration often of the order of 3 per cent. In further work [14, 15] experiments using ribonuclease in conjunction with quantitative ultraviolet photomicrography showed that the cytoplasmic nucleotides accumulating after irradiation were mainly ribo-polynucleotides closely resembling, and probably identical with, ribonucleic acid. Extraction methods showed characteristic nucleotide absorption with maximum at the wavelength 2,620 Å. It is important to note that the increase in ribonucleotide absorption occurs almost entirely after irradiation with doses less than the region of 750–1,000 r ; at higher doses usually there is no

accumulation of ribonucleotide absorption and often a definite decrease, presumably due to interference with less radio-sensitive processes in the disturbance of cellular nucleic acid metabolism (see also work to be published by L. D. Hamilton). Subsequent experiments have detected increased cytoplasmic absorption in a biopsy of a human basal-celled carcinoma of the skin taken immediately after delivery of 40 r of gamma radiation in 43 minutes.

Since 1940 I have been carrying out experiments on the irradiation of tissue cultures mainly of chick fibroblasts *in situ* on the stage of the ultraviolet microscope. Very little of this work has yet been published because of the difficulties of precise quantitative interpretation. It is of interest to include the result of an experiment (*Figure 2*) in which chick fibroblasts in culture (provided by Mrs. I. Simon-Reuss) received a dose of 435 r of X-radiation delivered in 6 minutes. There appears to be an increase in cytoplasmic absorption at 2,537 Å and also an increase in diffusible absorbing material in the photograph taken at between 2–6 minutes after the end of the X-ray exposure. At about 87 minutes after the irradiation the characteristic absorption is lost, the cells are breaking down and there appears to be gross damage to the chromosomes visible. A great many control experiments were carried out which

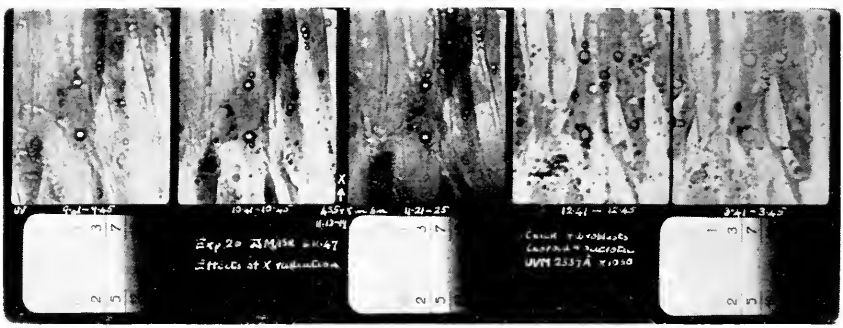


Figure 2. Serial ultraviolet photomicrographs of living chick fibroblasts before and after roentgen irradiation in situ *on the microscope stage at the times given. 6 mm quartz monochromat, N.A. 0·70, wavelength 2,537 Å. Roentgen ray dose was 435 r delivered in 6 minutes (87 kvp, no added filter; irradiation through quartz coverslip).*

showed that the ultraviolet radiation used for these exposures did not produce comparable effects.

Recently G. H. Hjort, working with me in Cambridge, has used the methods of ultraviolet photomicrography to study the effects of total body irradiation with doses between 100 and 600 r on the lymphocytes of lymphoid tissue in adult rats. The sections were prepared from frozen dehydrated tissue. It has been found that an increase in nucleotide type of absorption mainly in the cytoplasm can be detected as early as 15 minutes after irradiation and this biochemical change appears before any morphological changes can be detected. There is, however, wide variability in the behaviour of different cells in respect of these changes and much further quantitative examination of the experimental material is necessary. There is definite evidence that these early metabolic changes can be prevented by the administration of β-mercaptoethylamine before irradiation. Details of this investigation will be published later.

REFERENCES

[1] Bacq, Z. M. and Alexander, P. *Fundamentals of Radiobiology*. London, Butterworths, 1955. Liège, Thone ; Paris, Masson.

[2] Bacq, Z. M., Herve, A. et Scherber, F. *Arch. Intern. Pharmac. Thérap.* 1953, **94** 93.

[3] Bacq, Z. M., Fischer, P. et Beaumariage, M. L. *Bull. Acad. Méd. Belg.* 1954, in press.
[4] Mateyko, G. M. *et al.* *Amer. Journ. Physiol.* 1951, **167** 808.
[5] Brayer, F. T., Glasser, S. R. et Duffy, B. J., Jr. *Science*, 1954, **120** 112.
[6] Devik, F. *Brit. J. Radiol.* 1954, **27** 463.
[7] Devik, F. and Lothe, F. To be published.
[8] Patt, H. M. *et al.* *Amer. J. Physiol.* 1947, **150** 480.
[9] Patt, H. M. *et al.* *Science*, 1948, **108** 475.
[10] Edwards and Summers. *J. Lab. Clin. Med.* 1952, **40** 342.
[11] Schneider *et al.* Personal communication.
[12] Mitchell, J. S. *Nature, Lond.* 1940, **145** 105.
[13] Mitchell, J. S. *Brit. J. exp. Path.* 1942, **23** 285, 296, 309.
[14] Mitchell, J. S. *Brit. J. Radiol.* 1943, **16** 339.
[15] Mitchell, J. S. *Brit. Emp. Cancer Camp. 21st Ann. Rep.* 1944, 62.

CYTOLOGICAL EFFECT OF CHRONIC GAMMA IRRADIATION AND THE PROTECTIVE PROPERTY OF CERTAIN CHEMICALS AGAINST THE RADIATION INDUCED CHROMOSOME ABERRATIONS

KNUT MIKAELSEN

Institute of Genetics and Plant Breeding, Agricultural College of Norway, Vollebekk, Norway

MOST radiation effects are studied after acute radiation treatments over a short period of time. Effects of continuous or chronic radiation treatments over longer periods of time have been little investigated until recent years. Some results on effects of chronic gamma irradiation have been reported from gamma field radiation or other use of ^{60}Co[1, 2]. At the Agricultural College of Norway we had a ^{192}Ir isotope at work in 1953 and from 1954 a ^{60}Co source was operating in a gamma field. Some cytological results of chronic radiations and the protective property of certain chemicals against chromosome aberrations induced by irradiation will be presented.

Cuttings of *Tradescantia paludosa* were found most suitable for this study and were used as experimental material. Root development was initiated by placing the cuttings in tap water with continual aeration at ordinary greenhouse temperature.

When the cuttings showed good development of primary roots, they were transferred to specially designed lucite vessels which contained HOAGLAND and SNYDER's[3] nutrient solution. After 24 hours a fresh nutrient solution was substituted for the old and the plants were exposed to chronic gamma radiation from ^{60}Co. The roots were fixed immediately following the exposure period.

Two different experiments were performed. In the first experiment the plants were irradiated at dose rate of 12·5, 25, 50 and 100r/day. In another experiment the same dose rates were applied during a 48-hr exposure ; thus the roots received twice the total dose as in the previous experiment. Both acentric fragments and bridges were scored at anaphase, and the results are summarized in *Table I.*

Both fragment and bridge frequencies seem to be almost linearly proportional to dose. It is noteworthy, that the bridge frequencies are so low after chronic irradiation. The ratios between the frequencies of fragments and bridges is approximately 6 in all series, while after acute exposures to X-rays and neutrons the formation of bridges is much more frequent. Thus, restitutions resulting in chromatid exchanges seem to be difficult during the chronic radiation exposures.

By comparing the 24-hr and 48-hr exposure experiments, it seems obvious that the dose rate has been a more important factor in the yield of aberrations

than total dose given. The fragment frequencies were very similar at the same dose rate in the two experiments, while there is no correlation between fragments and total doses given.

This chronic radiation method is used to test certain chemicals for their protective ability against radiation-induced chromosome aberrations.

Table I.—Frequencies of chromosome aberrations in anaphase of meristematic root tissue of Tradescantia paludosa *after exposures to chronic gamma radiation from* ^{60}Co

Dose rate R/day	Total dose in R	No. of anaphase cells	No. of fragments	No. of fragments per 100 cells	No. of bridges	No. of bridges per 100 cells
(*a*) 48-hr exposure						
12.5	25	518	47	9·1	10	1·9
25	50	520	88	16·9	16	3·1
50	100	320	113	35·3	17	5·3
100	200	92	70	76·1	11	12·0
(*b*) 24-hr exposure						
12·5	12·5	396	26	6·8	5	1·3
25	25	388	61	15·7	9	2·3
50	50	417	122	29·3	22	5·3
100	100	293	175	59·7	15	5·1

Protective property of certain chemicals on chromosomes is reported by Mikaelsen[4, 5, 6], Riley[7], Forssberg and Nybom[8], Wollf[9] and Devik[10]. The results from my experiments are summarized in *Table II*, where glutathione, cysteine, thiourea, sodium hyposulphite ($Na_2S_2O_4$) and sodium cyanide (NaCN) proved to have protective property.

Table II.—Per cent reduction in fragment frequencies at different concentrations of the chemicals tested

Concentrations	Glutathione	Cysteine HCl low pH	Cysteine high pH	Thiourea	$Na_2S_2O_4$	NaCN
0	0	0	0	0	0	0
10^{-5}M	13	15			45	
5×10^{-5}M				7		10
10^{-4}M	36	44		44	41	11
3×10^{-4}M	50	60				
5×10^{-4}M			21			
1–1·5 $\times 10^{-3}$M	53	53		48	Toxic	25
$3 \cdot 10^{-3}$M	Toxic	Toxic				
$5 \cdot 10^{-3}$M			30			
10^{-2}M			35			

The chemicals were applied in the following manner. To the nutrient solution in which the *Tradescantia* cuttings were grown, the chemical compounds were added at certain concentrations. After one hour's preabsorption, the plants, still in these solutions, were exposed to chronic gamma radiation for 48 hours at a dose rate of 25 r/day which yielded a convenient

amount of fragments (*Table I*). By the use of the film badge method, it was indicated that the plants received a total dose of 50r in all experiments. The number of both acentric fragments and bridges per 100 cells at anaphase was determined. Conclusions regarding the effects of chemical treatments, however, were based upon the fragment data only, since bridges occurred in such low frequencies that it was difficult to evaluate the data (*Cf. Table I*).

Glutathione—In the presence of glutathione a marked and significant reduction in fragment frequency was obtained. The experiments included concentrations of glutathione ranging from 10^{-5}M to $1{\cdot}5 \times 10^{-3}$M. None of these concentrations seemed to cause any serious disturbances to the roots not subjected to radiation. The protective property increased with increasing concentrations and the maximum effect is obtained at $1{\cdot}5 \times 10^{-3}$M, where the fragment frequency was reduced 53 per cent. At a higher concentration (3×10^{-3}M) the roots became soft and were obviously injured. They were unsuitable for study.

Cysteine—Cysteine was applied as cysteine-HCl in two different series of experiments. The presence of cysteine-HCl, lowered the pH-value of the nutrient solution, particularly with the two strongest concentrations. One series of experiments was performed with these acid solutions. Thus, the plants were grown and exposed to cysteine under quite different pH-values. Judging by mitotic activity, the different pH-values did not seriously disturb the roots since equal numbers of dividing cells were present in all series.

The maximum effect of cysteine in acid solutions was 60 per cent reduction in fragment frequency at 3×10^{-4}M. A concentration of 3×10^{-3}M could not be tolerated and proved to be toxic to the roots.

In another series of experiments with cysteine-HCl, the solutions were neutralized with potassium hydroxide (KOH), so all cysteine and the control series had the same pH ($\sim$6) during the radiation exposure. Compared with the previous series, less reduction in fragment frequency was obtained. The maximum effect was a reduction of 35 per cent at 10^{-2}M.

In both series of experiments the protective effect of cysteine increased with increasing concentrations.

Thiourea—The chemical formula of thiourea is usually presented as NH_2—S—NH_2. In oxidation processes, however, it reacts[11] as a sulphydryl compound NH_2—SH=NH. Its effectiveness increased with concentrations and the maximum effect was 48 per cent reduction in fragment frequency at 10^{-3}M.

Sodium hyposulphite ($Na_2S_2O_4$)—Sodium hyposulphite, a strong reducing agent, showed a remarkable and optimal effect already at a concentration of 10^{-5}M, where the reduction in fragments amounted to 45 per cent. At 10^{-4}M no increase could be detected and 10^{-3}M proved to be toxic to the roots.

Sodium cyanide (NaCN)—With sodium cyanide a smaller but significant reduction of 25 per cent in fragment frequency was obtained at 10^{-3}M.

Conclusive evidence is presented of the modifying or protective property of glutathione, cysteine, thiourea, sodium hyposulphite, and sodium cyanide against chromosome fragmentation induced by gamma radiation. The mechanism by which these chemicals exert their protection is not clear. First, do these chemicals prevent chromosome breakage or do they heal broken

ends so that restitution of broken chromosomes is favoured ? A combination of both is an alternative possibility. Prevention of breakage is considered to be the most likely explanation. What arguments exist in favour of such an assumption ?

Barron and Flood[12] presume that such oxidizing agents are responsible for the oxidation of aqueous solution of certain thiols by ionizing radiation, as they have demonstrated. Therefore, it seems probable that the mechanism of the sulphydryl compounds, glutathione, cysteine and thiourea, may be due to their reactions with oxidizing agents produced by irradiation in the cell nuclei. These oxidizing agents may be inorganic or organic free radicals or peroxides. Thus, the sulphydryl compounds compete with the chromosomes for free radicals or other oxidizing reactants and less breaks will be produced.

It must also be considered that absorption of the sulphydryl compounds, as well as the other chemicals tested, may change the metabolic state of the cell. Patt[13] has reported that his data suggest perhaps that cysteine action is related to the availability of intracellular oxygen. It may be justifiable, at least, to conclude that the sulphydryl compounds exert their effects through their —SH groups since the results are so similar with the three compounds. It is reasonable to assume that sodium hyposulphite, which is a strong reducing agent and combines rapidly with molecular oxygen in aqueous solution, decreases the amount of dissolved oxygen in the tissue. It is assumed that by removing oxygen from the tissue, the production of the secondary formation of O_2H and H_2O_2 or other reactive products is prevented. King *et al*[14] claim, however, that in large measure oxygen exerts its action by altering the biological processes of the cell.

The protective ability of sodium cyanide may be more complicated and obscure than was the case with the other compounds tested. It appears, especially as regards the two strongest concentrations, that cyanide reduces the mitotic rate. Considerably fewer anaphases were found in these roots and the reduction in fragment frequencies was small compared with the concurrent experiments with the other chemical agents. These facts indicate that metabolic inhibition or metabolic changes may be the most reasonable explanation of the protective effect of sodium cyanide. Such metabolic alterations, involving complicated enzyme systems, initiated by KCN and CO are discussed by King *et al*[14]. The complicated effect of cyanide is demonstrated by D'Amato and Gustafsson[15] who showed that KCN treatment of seeds prior to X-irradiation increased visible mutations in barley with low concentrations. A stronger concentration of 10^{-2}M decreased the mutation rate, although the rate of chromosome breakage increased. Bacq[16] found that cyanide decreased the mortality in X-irradiated mice.

By comparing the effect of cysteine in acid and neutral medium, a marked difference in protection and tolerance is noticed. A similar response in the effect of cysteine, applied at different pH-values, on survival of mice after whole body X-irradiation was obtained by Patt *et al*[17, 18] and Goldie *et al*[19]. They state that the probable explanation of this difference may be the fairly rapid oxidation of cysteine to cystine in a neutral medium before its administration. Marshak[20] has shown that changes in intracellular

acidity alter the radio-sensitivity of the cell. It seems justifiable, however, in this case to put the main emphasis on the oxidation of cysteine to cystine.

Although the protective actions of these chemicals are not understood, indications of an indirect effect of radiation on chromosome fragmentation are demonstrated. This indirect effect seems to be of great importance in the production of chromosome breaks, since it is possible to reduce the radiation damage to about half in most of the cases. Unfortunately, however, it will be difficult to determine the exact relation between direct and indirect radiation effect by chemical means, because of their toxic effects on roots at certain concentrations.

REFERENCES

[1] Sparrow, A. H. *Science*, 1951, **114** 488.
[2] Sparrow, A. H. and Singleton, W. R. *Amer. Nat.* 1953, **87** 29.
[3] Hoagland, D. R. and Snyder, W. C. *Proc. Amer. Soc. Hort. Sci.* 1933, **30** 288.
[4] Mikaelsen, K. *Science*, 1952, **116** 172.
[5] Mikaelsen, K. *Proc. 9th Internat. Congress of Genetics*, 1953.
[6] Mikaelsen, K. *Proc. Nat. Acad. Sci.* 1954, **40** 171.
[7] Riley, H. P. *Rec. Genetics Soc. Amer.* 1952, **21** 62.
[8] Forssberg, A. and Nybom, A. *Physiol. Plant.* 1953, **6** 78.
[9] Wolff, S. *Genetics*, 1954, **39** 356.
[10] Devik, F. *Brit. J. Radiol.* 1954, **27** 463.
[11] Whitmore, F. *Organic chemistry*, New York, 1951.
[12] Barron, E. S. and Flood, V. *J. Gen. Physiol.* 1950, **33** 229.
[13] Patt, H. M. This Symposium.
[14] King, E., Schneiderman, H. and Sax, K. *Proc. Nat. Acad. Sci.* 1952, **38** 34.
[15] D'Amato, F. and Gustafsson, Å. *Hereditas*, 1948, **34** 181.
[16] Bacq, Z. M. *Science*, 1950, **111** 356.
[17] Patt, H. M., Tyree, E. B., Straube, R. L. and Smith, D. E. *Science*, 1949, **110** 213.
[18] Patt, H. M., Smith, D. E., Tyree, E. B. and Straube, R. L. *Proc. Soc. exptl. Biol. Med.* 1950, **73** 18.
[19] Goldie, H., Tarleton, G. T. and Hahn, P. F. *Proc. Soc. exptl. Biol. Med.* 1951, **77** 790.
[20] Marshak, A. *Proc. Soc. exptl. Biol. Med.* 1938, **39** 194.

THE EFFECT OF RADIATION ON FROZEN TUMOUR CELLS

ILSE LASNITZKI*

Strangeways Research Laboratory, Cambridge

IT has been shown by several observers that the response to radiation can be influenced by the metabolic activity of irradiated biological material. GLUCKSMANN and SPEAR[1] demonstrated that in tadpoles the radiation effect was delayed if the animals were chilled during and after exposure. GOLDFEDER[2], comparing the radio sensitivity of histologically similar mouse tumours found the radiation effect more marked in those showing a lower metabolic rate. A similar result was obtained by me[3] with the S37 tumour exposed as ascites and subcutaneous form. For the ascites form, which according to WARBURG and HIEPLER[4] functions at very low energy levels, the MLD found was one-fifth of that for the solid sarcomas.

In recent years CRAIGIE[5] and other workers have shown that certain tumours preserve their viability if frozen and kept at temperatures of dry ice and liquid air. This ability should make them a convenient material to study the effect of radiation in the frozen state at which metabolic activity is at a standstill, and in the present experiments the effects of radiation on tumours in the fresh and frozen state were compared.

EXPERIMENTAL

The material used was the Ehrlich ascites tumour. This tumour is frozen and stored on dry ice as a routine in our laboratory as an alternative to serial transplantations and samples stored, for instance, for several months proved to be viable on re-inoculation. The tumour cell suspensions were frozen at $-79°$ and kept at this temperature for 7 days prior to irradiation. Exposure took place *in vitro*. The dose was 1,500 r units at 75 r/min. Two experiments were made. In the first one the frozen cell suspension was exposed on the laboratory bench and gamma rays were used; in the second one it was irradiated on dry ice and X-rays were used. The frozen cells were thawed quickly immediately after exposure and injected subcutaneously into C3H mice. For comparison of the radiation effect fresh cells obtained from the same tumour sample were exposed in an identical manner and inoculated subcutaneously into the same strain of mice. Each set had its own unirradiated fresh and frozen control grafts. Each inoculum contained approximately 15×10^6 tumour cells. The criterion of radiation effect was the number and size of subcutaneous tumours obtained.

RESULTS

Figure 1 gives the percentage take obtained after inoculation of frozen and fresh unirradiated grafts. One week following inoculation it is 80 per cent for grafts derived from fresh and 90 per cent for grafts from frozen cells, an

* Sir Halley Stewart Fellow.

indication that freezing alone does not impair their viability. *Figure 2* shows the percentage take of fresh irradiated and control grafts. The appearance of tumours derived from irradiated grafts is delayed for one

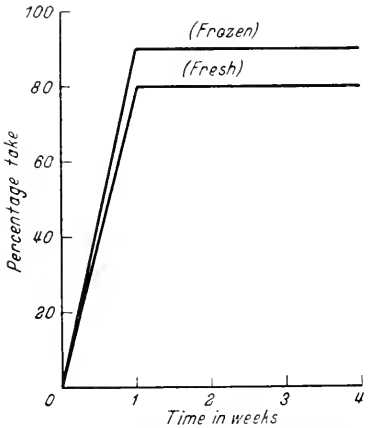


Figure 1. Percentage take of frozen and fresh unirradiated grafts (gamma ray experiment)

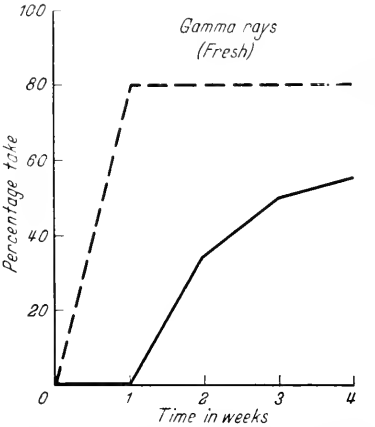


Figure 2. Percentage take of fresh irradiated (gamma rays) ——— and fresh control grafts – – – – –

week as compared with the unirradiated controls ; two weeks after inoculation it is 34 per cent ; at four weeks it is 56 per cent. *Figure 3* gives the results obtained with frozen irradiated grafts. In contrast to the fresh

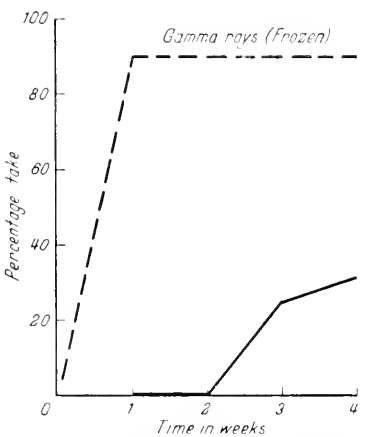


Figure 3. Percentage take of frozen irradiated (gamma rays) ——— and frozen control grafts – – – – –

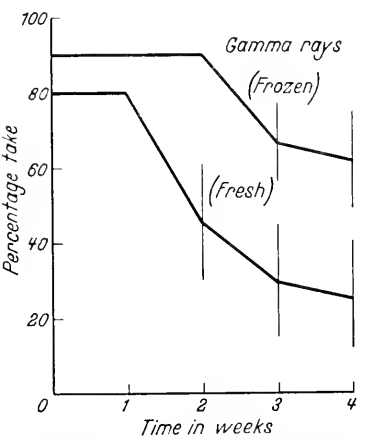


Figure 4. Comparison of the inhibition of tumour takes from irradiated (gamma rays) fresh and frozen grafts expressed as the difference from the control percentage take

irradiated cells the first appearance of tumours is delayed longer and the percentage take is significantly smaller. At three weeks after grafting it is 24 per cent and at four weeks 31 per cent. *Figure 4* gives a direct comparison

of the radiation effect on cells exposed in the fresh and frozen state expressed as the difference in percentage take from their respective controls. The upper line gives the difference of tumours derived from frozen, the lower line that obtained from fresh cells. Taking the results at 3 and 4 weeks following grafting, the effectiveness of radiation is increased for the frozen cells by a factor of two.

The greater effectiveness of radiation on frozen cells is further borne out by measurements of tumour sizes. *Figure 5* gives the tumour volume in mm^3 for irradiated frozen and fresh grafts. Four weeks following inoculation

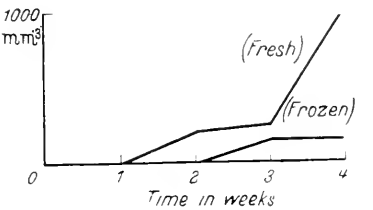


Figure 5. Tumour volume in mm^3 *obtained from irradiated (gamma rays) fresh and frozen grafts*

tumours derived from fresh cells have reached a size of 1,000 mm^3 as against 170 mm^3 for those obtained from frozen cells.

Cell counts made in the growing edge of established tumours show that the mitotic rate is similar whether the tumours are derived from fresh or frozen irradiated cells but that the proportion of abnormal meta- and ana-phases is increased in tumours obtained from frozen grafts. *Figure 6* illustrates the results of cell counts made on tumours four weeks after implantation. The two columns on the left represent mitosis as a percentage of

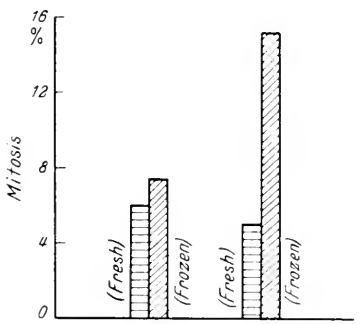


Figure 6. Mitosis expressed as percentage of resting cells (left-hand columns) and abnormal meta- and ana-phases expressed as the percentage of total mitosis (right-hand columns)

resting cells. It is 6 per cent of resting cells for tumours derived from irradiated fresh cells, and 7·2 per cent for tumours derived from irradiated frozen cells. The two columns on the right give the proportion of abnormal meta- and ana-phases as a percentage of the total mitotic count. The figures are : 5 per cent for fresh and 15·3 per cent for frozen tumours. It is reasonable to assume that some of these abnormalities do not produce viable daughter cells and that this loss in viability may, at least partly, be responsible for the slower growth rate in spite of an equal mitotic index.

The greater effectiveness of radiation in the case of frozen tumours was rather contrary to expectation. Exposure, in these experiments, took place on the bench immediately after the removal of the frozen tumour from the

dry ice. A rise in temperature was therefore possible at the time of irradiation. Normally, a rise in temperature up to —20° for short periods was found not to have any injurious effects on cell survival, but the possibility

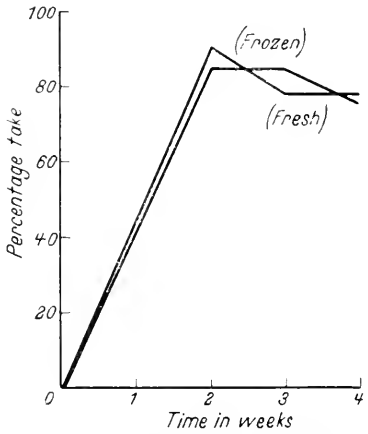


Figure 7. Percentage take of frozen and fresh control grafts. (X-ray experiment)

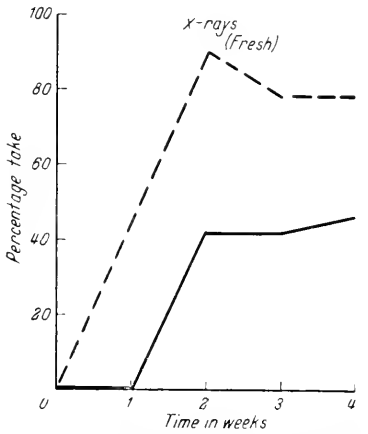


Figure 8. Percentage take of irradiated (X-rays) fresh ——— and fresh control – – – – – grafts

could not be excluded that the existence of a temperature change may have increased the radiation damage. To settle this point, the experiment was repeated with the modification that the frozen material was kept on dry ice during exposure and that X-rays instead of gamma radiation was used.

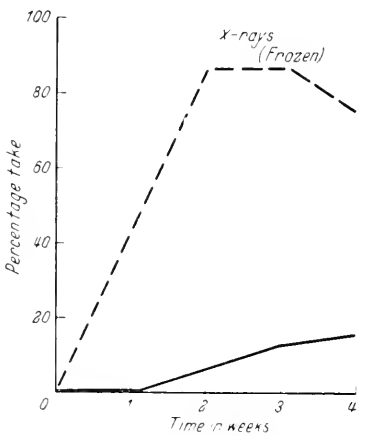


Figure 9. Percentage take of irradiated (X-rays) frozen ——— and frozen control grafts – – – – –

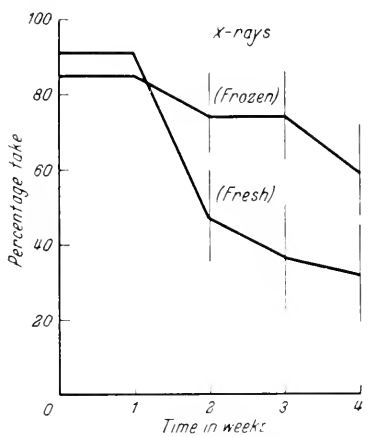


Figure 10. Comparison of inhibition of tumour takes from irradiated (X-rays) frozen and fresh grafts expressed as the difference from the control percentage take

Figure 7 gives the percentage take for the second series of fresh and frozen controls which is similar for both types of cells and shows again that viability is unimpaired by freezing alone. *Figure 8* shows the percentage takes of

fresh control and irradiated grafts following a dose of 1,500r of X-rays. The reduction is of the same order as in the gamma ray experiment : it is 42 per cent at two weeks and 46 per cent at four weeks following implantation. *Figure 9* gives the results obtained with frozen control and irradiated cells. The take is 13 per cent at two weeks, and 18 per cent at four weeks after grafting. *Figure 10* gives a direct comparison of the radiation effect expressed again as the difference in percentage take from that of the controls. Taking the points at three and four weeks the radiation effect for frozen tumour is here again increased by a factor of approximately two.

The second experiment thus confirms the results obtained with the gamma ray and shows that the rise in temperature cannot have been responsible for the increased radiation effect on frozen cells. The greater effectiveness of radiation on frozen tumour cells seems, at this stage at least, difficult to interpret. According to current views (Craigie[5]), freezing involves a certain degree of cytoplasmic dehydration. The ensuing intracellular increase in salt concentration may render the cells more vulnerable to ionizing radiations although it may not impair cell survival under normal conditions. Another alternative, suggested tentatively, is that at the temperature prevailing during exposure the movement of free radicals and ions produced by radiation may be restricted more for one species than for another : in this case less recombination would occur and more free radicals remain available for interaction with biological structures which under the conditions of the experiments return to normal temperature and activity shortly after exposure.

It is hoped to throw more light on this matter by further experiments in which thawing and reinoculation after irradiation will be delayed.

REFERENCES

[1] Glucksmann, A. and Spear, F. G. *Brit. J. Radiol.* 1939, **12** 486.
[2] Goldfeder, Anna. *Radiology*, 1951, **57** 845.
[3] Lasnitzki, Ilse. *Brit. J. Radiol.* 1953, **27** 228.
[4] Warburg, Otto and Hiepler, E. *Z. Naturforsch*, 1952, **7b** 3, 193.
[5] Craigie, J. *Advances in Cancer Research*, 197, New York, Academic Press, 1954.

MECHANISMS OF MUTATION PRODUCTION IN MICRO-ORGANISMS

HOWARD B. NEWCOMBE

Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

A PECULIARITY in the action of X-rays on the spores of one of the *Actinomycetes* was noted by KELNER[1] in 1948. It appeared that high doses of X-rays altered the capacity of the spore suspension for further mutational response, so that still higher doses caused no corresponding increase in the proportions of mutant spores found among the survivors. In some experiments the proportion of mutants reached a plateau and remained unaltered as the dose was increased, while in other experiments the mutant frequency rose to a maximum and then declined as the dose was raised still further.

This observation was of interest because it indicated a similarity in the actions of X-rays and ultraviolet light which had not previously been suspected. The dose-mutation curves for ultraviolet had been known for some time to be non-linear in a wide range of experimental organisms, showing either a plateau, or a peak followed by a decline ; but in the same materials, where the comparison was made, the corresponding response to X-rays was notably linear. *Streptomyces* is exceptional in that it is the one organism so far studied in which a similar saturation or impairment of the capacity for induced mutation has been observed at high doses, with both ultraviolet and X-rays.

For this reason we decided to carry out a further study of the radiation response relationships in another strain of this organism, and I shall describe certain of the experiments into which this has led us, and the bearing which the results appear to have on the problem of the origin of the induced mutations.

MATERIALS

It should be emphasized that *Streptomyces* is exceptionally well suited to quantitative experimentation. The spores are uninucleate, and suspensions can be prepared in which there is no clumping. The induced mutations, which affect both colony morphology and colour, are numerous and clearly recognizable ; and to observe them one has only to plate suitable dilutions of the treated suspensions and examine the resulting colonies. It is thus possible to plan experiments in which large numbers of mutants can be scored, and where necessary the experiments can be repeated many times, often on successive days with the scoring being done the following week. (For media, and methods of handling, see NEWCOMBE[2].)

The induced changes do not revert to the wild type, except as rare events in certain mutant lines. However, many of them give rise to a range of further mutational changes with very high frequencies, and for this reason the initial changes might be described as mutations to a 'variegated' form.

There seems to be an almost limitless variety of these subsequent changes, occurring apparently in a stepwise manner ; most of these are associated with still further instabilities, and so on indefinitely. The result is an apparently limitless variety of mutant types which it would be very difficult to account for in terms of a cytoplasmic origin. For this reason it seems altogether probable that the initial changes are either genic or gross chromosomal in nature, and it is quite possible that both kinds of change occur. It should be noted that even the unstable variant lines can be subcultured almost indefinitely without apparent change, provided one selects at each subculture a colony of a certain appearance ; thus the alterations are clearly heritable.

DOSE-RESPONSE RELATIONSHIPS

Our initial experiments confirmed Kelner's finding ; the X-ray dose-mutation curve reached a peak in the vicinity of 8,000r, and the proportion of mutants fell to approximately one-half when this dose was doubled. A similar peak was found for the corresponding ultraviolet curve at about 400 ergs per mm^2. Further, it appeared that the non-linearities must have a common cause, since the mutagenic effects of ultraviolet and ionizing radiation were less than additive in combined treatments (NEWCOMBE and MCGREGOR[3]).

FRACTIONATION EXPERIMENTS

When it is found that a certain dose of radiation causes some change in the sensitivity to a second and similar exposure, it is of interest to know whether this sensitivity change is permanent or whether, when the second exposure is sufficiently delayed, there is some recovery of the original capacity to respond so that the effects of the two exposures become more nearly additive. Fractionation experiments of this nature were carried out, the spores being incubated in medium for as long as eight hours between two exposures of 8,000r each. No appreciable change was found in the mutational response to the second irradiation (*see Table I*).

When spores which had received the first exposure and had been incubated for the eight hours were stained, about 2 per cent of them were found to have undergone a single nuclear division. Also, when the suspensions were plated at this stage about 2 per cent of the spores grew into visible colonies. Since these proportions are very nearly the same, it seems likely that the spores in which nuclear division was observed were in fact the survivors. If this was the case it would appear that the capacity to respond to the mutagenic effect of a second exposure must remain impaired even after a nuclear division has intervened.

A further inference, likewise relevant to the nature of the non-linear response relationship, can be drawn from this experiment. It will be noted that with an eight-hour incubation between the two irradiations, there was almost no additional killing from the second exposure. (Two per cent of the spores survived the first irradiation, while 95 per cent of these survived the second.) It has been suggested that the plateau or decline in proportion of mutants with increasing dose could be due to differential killing of the induced mutant cells. However, in this experiment, where the second

exposure caused almost no additional killing, the proportion of mutants still failed to rise and in fact declined appreciably.

Differential killing of those cells which would otherwise have developed into mutant colonies can be ruled out with an even greater degree of certainty, as the explanation of a similar non-linear response found in a different type of cell. As a part of the above experiment, spore suspensions were also incubated without any preliminary irradiation, and were then

Table I.—Effect of fractionation on the consequences of doubling the dose of X-rays

A non-linear mutagenic response to the second irradiation, in the absence of altered survival (Dose : x—8000r. Incubation in medium)

Treatment (Irr.-Inc.-Irr.)	*Mutation*			*Survival* per cent
	Mutant colonies	Total colonies	Per cent mutation	
4-hour incubation				
x-inc-o	1,160	4,598	25·2	3·41
xx-inc-o	187	1,055	17·7	0·78
x-inc-x	249	1,439	17·3	1·07
6-hour incubation				
x-inc-o	409	1,822	22·5	2·26
xx-inc-o	109	517	21·1	0·64
x-inc-x	149	944	15·8	1·17
8-hour incubation				
x-inc-o	249	1,158	21·5	1·95
xx-inc-o	60	265	22·6	0·45
x-inc-x	202	1,104	18·3	1·86

Growth of 'x-inc' suspensions : 4 hours, no germination, no nuclear division ; 6 hours, 7·8 per cent germination, 4 binucleate cells out of 380 ; 8 hours, 8·0 per cent germination, 4 binucleate cells out of 187.

Controls : untreated, 9 mutation colonies out of 814=1·1 per cent ; o-inc-o with 4 hours incubation 45/1,225=3·7 per cent, with 6 hours 24/732=3·3 per cent, and with 8 hours 16/541=3·0 per cent

Note : All incubations carried out in closed metal boxes with little or no air-space above suspension.

exposed to the single and the double dose of X-rays (*see Table II*). Where this incubation was for an eight-hour period, the spores grew into short mycelial strands containing an average of about eight nuclei apiece. These strands, as might be expected, were very resistant to the lethal action of X-rays, the single and the double exposures both resulting in a net survival of 50 per cent. The proportion of cells which developed into mutant colonies was likewise similar at the two doses (34 and 39 per cent respectively). We cannot in this case suppose that the second exposure induced as many mutations as the first (but killed a disproportionate number of the mutant

cells) since this could not have occurred without a noticeable change from the 50 per cent survival.

It is conceivable, of course, that in a suspension of multinucleate strands the shape of the dose-mutation curve might be influenced by factors other than those operating in the uninucleate spores. However, the direction and extent of the deviation from a linear response are very similar in uninucleate spores, binucleate spores, and 8-nucleate strands ; and the evidence against a selective killing of cells which would otherwise give rise to induced mutant colonies, is likewise consistent throughout.

Table II.—Effect of pre-incubation on the consequences of doubling the dose of X-rays

A non-linear mutagenic response to the second irradiation, in the absence of altered survival
(Dose : x=8,000r. Incubation in medium)

Treatment (Irr.-Inc.-Irr.)	*Mutation*			*Survival* per cent
	Mutant colonies	Total colonies	Per cent mutation	
4-hour incubation				
o -inc-x	708	3,178	22·3	2·36
o-inc-xx	302	1,237	24·4	0·92
6-hour incubation				
o-inc-x	3,277	5,709	57·4	7·09
o-inc-xx	2,125	3,129	67·9	3·89
8-hour incubation				
o-inc-x	924	2,709	34·1	50·07
o-inc-xx	1,073	2,733	39·3	50·52

Growth : no nuclear division at 4 hours ; spores mostly binucleate at 6 hours ; approximately 8-nucleate strands at 8 hours.

Controls : as in previous table.

Note : All incubations carried out in closed metal boxes with little or no air-space above suspensions.

The non-linearity might perhaps be accounted for in terms of a grossly heterogeneous population, but we would have to make some very improbable assumptions : not only that the part of the population which resists killing consists of two fractions (one mutation-resistant and one mutation-sensitive), but also that the difference between resistance and sensitivity is extreme with respect both to killing and to induced mutation, and that intermediate degrees of resistance are virtually absent.

Since a differential killing of induced mutants can be ruled out, and an interpretation in terms of a population heterogeneity is only remotely possible, it seems probable that high doses of radiation saturate (or otherwise interfere with) some essentially intracellular response on which most of the induced mutation, and apparently a part of the killing, depend.

SENSITIVITY TO INDUCED MUTATION AT DIFFERENT STAGES OF GROWTH

It was also noted in these experiments, that the sensitivity to induced mutation varied with the period of preliminary incubation in medium (*see Table II*), and this suggested that such changes might be related in some way to the different stages in the nuclear division cycle as had been found in higher organisms (for review see SPARROW[4]). Fortunately, the first nuclear division is nearly synchronous in *Streptomyces* spore suspensions (McGregor), and with the aid of parallel cytological observations it has been possible to study in detail the sensitivity changes occurring during this division and the subsequent stages of germination and mycelial growth.

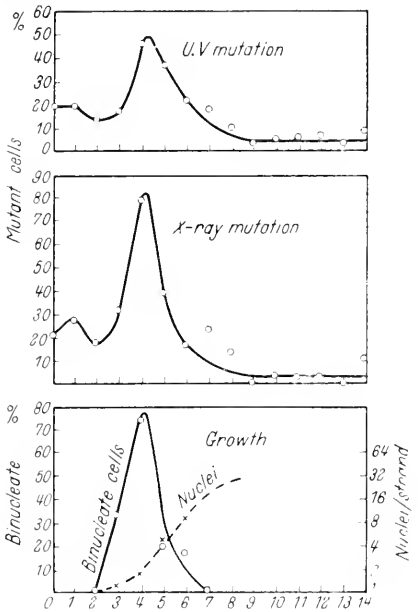


Figure 1.

Inoculum : $1{\cdot}2 \times 10^7$ spores per ml of medium.

Incubation : 7 hours first day, refrigerated overnight, 7 hours second day ; samples from 7-hour suspension irradiated both before and after the refrigeration (with ultraviolet the storage caused an increase from 18·5 to 20·4 per cent mutants, with X-rays a decrease from 24·0 to 9·4 per cent) ; suspension agitated at intervals throughout incubation.

Growth : peak proportions of strands with,
3 to 5 nuclei = 37 per cent at 5 hours,
6 to 10 nuclei = 58 per cent at 6 hours (40 per cent at 5 hours) ;
higher numbers difficult to score with accuracy.

Irradiation : samples diluted $1/10^2$ with saline before irradiation ; ultraviolet 200 ergs per mm^2, X-rays 8,000 r.

Survival : per cent at 0, 1, 2, ... 14 hours,
for ultraviolet—3, 5, 3, 3, 8, 39, 50, 78, 69, 87, 85, 91, 81, 84, 100.
for X-rays—17, 8, 3, 4, 18, 42, 58, 10, 51, 64, 73, 67, 59, 92, 66.

Unirradiated controls showed 0·5 per cent mutation and 100 per cent survival at 0, 7, and 14 hours.

Each point in the curves for ultraviolet and X-ray mutation curves was based on approximately 400–1,200 colonies.

The results of a typical experiment are shown in *Figure 1*. A spore suspension was incubated in medium over a 14-hour period, samples being taken at hourly intervals. One part of each sample was irradiated with X-rays (approximately 8,000 r) and another with ultraviolet (200 ergs per mm^2) these being plated at a range of dilutions, while a third was fixed and later stained for cytological study. For the sake of convenience the incubation was interrupted after 7 hours and the suspension stored in the cold overnight. Samples taken before and after the storage, and treated in the usual manner, showed that the interruption had not caused any major change in response.

The proportion of induced mutations was observed to increase to more than double when the spores had become binucleate at the time of irradiation (from 22 up to 79 per cent for X-rays, and from 19 up to 46 per cent for ultraviolet), and to decline to 10 per cent or less as multinucleate strands developed. As the two curves are based on approximately 6,000 mutants scored, out of a total of approximately 30,000 colonies observed, they are relatively accurate. Also, this general relationship between mutability and number of nuclei has been found consistently in other similar experiments. It should be noted also that when the cells are most sensitive to the mutagenic effects of the irradiation, they tend in addition to be most sensitive to the lethal effects.

Table III.—Sensitivity to X-ray induced mutation

Effect of pre-incubation in the absence of nutrient

(Spores harvested in saline ; diluted $1/10^2$ in saline before incubation. Combined data from two experiments. Dose, 8,000r)

Pre-incubation (hours)	*Induced mutation*		
	Mutant colonies	Total colonies	Per cent mutation
0	511	2,761	18·5
2	818	4,145	19·7
4	590	3,035	19·4
6	746	3,320	22·5
24	818	4,406	18·6
48	505	3,094	16·3

Growth during pre-incubation : at 3 days, out of 68 spores examined, 48 were still uninucleate, 2 binucleate, and 22 were very much shrunken and appeared dead.

Unirradiated control : 0·5 per cent mutation (18/3,439) ; no apparent difference between 0 hour and 9–11 day incubation.

Survival : in unirradiated controls, 40–50 per cent after 9–11 days' incubation ; in irradiated samples, between 0·06 and 0·18 per cent throughout.

The frequency of induced mutation eventually declines to 9·4 per cent (232/2,479) when irradiated after 9–11 days' incubation.

Certain similarities in the actions of X-rays and ultraviolet should be noted. The peak sensitivity coincides in both cases with the binucleate state ; the extent of the increase is similar ; and so also is the extent of the subsequent decline in sensitivity. In addition there are minor irregularities so that certain of the points do not fall precisely on a smooth curve ; these are probably significant, and they deviate in the same directions for the two agents. The X-ray and ultraviolet survivals are also similar in their major features ; that is, sensitivity is high during the binucleate stage, and strikingly low when many nuclei are present.

These variations in response could be due directly to the observed nuclear changes, or alternatively, the association could be fortuitous, and the result of some loosely associated physiological changes. However, our early attempts to separate the two failed. It was found that spores incubated in saline which lacked nutrient, showed neither the nuclear divisions nor

the characteristic (X-ray) sensitivity changes, over a 48-hour period (*see Table III*). The test was carried out using a stock saline suspension of spores (which contained traces of nutrient leached from the agar on which the spores had been grown) and diluting by a factor of 1/100 with pure saline to reduce the amount of nutrient. When a parallel experiment was carried out without dilution, a proportion of the spores (between a quarter and a half of them) were able to germinate and grow into short multinucleate strands, while the rest remained uninucleate. Under these conditions the characteristic rise and decline in sensitivity to X-rays could be observed, but was less striking than under the standard conditions (*see Table IV*).

It seemed important at this stage to determine whether, as we supposed, the sensitivity changes were peculiar to the growing spores, and absent in

Table IV.—Sensitivity to X-ray induced mutation

Effect of pre-incubation in the presence of traces of nutrient
(Spores harvested from nutrient agar culture by washing with saline ; incubated undiluted. Combined data from two experiments. Dose, 8,000r)

Pre-incubation (hours)	*Induced mutation*		
	Mutant colonies	Total colonies	Per cent mutation
0	630	2,429	25·9
2	573	2,099	27·3
4	391	1,310	30·1
6	567	1,588	35·7
24	658	7,615	8·7
48	225	2,184	10·0

Growth during pre-incubation : at 1 day 20–24 per cent germination, average approximately 10 nuclei per germinated spore ; at 2 days, no detectable change.

Unirradiated control : 0·4 per cent mutation (3/857).

Survival : 0·07–0·27 in one experiment ; 0·02–0·04 in the other. (There was no appreciable difference between the two experiments with respect to mutation.)

The frequency of induced mutation remains at a low level, 9·9 per cent (325/3285), when irradiated after 6–14 days' incubation.

those which remained uninucleate. To confirm this, a suspension which had been incubated in this manner for 28 hours, and which had become relatively insensitive to the mutagenic effects of X-rays, was passed through very fine filter paper (Watman No. 3) to remove the mycelial strands. This filtrate, which contained mainly the ungerminated spores, showed a threefold increase in sensitivity to X-ray induced mutation (*see Table V*).

A similar increase would also be expected as the result of filtering the suspension after it had been irradiated, and this was in fact observed (*see Table V*). Thus the effect could not be due to some subtle change in the condition of the suspension at the time of irradiation, and it is clear that the greater part of the sensitivity changes observed during the incubation must be associated with the growing cells, and that they are not a function of the suspension as a whole.

Somewhat similar evidence was obtained from the changes which occurred under more nearly normal conditions. In most of the experiments where the spores were incubated in medium prior to irradiation, the sensitivity fell off rapidly as the average number of nuclei increased, to four or eight per strand. In one experiment, however, the decline was much more gradual in relation to the nuclear increase, and this was found to be associated with a more poorly synchronized growth, due apparently to an unusual degree of crowding ; the spores passed through the first nuclear division together, but a proportion of them lingered much longer than usual in the sensitive binucleate state.

Thus all of the evidence so far, emphasizes a close association between nuclear number and response to radiation, which can be observed over a wide range of conditions of growth. The sensitivity changes do not seem

Table V.—Sensitivity to X-ray induced mutation

Effect of pre-incubation in the presence of traces of nutrient
Effect of removal of germinated spores by filtration
(Spores harvested from nutrient agar culture by washing with saline ; incubated without dilution. Dose, 8,000r)

Pre-incubation	*Treatment*	*Induced mutation*		
		Mutant colonies	Total colonies	Per cent mutation
0 hour	Irradiated	586	2,425	24·2
28 hour*	Irradiated†	45	956	4·7
28 hour*	Irradiated† and Filtered	146	1,049	14·0
28 hour*	Filtered and Irradiated	169	1,063	15·9

* Same incubated suspension. † Same irradiated suspension.

Unirradiated controls : 0-hour, 0·7 per cent mutation (15/2,015) ; 28-hour, 0/9 per cent (13/1,509).

Survival : in unirradiated control, 75 per cent after 28 hours' incubation ; in 0-hour irradiated 0·12 per cent ; 28-hour irradiated 0·5 per cent ; irradiated-filtered and filtered-irradiated 0·05 per cent each.

to be influenced by the particular stage in the nuclear division cycle, since the period of maximum sensitivity apparently extends over the whole of the binucleate state and not just a part of it, and the nuclear divisions subsequent to the first are almost completely unsynchronized. But it is still not certain that nuclear number directly determines the response, since changes in both nuclear number and response might have a common physiological cause.

SENSITIVITY DIFFERENCES WHICH ARE NOT DUE TO NUCLEAR NUMBER

It also appeared that other differences in the state of the cell at the time of irradiation could influence the proportions of induced mutant colonies, even where the nuclear number was constant. This was indicated by minor irregularities in certain of the experimental results, such as those

noted in *Figure 1.* In addition the effects of various treatments which might influence the physiological state were tested using both multinucleate strands and uninucleate spores. Three such tests will be described.

In the first, spore suspensions containing $6 \cdot 5 \times 10^7$ spores per ml of medium were grown under the following sets of alternative conditions : (*a*) with 5 ml of air, and with 20 ml of air, in a 30 ml tube ; (*b*) incubating continuously for 32 hours, and incubating for four 8-hour periods with storage in the cold after each ; and (*c*) sealing the tubes lightly with plastic screw caps, and covering with loose aluminium caps ; these being applied in all combinations to make a total of eight different treatments. The amount of growth was very similar in all cases, the resulting strands being

Table VI.—Effect on frequency of spontaneous mutant colonies of amount of air available during preliminary growth

Amount of Air (in 30 ml tube)	*Other treatments*	*Mutation*			
		Mutant colonies	Total colonies	Per cent	Factor increase
5 ml	A (CT5)*	10	644	1·6	
	B (CL5)	7	633	1·1	
	C (IT5)	16	591	2·7	
	D (IL5)	8	345	2·3	
20 ml	A (CT20)	52	790	6·6	4·1
	B (CL20)	45	378	12·0	10·9
	C (IT20)	29	591	4·9	1·8
	D (IL20)	34	430	7·6	3·3

* C=continuous incubation (32 hours).
I=intermittent incubation (4 eight-hour periods, at 24-hour intervals).
T=tube tightly capped (plastic screw cap).
L=tube loosely capped (aluminium cap).

Inoculum $6 \cdot 5 \times 10^7$ spores per ml of medium.

Growth : CT5 and CL5, strands 4–10 diameters in length ; in other suspensions 6–14 diameters in length. Nuclei stained clearly where the incubation was intermittent ; where the incubation was continuous, the nuclei were obscured by stainable material in the form of much larger cylindrical bodies which nearly filled the strands.

for the most part more than four times their own diameter in length, and less than sixteen times. No difference was observed as the result of the two different kinds of caps used, and these treatments can be considered as replicates.

Samples from all eight treatments were plated without irradiation, and irradiated with standard doses of X-rays and of ultraviolet. The greater quantity of air resulted throughout in a considerably higher spontaneous mutant frequency (5 to 12 per cent, as compared with 1 to 3 per cent ; *see Table VI*) ; the numbers of ultraviolet induced mutants was not noticeably affected, but the X-ray induced mutants were reduced to about one half. Intermittent incubation on the other hand reduced the numbers of ultraviolet induced changes to half, but had little effect on the X-ray changes. There was also a consistent effect of intermittent incubation on the staining

of the cells, the nuclei being clearly visible in a lightly stained cytoplasm, whereas with continuous incubation the stainable material nearly filled the cell.

Table VII.—Effect on X-ray mutability of amount of air available during preliminary growth
(X-ray dose 16 min at 50 cm)

Amount of Air (in 30 ml tube)	*Other treatments**	*Mutation*				
		Mutant colonies	Total colonies	Per cent	Minus control*	Factor decrease
5 ml	A (CT5)	40	475	8·4	6·9	
	B (CL5)	29	386	7·5	6·4	
	C (IT5)	39	362	10·8	8·1	
	D (IL5)	20	242	8·3	5·9	
20 ml	A (CT20)	59	547	10·8	4·2	0·61
	B (CL20)	41	297	13·8	1·8	0·28
	C (IT20)	49	394	12·4	7·5	0·93
	D (IL20)	35	314	11·2	3·6	0·61

* For unirradiated controls, size of inoculum, other treatments, and amount of growth see *Table VI.*

Per cent survival = 73·7, 58·2, 61·2, 70·1, 69·4, 78·5, 66·7, 69·8, respectively.

In a second experiment, spores were grown into somewhat longer strands, having lengths of about thirty times their own diameter and probably containing in the vicinity of thirty nuclei. The spores were incubated intermittently, that is for 7 hours each day up to a total of 35 hours,

Table VIII.—Effect on ultraviolet mutability of continuous versus intermittent incubation during preliminary growth
(Ultraviolet dose, 200 ergs per mm²)

Continuous or intermittent	*Other treatments**	*Mutation*				
		Mutant colonies	Total colonies	Per cent mutation	Minus control*	Factor decrease
Continuous	A (CT5)	52	414	12·6	11·0	
	B (CL5)	83	442	18·8	17·7	
	C (CT20)	123	628	19·6	13·0	
	D (CL20)	68	377	18·0	6·0	
Intermittent	A (IT5)	61	629	9·7	7·0	0·64
	B (IL5)	24	319	7·5	5·2	0·28
	C (IT20)	54	475	11·1	6·5	0·50
	D (IL20)	41	387	10·6	3·0	0·50

* For unirradiated controls, size of inoculum, other treatments, and amount of growth, see *Table VI.*

Per cent survival = 64·3, 66·5, 79·5, 99·7, 100, 92·5, 80·4, 86·0, respectively.

Note: With intermittent incubation, the nuclei were small and clearly stainable, whereas with continuous incubation, the cytoplasm seemed divided into large deeply staining bodies which nearly filled the mycelial thread.

storing overnight in the cold. Samples taken each day were tested for spontaneous mutation and for sensitivity to X-ray and ultraviolet induced change. Most of the growth was probably completed in the first 14 hours.

Under these conditions the spontaneous mutations increased continuously with incubation (*see Table IX*). X-irradiation however induced only 1 or 2 per cent of additional mutants whereas ultraviolet was a remarkable effective mutagen despite the multinucleate state, inducing from 15 to

Table IX.—Sensitivity to X-rays and to ultraviolet in the uninucleate and multinucleate states
(Dose, 8,000 r. and 400 ergs per mm² respectively)

Hours Pre-Incubation	*Mutation*			*Survival*
	Mutant colonies	Total colonies	Per cent mutation	Per cent
Incubated and X-irradiated (diluted 1/10 in medium before X-irradiation)				
0	152	700	21·7	2·1
7	126	3,563	3·5	10·7
14	94	3,160	3·0	9·5
21	142	2,821	5·0	8·5
28	128	3,890	3·3	8·7
35	187	2,490	7·5	7·5
Incubated and ultraviolet irradiated (diluted 1/10 in saline before ultraviolet)				
0	75	298	25·2	6·7
7	649	2,196	29·6	8·9
14	388	2,485	15·6	7·6
21	243	435	55·9	1·0
28	294	749	39·3	1·6
35	389	911	42·7	1·8
Control (incubated and plated without irradiation)				
0	62	7,813	0·8	
7	53	6,189	0·9	
14	128	7,072	1·8	
21	152	3,957	3·8	
28	147	3,376	4·4	
35	213	4,055	5·3	

Inoculum : 2×10^7 spores per ml.

Incubation : in medium ; intermittent 7 hours out of each 24 ; stored in cold between incubations.

Growth : all spores have germinated and grown into a multicellular mycelium (about 30 nuclei per infective unit) by 7 hours. The suspension probably reaches saturation shortly after.

55 per cent mutants (*see Table IX*). Thus, a state seems to have been achieved in which the sensitivity to the mutagenic action of the two agents differed widely. In addition there appear to have been significant fluctuations in sensitivity to ultraviolet induced mutation, and it would seem that such sensitivity must have been very much dependent upon uncontrolled physiological variables.

Finally, spore suspensions in medium were bubbled with nitrogen and incubated, over a 24-hour period. During the treatment almost all nuclear

division was suppressed so that few of the cells became binucleate, and a negligible proportion had more than two nuclei. These limited divisions might have been expected to increase the sensitivity to radiation induced mutation and killing, but instead the net effect of the treatment was to reduce both the ultraviolet and the X-ray mutability to about half, and to increase survival by 2- to 3-fold for X-rays and by 8- to 10-fold for ultraviolet (*see Table X*). The effects could not have been due to any differences in dissolved oxygen at the time of irradiation since all suspensions were diluted with the same chilled saline before being exposed to X-rays or ultraviolet.

Table X.—Physiological control of sensitivity in the uninucleate state

Effect of bubbling with nitrogen during 24-hour incubation in medium

Experiment (Suspension)	*Hours' Incubation with medium and* N^2	*Mutation* Mutant colonies	/	Total colonies	Per cent mutation	*Factor reduction* (24/0)	*Survival* Per cent
Unirradiated							
A	0	2	/	245	0·82		
	24	5	/	355	1·41	No detectable killing with incubation.	
B	0	3	/	265	1·13		
	24	38	/	1,200	3·12		
Ultraviolet							
A	0	249	/	966	25·8		4·0
	24	175	/	1,152	15·2	0·59	35·0
B	0	249	/	1,109	22·5		4·2
	24	55	/	473	11·1	0·52	38·7
X-rays							
A	0	90	/	90	26·2		14·1
	24	169	/	993	17·0	0·65	30·0
B	0	88	/	317	27·8		13·3
	24	66	/	458	14·4	0·52	37·8

Note : Some of the spores were clearly uninuncleate (8 out of 49) ; some were uninucleate but the nuclei appeared 'dumb-bell' shaped as if incompletely divided (32/49) ; and a very few were binucleate (6/49) ; three out of 49 had 3–8 nuclei.

Thus it is clear that various changes in the physiological state of a cell can influence the likelihood that it will produce a mutant colony as a result of irradiation. In the case of irradiated multinucleate cells it is altogether likely that mutant and non-mutant nuclei compete, and differences in the physiological state at the time of irradiation might persist long enough to influence the outcome of such internal competition. It is therefore difficult to show conclusively, using multinucleate cells, that the physiological state can influence the susceptibility of individual nuclei to induced mutation ; although certain of the present results could be most simply interpreted on this assumption.

However it is quite certain that the mutability of individual nuclei must have been reduced to about one half in the experiments using nitrogen treated uninucleate (and binucleate) spores. And it must also be that the sensitivity of the individual nuclei to induced mutation is at least doubled in the binucleate cells arising under normal conditions of growth.

Thus physiological differences are capable of resulting in very considerable variations in sensitivity to induced mutation. And furthermore, in the experiments demonstrating this, lethality and mutagenesis were similarly affected, indicating that the modifiable processes are in part common to both types of response.

CONCLUSIONS

Originally it was hoped that these experiments would yield information regarding the time of the induced mutations, and whether such changes were in any way dependent on the process of gene (and chromosome) replication. Both X-ray and ultraviolet induced mutations had appeared to be delayed until the time of gene replication in *Escherichia coli*, since irradiated cells gave rise to colonies sectored for induced changes, even where the so-called ' double selection ' technique had been used in an attempt to insure that each colony came from a single irradiated gene complement (NEWCOMBE[6, 7] ; WITKIN[8]). Also, the mutagenic effects of ultraviolet in *Streptomyces* had been found to lose their capacity for photoreversal only under conditions favourable to nuclear division, and at a time shortly before the nucleus becomes visibly double (reported elsewhere).

However, we have not been able to add to these observations any evidence of a critical period in the nuclear division cycle during which mutation might be taking place. Exceptional sensitivity to induced mutation appears to extend over the whole of the binucleate stage, and not just a part of it ; and the fractionation experiments appear to indicate that nuclear division brings no release from an earlier saturation (or impairment) of the capacity for induced mutation.

What has been shown is that the physiological state of a cell at the time of irradiation can influence the likelihood that it will subsequently develop into a mutant colony. Where the cells are multinucleate at the time of irradiation it is not entirely certain whether such differences might not arise through effects influencing subsequent competition between mutant and non-mutant nuclei within the strands. However, where only one or two nuclei are present it is clear that the sensitivity of individual nuclei to induced mutation is subject to very considerable physiological control.

This suggests the possibility that the non-linear dose-response relationships may also be physiological in origin. In support of this view, examples of non-linearity have been described which could not be the result of differential killing of induced mutants, and which would be very difficult to explain in terms of an initial population heterogeneity.

In view of the evidence for a physiological control over the response of the genetic material of *Streptomyces* to mutagenic agents, it seems altogether possible that the physiological changes produced by high doses of the mutagens themselves may be effective in altering the capacity to respond to a further exposure.

Two observations support the view that the non-linear X-ray dose-mutation curves observed in *Streptomyces* are the result of a genuine saturation or impairment of the capacity of individual spores to respond when the dose is high :

First, at doses where a further increase is ineffective in producing mutations, it is also relatively ineffective in producing lethal changes. Second, cell suspensions have been obtained by pre-incubation which are very resistant to X-ray killing ; and in these the less-than-linear response to increasing doses could not be accounted for in terms of differential killing of cells which would otherwise produce mutant colonies.

In addition, very considerable changes in sensitivity to X-ray induced mutation have been observed following the first nuclear division, and also as the result of various treatments affecting the physiological state at the time of irradiation. As the capacity for induced mutation is evidently dependent on the physiological state of the organism, it is not surprising that high doses of radiation should alter this capacity for mutagenic response.

REFERENCES

[1] KELNER, A. *J. Bact.* 1948, **56** 457.
[2] NEWCOMBE, H. B. *J. Gen. Microbiol.* 1953, **9** 30.
[3] NEWCOMBE, H. B. and MCGREGOR, J. F. *Genetics*, 1954, in press.
[4] SPARROW, A. H. *Ann. N.Y. Acad. Sci.* 1951, **51** 1508.
[5] MCGREGOR, J. F. *J. Gen. Microbiol.* 1954, in press.
[6] NEWCOMBE, H. B. *Genetics*, 1951, **36** 570.
[7] NEWCOMBE, H. B. *ibid*, 1953, **38** 134.
[8] WITKIN, E. *Cold Spring Harbor Symp. Quart. Biol.* 1931. **16** 357.

DISCUSSION

R. LATARJET : La chute du taux des mutations induites par les fortes doses de rayons ultraviolets a un caractère très général, conformément aux indications du Newcombe. Aux cas qu'il a mentionnés, je puis ajouter celui que j'ai observé en obtenant les premières mutations qui aient été induites chez un virus* (*Figure 2*). Il s'agissait de mutations 'host range' obtenues chez le bactériophage T2 en irradiant celui-ci pendant qu'il se multiplie au sein de son hôte Escherichia coli souche B.

La courbe ci-jointe groupe les points expérimentaux obtenus. Elle montre la chute très brusque des mutations induites à partir d'une dose de 5.000 ergs mm^{-2}.

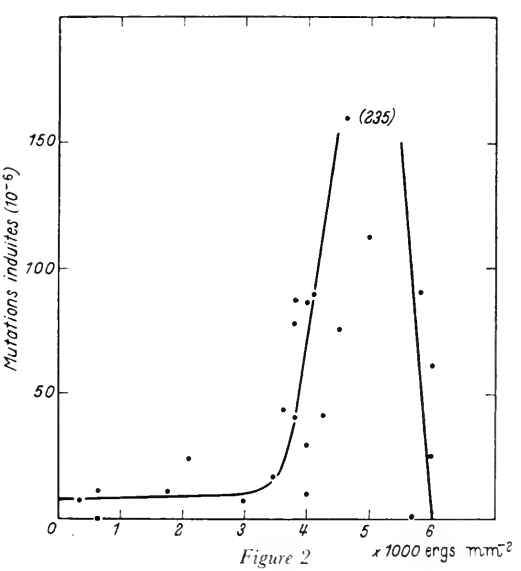


Figure 2

* *Compt. rend. Acad. Sci. Paris*, 1949, **228** 1354.

INFLUENCE DES DOSES ÉLEVÉES DE MÉTHYL-BIS-(2-CHLOROÉTHYL)AMINE SUR L'OVAIRE DE LAPINE ADULTE, AVEC OU SANS PRÉPARATION PAR LA BÉTAMERCAPTOÉTHYLAMINE

PAUL DESAIVE

Laboratoire de Pathologie Chirurgicale Générale de l'Université de Liège et du Centre National Belge pour l'Etude de la Croissance Normale et Pathologique

L'APPAREIL folliculaire de l'ovaire adulte constitue, à notre avis, un excellent test biologique de l'action des radiations ionisantes et de celle des substances dites radiomimétiques ; si les effets des premières sur ce système ont fait l'objet de nombreux et intéressants travaux (parmi lesquels nous citerons tout spécialement les remarquables publications de LACASSAGNE[1] et de GRICOUROFF[2]), et nous ont personnellement servi pour établir notre conception du mécanisme de l'évolution et de l'involution folliculaires[3], l'influence des secondes n'a, par contre, suscité qu'un petit nombre de travaux originaux ; or ce problème est important, en raison de l'extension croissante, en thérapeutique humaine, de l'emploi des dérivés bi- ou tri-substitués du gaz moutarde ; c'est pourquoi nous l'avons abordé dans une note préliminaire récente[4], dans laquelle nous avons signalé que 'nitrogen mustard (Boots)' (HN_2), à la dose unique de 0,3 mmg/kg ou à cette même dose suivie, à 8 jours d'intervalle, d'une nouvelle injection de 0,5 mmg/kg, provoque une nette atrésie des follicules mûrissants préovulatoires et surtout favorise l'apparition de formes atrétiques anormales et d'un nombre inhabituel de follicules à ovocytes multiples et de complexes primordiaux polynuclées.

Par ailleurs, les excellents résultats radioprotecteurs obtenus par BACQ et collaborateurs[5] avec la bétamercaptoéthylamine (bécaptan) nous ayant incité à étudier le comportement de cette substance à l'égard des effets de l'ypérite à l'azote (HN_2) sur les cellules génératrices de l'intestin grêle du rat, nous avons pu constater[6] que le bécaptan, à la stricte condition d'être introduit dans l'organisme quelques minutes avant HN_2, limite, dans une certaine mesure, les effets immédiats de ce toxique. C'est pourquoi, nous avons tenté d'établir dans quel sens, la préparation de la lapine par le bécaptan est capable de modifier la réponse de l'ovaire à l'action de HN_2.

MATÉRIEL ET TECHNIQUES

Nous avons utilisé des lapines adultes d'un poids moyen de 2 kg, isolées en cage au moins un mois avant le début des expériences.

Six lapines ont reçu dans la veine marginale de l'oreille 2 mmg de methyl-bis-(2-chloroethyl)amine (HN_2), dilués extemporanément dans 3 cm³ de sérum physiologique. Cette dose de 2 mmg (1 mmg/kg) a été bien supportée par les animaux ; elle représente cependant, à l'échelle humaine, une

quantité de 70 mmg par kg, qui n'est tolérée en une fois que par la seule voie intra-artérielle.

Six autres lapines ont été soumises au même traitement, mais trois minutes *après* avoir reçu par voie intrapéritonéale, 50 mmg de bécaptan (soit 0,5 cm³ de la solution à 10 pour cent de bécaptan de Labaz).

Dans chacun de ces deux groupes expérimentaux, les animaux ont été sacrifiés après 12, 24, 36, 48, 72 et 96 heures. Les deux ovaires de chaque sujet ont été prélevés, fixés au liquide de Bouin, inclus dans la paraffine, débités en coupes sériées de 6 μ et colorés soit à l'Azan pour l'étude topographique de l'appareil folliculaire, soit à l'hématoxyline ferrique–éosine pour l'examen cytologique. Puis, pour chacun des ovaires, une coupe sur vingt a été dessinée au projecteur de profil SIP ; et sur le 'puzzle' constitué par l'ensemble de ces dessins, les différents types folliculaires évolutifs et involutifs, ont été soigneusement comptés.

Rappelons à ce sujet, que nous distinguons histologiquement[3] 8 stades folliculaires successifs : 1 stade primordial (de réserve) *P*, 4 stades *A*, *B*, *C* et *D* correspondant à la période de croissance de l'ovocyte, et 3 stades *E*, *F* et *G* contemporains de la préparation du follicule à l'éventuelle rupture (qui, chez la lapine, est provoquée par le coït). Les résultats de ces numérations ont été réunis en tableaux permettant d'établir une comparaison aisée entre les états folliculaires des différents ovaires étudiés.

Enfin, par une technique particulière de comparaison de deux ovaires d'une même paire, nous avons recherché les effets éventuels du bécaptan employée isolément.

RÉSULTATS EXPÉRIMENTAUX

Nos observations ont porté sur les points suivants :

(*1*) L'aspect histologique et cytologique des ovaires et plus spécialement la fréquence des processus atrétiques.

(*2*) La formule folliculaire, c'est-à-dire la répartition des follicules en 7 stades successifs de *A* à *G*.

(*3*) L'aspect et la fréquence des organites atypiques, et plus particulièrement, des groupements anormaux de follicules primordiaux, des follicules à ovocytes multiples et des ruptures folliculaires intra-ovariennes.

(*A*) *Etude des ovaires de lapines traitées par une seule injection intrapéritonéale de* 50 mmg *de bécaptan*

Avant tout essai d'interprétation de l'action du bécaptan sur les effets ovariens de HN_2, il était évidemment indispensable de déterminer l'influence éventuelle du bécaptan seule.

Deux lapines ont subi l'ablation de l'ovaire gauche, utilisé comme témoin, puis ont reçu 8 heures après, en injection intrapéritonéale, 50 mmg de bécaptan.

Les ovaires droits ont été prélevés 12 heures et 36 heures après cette injection (soit 20 heures et 44 heures après l'ovaire témoin).

Voici *Tableau I*, la comparaison des formules folliculaires de ces ovaires et de leurs témoins.

A la lecture de *Tableau I*, on constate que les différences entre les deux ovaires traités et leurs témoins sont légères et répondent à la loi normale

de la compensation (visible, à la 36ème heure, au niveau des stades, *B*, *C* et *D*).

D'autre part, les ovaires prélevés après bécaptan ne présentent aucune anomalie de structure ; les figures d'atrésie s'y rencontrent en proportion normale et les follicules primordiaux y sont disposés comme d'habitude ; on n'y rencontre pas de grandes formes atrétiques anormales et enfin les follicules à ovocytes multiples qu'ils contiennent sont en nombre à peu près égal à celui des témoins.

En résumé, le bécaptan ne parait exercer par lui même aucune action sur l'appareil folliculaire, constatation qui exclut pratiquement toute chance de provoquer par ce produit—à la dose employée—des lésions cytologiques sérieuses de la glande génitale femelle. Cette observation est d'autant plus interessante que d'autres substances radioprotectrices sont, au contraire,

Tableau I

	Follicules									*Follicules*							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*Totaux*		*A*	*B*	*C*	*D*	*E*	*F*	*G*	*Totaux*
I *Ovaire gauche témoin*	2.485	612	98	67	44	11	5	3.322	*Ovaire dt prélevé* 12 h *après bécaptan*	2.378	484	85	73	38	20	5	3.083
II *Ovaire gauche témoin*	2.188	368	48	48	31	12	5	2.700	*Ovaire dt prélevé* 36 h *après bécaptan*	2.367	647	88	72	16	8	7	3.205

nettement toxiques pour l'ovaire : par exemple, chez une lapine soumise à trois injections sous-cutanées journalières de 2 mmg de cyanure de sodium, nous avons compté, dans un seul ovaire, 3 ruptures intra-ovariennes traduisant un bouleversement important de la physiologie folliculaire.

(B) Etude des ovaires de lapines traités par HN_2 seul ou par HN_2 précédé de bécaptan

I. Aspects histologique et cytologique des ovaires traités—Outre une très légère diminution du poids ovarien moyen, par rapport à la normale et une congestion assez considérable des hiles, nous avons, comme dans notre travail précédent, noté une chute rapide du nombre de grands follicules préovulatoires, et surtout une modification importante de l'état des follicules primordiaux.

Dans les toutes premières heures (12° et 24°), ceux-ci montrent des lésions ovocytaires et folliculeuses importantes ; les minces cellules granuleuses se vacuolisent et se désunissent ; le cytoplasme de l'ovocyte gonfle, devient plus colorable et prend un aspect pommelé ; la chromatine nucléaire—à

l'état diplotène de H. de Winiwarter—se désagrège, se ratatine et se disperse finalement en grains pycnotiques.

La fréquence de ces images est beaucoup plus considérable que dans un ovaire normal, où elles n'apparaissent que d'une manière discrète et très fugace ; dans l'ovaire ypérité non protégé par le bécaptan, la proportion habituelle de 2/1.000 monte à la douzième heure à 369/1.000 et à la 24° heure à 549/1.000, puis s'abaisse progressivement à 180/1.000 à la 96° heure, en passant aux 36°, 48° et 72° heures par les valeurs 240/1.000, 261/1.000 et 251/1.000 ; dans l'ovaire protégé, elle n'est à la douzième heure que de 289/1.000 mais elle se relève à 542/1.000 à la 24° heure pour s'atténuer ensuite, en passant par les valeurs de 345/1.000 à la 36° heure, de 279/1.000 à la 48° heure, de 260/1.000 à la 72° heure et de 182/1.000 à la 96° heure.

Nous signalons ici que l'atrésie des follicules primordiaux se maintient à un taux relativement élevé jusqu'au huitième jour, sans doute en raison du phénomène de 'mort retardée' qui frappe les follicules primordiaux non immédiatement tués par le toxique, mais suffisamment atteints pour être incapables de poursuivre leur évolution.

Notons pour mémoire que dans un ovaire irradié à 2.500 r, l'atrésie des follicules primordiaux, déjà très nette après 2 heures, atteint 380/1.000 à la 16° heure et 824/1.000 à la 54° heure pour devenir pratiquement complète à partir de la 72° heure.

Pour ce qui est des autres types folliculaires (de *A* à *G*) la comparaison des rapports : nombre de formes atrétiques/nombre de formes évolutives, calculés dans les ovaires normaux d'une part et les ovaires ypérités d'autre part, est extrêmement malaisée en raison du fait que, même dans les conditions physiologiques, la valeur de chacun de ces rapports varie avec le moment du prélèvement de l'ovaire par rapport à la durée de son cycle ; si ce moment est proche du début de l'intervalle de 32,8 heures qui, selon nous, sépare deux poussées évolutives successives, on trouve peu d'atrésies ; si, au contraire, le prélèvement a eu lieu à un instant proche du début d'une nouvelle poussée, les seules follicules évolutifs persistants sont ceux destinés, soit à passer au stade suivant, soit (s'il s'agit de follicules du stade *G*) à involuer définitivement, à moins que l'acte génital ne les ait préparés à l'ovulation.

Nous avons réuni sur le *Tableau II* ci-après les incidences atrétiques moyennes relevées dans les deux séries d'ovaires que nous étudions ici, en les rapprochant des résultats obtenus dans les deux séries précédentes, ainsi que dans un groupe important d'ovaires normaux.

De la lecture de ce *Tableau II*, il ressort immédiatement que dans l'ovaire normal, le taux atrétique est sensiblement le même à chacun des 7 stades : l'appareil folliculaire est en équilibre, et les réductions numériques parfaitement calquées sur le rythme des poussées de développement, se font sans heurt pour produire, à partir d'environ 1.600 follicules *A*, 6 follicules *G* prêts à l'ovulation. Au contraire, l'ovaire ypérité est le siège de bouleversements importants : atrésie massive initiale des follicules jeunes et des follicules mûrissants, suivie d'une hyperproduction réactionnelle d'éléments jeunes et d'un retour progressif de l'appareil noble à une dynamique normale; dès lors, les taux atrétiques aux différents stades sont évidemment dissemblables, et comme la régénération—qui débute à la douzième heure—est

nécessairement dominante, la moyenne atrétique générale, pour l'ensemble de nos observations, est inférieure à la moyenne classique (tout au moins pendant la phase folliculaire du cycle). Notons en passant qu'après préparation par le bécaptan, cette moyenne atrétique est très proche de la normale ; ce fait dont la valeur absolue ne doit pas être exagérée, en raison des réserves que nous avons émises plus haut, semble cependant indiquer que la radioprotection favorise un retour plus rapide de l'ovaire à son équilibre physiologique.

Sur le plan de la simple observation microscopique, nous ajouterons ici, en confirmation de nos constations précédentes, que les éléments immédiatement touchés par l'atrésie sont, outre les follicules primordiaux, les types

Tableau II

Rapports : $\frac{\text{Nombre de formes atrétiques}}{\text{Nombre de formes évolutives}}$

	A	*B*	*C*	*D*	*E*	*F*	*G*	*Moyennes générales*
Lapines normales	0,58	0,61	0,73	0,84	0,53	0,37	0,20	*0,551*
Lapines ypéritées								
(a) *Dose unique :* 0,6 mmg (*de la* 24° *à la* 192° *heure*)	0,325	0,325	0,381	0,451	0,391	0,281	*pas de G ou pas de G atrétique*	*0,359*
(b) *Dose répétée* : 0,6 *puis après* 8 jours 1 mmg (*de la* 24° *a la* 168° *heure après la seconde injection*)	0,325	0,325	0,365	0,490	0,348	0,210	*pas de G ou pas de G atrétique*	*0,343*
(c) *Dose unique :* 2 mmg (*de la* 12° *à la* 96° *heure*)	0,170	0,176	0,949	0,610	0,390	0,337	0,047	*0,383*
(d) *Dose unique :* 2 mmg (*précédée de* 50 mmg *de bécaptan* (*de la* 12° *à la* 96° *heure*)	0,158	0,161	0,570	0,648	0,905	1,040	0,233	*0,530*

les plus avancés de la chaîne folliculaire, c'est-à-dire les follicules *F* et *G* ; les formes intermédiaires—*D* et *E* particulièrement—sont relativement épargnées.

Enfin, notons encore que l'effort de réparation de l'ovaire est extrêmement précoce : à la douzième heure, on voit apparaître sous l'épithélium germinatif, d'importants groupes de follicules jeunes en pleine activité. Nous reviendrons plus loin sur cette intéressante constatation ; mais nous retenons dès à present que les ovaires de lapines traitées par le bécaptan avant de recevoir l'injection de HV_2, se caractérisent par une destruction moins brutale des follicules primordiaux et ensuite par une tendance de l'appareil folliculaire à reprendre plus rapidement son comportement involutif habituel.

II. Formules folliculaires—Nous avons groupé dans le *Tableau III* les résultats des numérations folliculaires aux différents stades *A* à *G*, dans chacune des deux séries ovariennes étudiées, et nous les avons fait suivre des résultats

moyens des numérations correspondantes obtenues à partir de 19 ovaires normaux prélevés à des phases différentes du cycle.

Dans les deux groupes expérimentaux, nous relevons un premier fait important (que nous avions déjà observé lors de nos expériences à doses plus faibles d'ypérite) : à savoir, par rapport aux témoins, l'extrême élévation, dès la douzième heure, des nombres de follicules jeunes, *A* et *B* en ordre principal, en conséquence de l'accroissement réactionnel de la quantité de

Tableau III. Numérations Folliculaires

A. Lapines traitées par 2 mmg *d'*HN_2

Délais de prélèvement	*Types folliculaires :* *A*	*B*	*C*	*D*	*E*	*F*	*G*	*Formes atrétiques spéciales*
12 h	6.432	1.076	46	6	3	1	1	1 *RO*
24 h	3.541	593	89	23	5	5	4	2 *GTd et* 1 *GMd*
36 h	2.427	407	96	72	46	5	2	—
48 h	3.176	532	68	17	12	8	4	—
72 h	2.174	365	56	14	11	7	5	1 *GH*
96 h	4.578	762	334	124	76	16	7	—
Moyennes	3.721	622	115	43	25	7	4	0,83

B. Lapines traitées par 2 mmg *d'*HN_2, *après* 50 mmg *de bécaptan*

12 h	7.204	1.200	140	47	47	21	6	—
24 h	2.465	411	54	13	8	5	4	2 *GMd*
36 h	2.789	462	55	43	34	7	3	—
48 h	4.500	750	84	26	9	4	3	—
72 h	1.878	312	124	87	60	24	6	1 *RO*
96 h	2.620	432	234	53	23	11	6	—
Moyennes	3,576	594	115	45	30	12	5	0,5

C. Lapines normales à différentes phases du cycle (19 *ovaires*)

Moyennes	1.569	178	85	35	23	11	6	*Pas de formes atrétiques spéciales*

follicules primordiaux sortis de la réserve quiescente et prêts à évoluer ; nous n'avons pu, dans ce domaine, distinguer les ovaires protégés de leurs congénères non protégés.

Par contre, nous constatons que les follicules mûrissants des stades *F* et *G*, à l'autre extrémité de la chaîne évolutive, restent en quantité sensiblement normale dans les premiers, alors qu'ils diminuent considérablement dans les seconds ; il semble donc que le bécaptan soit capable de protéger, pendant un temps assez long pour leur permettre d'évoluer sans encombre, les

follicules qui, au moment du traitement, se trouvaient aux stades intermédiaires *D* et *E*, correspondant à la fin de la période de croissance de l'ovocyte et au début de la préparation à l'ovulation.

D'ailleurs si nous comparons—pour éviter l'erreur due à d'éventuelles différences individuelles—les moyennes sur quatre jours de nos deux séries et les moyennes normales, nous constatons effectivement que, malgré le retour à l'état physiologique qui s'annonce dès le troisième jour dans les

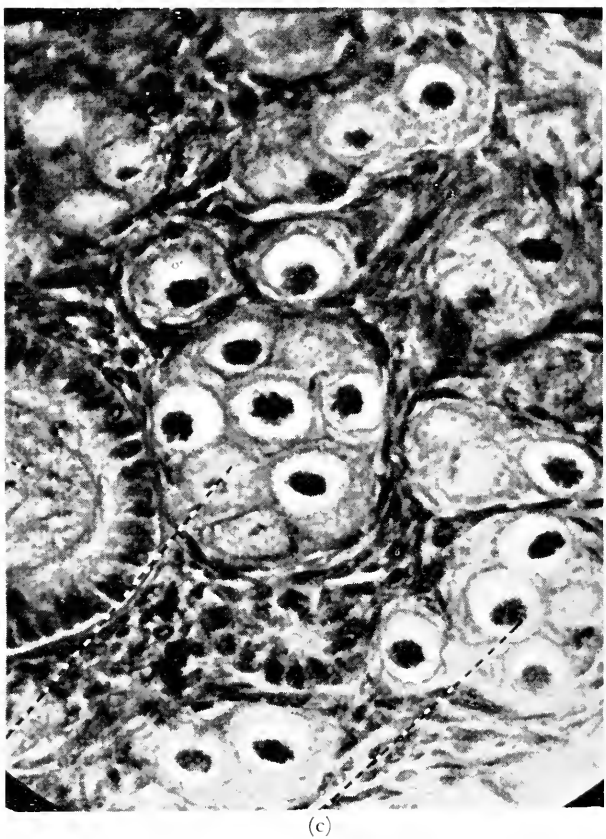


Figure 1. (a) *Cortex ovarien (après* HN_2*).* (b) *Foll. A.* (c) *Amas, d'allure plasmodiale, de follicules primordiaux*

ovaires intoxiqués par l'ypérite, les nombres moyens de follicules *F* et *G* sont, dans ce cas, nettement inférieurs à la quantité ordinaire, alors qu'après intervention de bécaptan, ils lui sont égaux et même supérieurs.

En conclusion, l'appareil folliculaire évolutif réagit à l'injection intraveineuse de 2 mmg d'HN_2, dans le même sens mais avec plus d'intensité qu'après l'introduction de 0,6 mmg ; nous assistons en effet, à la même atrésie hâtive des grands follicules mûrissants, avec économie relative des stades intermédiaires, mais avec déclenchement d'une poussée plus importante encore de follicules jeunes.

D'autre part, la préparation de la lapine par injection intrapéritonéale de 50mmg de bécaptan a pour conséquence d'élever le nombre moyen observé de grands follicules mûrissants, ce qui semble conférer à cette substance une action protectrice immédiate, mais limitée, vis à vis des stades les plus avancés de l'échelle folliculaire.

III. Aspect et fréquence des formations atypiques, à point de départ folliculaire—(*1*) A la suite de nos précédentes recherches, nous avons signalé que la caractéristique essentielle de l'ovaire de lapines soumises à l'injection intraveineuse de 0,3mmg/kg de $H.V_2$ est l'apparition dans le cortex ovarien, de groupements de follicules primordiaux, *en cordons ou amas d'allure plasmodiale* (voir *Figure 1*) ; arrondies ou polygonales, limitées par un seul rang de cellules folliculeuses aplaties, ces formations contiennent dans un cytoplasme finement granuleux un nombre variable de noyaux au stade diplotène, caractéristique des ovocytes arrivés à la fin de leur préparation prépubérale.

Nullement spécifiques de l'ypérite, ces groupements—que nous avons rencontrés—en quantité réduite—dans quelques ovaires pathologiques après administration de doses élevées de gonadotrophines ou après destruction de l'hypophyse par le radon, frappent ici par leur abondance et leur volume. Nous les avons observés à partir du troisième ou du quatrième jour après 1mmg d'ypérite et dès la 24° heure lorsque l'injection a été répétée après huit jours ; dans les deux modes expérimentaux, ces groupements particuliers se maintiennent pendant quatre jours puis disparaissent à une époque qui correspond approximativement au retour de l'ovaire à la normale.

Dans les expériences relatées ici, nous avons retrouvé ces groupements à la 96° heure après 2mmg d'ypérite ; mais, fait remarquable, nous en avons notés dès la 12° heure dans les ovaires protégés, en petit nombre il est vrai et assez pauvres en ovocytes (trois ou quatre en moyenne) ; il semblerait donc que le radioprotecteur utilisé favorise la production de ces organites, qui est vraisemblablement liée à un processus régénératif, interprétation sur laquelle nous reviendrons bientôt.

(*2*) Une autre observation a traité à la fréquence élevée dans les ovaires ypérités, des follicules à ovocytes multiples. L'ovaire de lapine est d'ordinaire pauvre en ces élements particuliers ; nous en avons trouvé dans 102 ovaires sur 159 (soit dans 64,16 pour cent de ceux-ci) en quantité variant de 1 unité à 121 par organe et dans la proportion de 1 follicule multiovulaire pour 200 follicules mono-ovulaires. Or l'examen de 13 ovaires traités par $H.V_2$ à faible dose nous a permis de découvrir chez chacun d'entre eux, des organites pluri-ovocytaires à l'un ou l'autre des sept stades folliculaires évolutifs, avec une fréquence de 1 pour 180 et des nombres totaux de 1 à 58 unités.

Nous avons fait la même constatation dans nos deux séries actuelles et nous reproduisons ici nos numérations à ce sujet (*Tableau IV*).

Non seulement, tous les ovaires enlevés chez des lapines traitées par 2mmg d'$H.V_2$ renferment des follicules multiovulaires, mais encore, est-ce parmi eux que nous avons trouvé celui qui, de tous les ovaires de notre collection, en contient le plus grand nombre : 169.

Signalons en outre que, dans ces deux groupes, la valeur moyenne des rapports numériques entre les follicules multiovulaires et les follicules ordinaires dépassent de loin celles observées dans nos autres séries d'ovaires ;

dans le groupe des non protégés, le nombre moyen de follicules pluriovocytaires est de 30,5 pour un nombre folliculaire total moyen de 4.528 (soit un follicule multiovulaire pour 145 follicules mono-ovulaires) et dans le groupe des protégés, ce nombre s'élève à 45,3 pour 4.376 (soit un follicule pluriovulaire pour 96 follicules ordinaires).

Ces faits confirment d'une part, la fréquence élevée des follicules multiovulaires dans les ovaires ypérités et, d'autre part, l'influence favorable que le bécaptan exerce sur la génèse de ces éléments qui, au même titre que les formations plasmodiales, nous semblent constituer un indice de régénération ovocytes ovarienne hyperactive.

Enfin, signalons en passant que les deux ruptures intra-ovariennes (*RO*) que nous avons observées, l'une à la douzième heure, chez un non protégé,

Tableau IV

A. Lapines traitées par 2 mmg HN_2		*B. Lapines traitées par* 50 mmg *de bécaptan puis par* 2 mmg *d'*HN_2	
	Follicules multiovulaires		*Follicules multiovulaires*
12° h	98 [56 *A*, 31 *B* (*dont* 1 *triple*), 9 *C*, 1 *D*, 1 *RO*]	12° h	169 [19 *A* (*dont* 7 *triples*), 50 *B* (*dont* 5 *triples*), 23 *C* (*dont* 2 *triples*), 2 *D*, 1 *E*, 1 *F*]
24° h	3 (2 *A*, 1 *B*)	24° h	3 (2 *A*, 1 *B*)
36° h	2 (1 *A*, 1 *B*)	36° h	3 (2 *A*, 1 *B*)
48° h	6 (4 *A*, 2 *B*)	48° h	22 (10 *A*, 6 *B*, 5 *C*, 1 *D*)
72° h	13 (8 *A*, 5 *B*)	72° h	33 [10 *A*, 7 *B*, 5 *C*, 4 *D* (*dont* 1 *triple*), 3 *E*, 3 *F* (*dont* 1 *triple*) 1 *RO* (*quadruple*)]
96° h	61 [37 *A* (*dont* 1 *triple*), 19 *B*, 2 *C* (*dont* 1 *triple*), 1 *D*, 1 *E*, 1 *F*]	96° h	42 (23 *A*, 10 *B*, 5 *C*, 2 *D*, 1 *E*, 1 *F*)
Moyenne :	30,5	*Moyenne* :	45,3

l'autre à la soixantedouzième heure chez un protégé, concernaient des follicules *G* à ovocytes multiples : 1 biovocytaire et un quadriovocytaire.

(*3*) Il nous reste un dernier point à envisager ; celui de la présence dans les ovaires ypérités de types atrétiques anormaux, et en particulier de ces ruptures intra-ovariennes auxquelles nous venons de faire allusion et que nous avons été le premier à signaler chez la lapine.

Il est certain que l'intoxication brutale de l'ovaire par l'ypérite précipite de manière excessive l'atrésie des follicules préovulatoires : nous avons vu, à la 24° heure, aussi bien après que sans protection, apparaître de grands follicules très dilatés (*GT*, qui ne se voient habituellement que dans les ovaires de lapines gonadotropisées ou dans les ovaires greffés dans la rate après castration), ainsi que des méroxanthosomes (*GM*) exceptionnellement rencontrés dans les ovaires normaux ; nous avons même observé, à la 72° heure, chez un non protégé, un follicule hémorragique (*GH*).

Nous avons également noté, surtout chez les lapines non protégées, la naissance prématurée de l'antrum dans les follicules des stades jeunes *C* et même *B*.

Mais le fait avant tout remarquable est la *fréquence réellement anormale, après ypérite, des ruptures folliculaires intra-ovariennes*. On sait que chez la lapine, comme chez la chatte, l'ovulation n'est provoquée que par le coït, stérile ou non, par l'excitation électrique du tractus génital externe, ou par l'administration d'une quantité importante de gonadostimuline. Il peut cependant arriver qu'un follicule se rompe à l'intérieur de l'ovaire ; normal chez les monotrèmes et certains oiseaux, ce processus atrétique est considéré comme rarissime chez les mammifères. Nous l'avons observé 24 fois sur 358 ovaires (soit dans 6,7 pour cent des cas) et seulement dans 2 ovaires sur 56 appartenant à des lapines n'ayant subi aucune influence expérimentale[8] (soit dans 3,5 pour cent des cas). Or sur les 13 ovaires ypérités à dose faible, nous avons observe deux fois ce phénomène ; et sur les 12 des présentes séries nous l'avons également constaté deux fois (1 à la douzième heure chez un ovaire non protégé et 1 à la 72° heure chez un ovaire protégé).

Nous obtenons ainsi un total de 4 *RO* sur 25 ovaires ypérités, soit un pourcentage de 16, de loin supérieur à la proportion normale et qui n'est égalé que par ceux—15,70 et 21,5 pour cent—que nous avons établis chez des lapines, respectivement hypophyséoprives et thyréoprives.

Les *RO* que nous avons vues dans la série actuelle sont, en outre, très particuliéres ; la première, à la douzième heure, concerne un follicule gauche biovulaire qui s'est rompu en deux endroits ; l'un des ovocytes est déjà accolé à l'une des brêches par laquelle s'est engagée sa corona radiata ; l'autre semble se diriger vers la seconde ouverture, plus largement créée en direction du stroma voisin. Quant à la seconde *RO*, à la 72° heure, elle est plus extraordinaire encore ; le follicule mûr devenu dehiscent contenait quatre ovocytes : le premier est situé au fond de la néocavité créé dans le pourtour folliculaire, le second et le troisième (celui-ci atrétique) sont dans un chenal qui se dirige vers le cortex, et le quatrième est sorti de l'ovaire, et est attaché à la séreuse ovarienne par un reste de corona.

La nature atrétique de ce phénomène est indéniable ; sa cause principale réside dans une altération de l'enveloppe folliculeuse dont certains éléments peuvent avoir subi spécifiquement l'action nécrosante du $H.N_2$, à moins que cette lésion ne soit indirectement le résultat d'une modification vasculaire localisée, en relation avec l'altération, sous l'influence du toxique, du métabolisme ovarien de l'acide ascorbique.

Les ovaires des animaux traités par le bécaptan ne sont pas très différents de leurs congénères soumis à l'action de $H.N_2$ seul ; nous y retrouvons en effet, quelques formes atrétiques atypiques—méroxanthosomes et ruptures intra-ovariennes—mais en nombre moindre (3 au total, au lieu de 5) ; cette dernière constatation serait de peu d'interêt (des différences individuelles quant au nombre de follicules susceptibles de subir ces formes d'atrésie pouvant exister, au départ de l'expérience, entre les ovaires utilisés), si elle ne coïncidait avec un comportement inverse des grands follicules évolutifs, qui sont nettement plus nombreux chez les lapines protégées ; dès lors, le bécaptan peut être considéré comme très probablement capable de limiter l'atrésie des follicules mûrissants.

En conclusion—L'injection intraveineuse, chez la lapine, de 2mmg de HN_2 apporte une confirmation des altérations que nous avons antérieurement observées, avec les doses plus faibles, au niveau de l'ovaire, et qui sont en ordre principal :

(*1*) La perception d'un trouble profond dans le mécanisme—rythmé par la succession, à intervalles de 32,8 heures, des poussées folliculaires—de l'évolution et de l'involution de l'appareil noble de l'ovaire.

(*2*) L'accroissement considérable, au cours des premières heures, de la proportion (ordinairement très faible) des formes atrétiques par rapport aux formes normales de follicules primordiaux *P*.

(*3*) La diminution du nombre moyen des grands follicules mûrissants et leur passage prématuré en atrésie par des mécanismes brusqués—tels que transformation méroxanthosomiale ou rupture intra-ovarienne—exceptionnels dans les conditions normales.

A côté de ces phénomènes dégénératifs, s'inscrivent des manifestations réparatrices, parmi lesquelles nous citerons essentiellement :

(*1*) Une brutale mise en chantier, dès la douzième heure, de follicules jeunes de types *A* et *B* surtout, issus d'un important groupe d'éléments primordiaux qui normalement aurait suffi à alimenter trois ou quatre poussées successives.

(*2*) La présence d'une quantité excessive de follicules pluriovocytaires.

(*3*) L'apparition, dans le cortex ovarien, de groupes polynucléés, formés d'ovocytes de type primordial, au noyau diplotène, logés dans une enveloppe folliculeuse commune, et qui, bien que non spécifiques de l'action de l'ypérite, frappent ici par leur abondance et leur volume.

Si l'injection intraveineuse d'ypérite à l'azote est précédée de l'introduction intrapéritonéale de 50mmg de bécaptan, les lésions ci-dessus décrites perdent de leur acuité, tandis que les manifestations régénératrices s'accentuent ; cette 'protection' relative s'extériorise par les faits suivants :

(*1*) Une allure d'ensemble plus normale des processus folliculaires et notamment une moyenne atrétique générale proche de la moyenne habituelle.

(*2*) Une légère diminution, au cours des toutes premières heures, du taux d'atrésie des follicules primordiaux.

(*3*) Le maintien à un niveau plus élevé du nombre moyen des grands follicules du type de Graaf.

(*4*) La même considérable hyperproduction de follicules jeunes que dans l'ovaire simplement ypérité, mais, avec en plus,

(*5*) la découverte d'un nombre plus important encore de follicules multiovulaires et enfin,

(*6*) la précocité extrême de l'apparition des groupements primordiaux polynuclées, dont des exemples sont déjà observables à la douzième heure.

REVUE

La connaissance des effets biologiques du gaz moutarde (β β′-dichlorodiéthyl sulfide) remonte aux travaux d'AUERBACH et ROBSON[9] qui ont pour la première fois signalé, en 1942, les altérations chromosomiales que cette substance provoque, *in vivo*, chez la Drosophile. Plus tard, KOLLER[10], puis

Darlington et Koller[11], employant le méristème de la racine d'Allium et le pollen de Tradescantia, ont démontré que la centromère des chromosomes en méiose ou en mitose était particulièrement sensible au gaz moutarde, et que le pourcentage des fragmentations chromosomiales s'accroissait de la 6 heure à la 72° heure aprés le traitement. En utilisant comme test des embryons d'amphibien, Gillette et Bodenstein[12], puis Bodenstein[13] ont observé au niveau de la crête épidermique un arrêt des mitoses à l'interphase, mais avec maintien de la croissance et de la différenciation de certaines cellules qui deviennent anormalement grandes puis se détruisent. Gilman et Philips[14] ont considéré que l'ypérite et ses dérivés stoppent les cellules en interphase tout en permettant aux cellules en mitose de poursuivre le cycle de celle-ci.

Ross[15], partant de la similitude des effets mutagènes et chromosomiaux produits sur *Vicia faba*, d'une part par les diépoxydes et les ypérites (Loveless et Revell[16]) qui réagissent par l'intermédiaire d'un ion carbonium C^+, et d'autre part, par les rayons X et les peroxydes, qui interviennent par le moyen de radicaux libres, a établi différents points d'analogie entre les deux modes d'action.

La similitude entre ces mécanismes n'est cependant pas absolue. Ford[17] a pu préciser, sur les cellules du méristème *Vicia faba*, que la méthyl-bis-(2-chloroéthyl)amine ($H.N_2$) agit électivement sur l'héterochromatine alors que celle des radiations ionisantes (des photons gamma en l'occurence) se distribue de manière égale aux chromosomes courts ; Ford a de plus constaté que les effets mitoclasiques de $H.N_2$ sont légèrement plus tardifs que ceux des rayons gamma ; cette différence a été également observée par Koller et Casarini[18] au niveau de la moëlle osseuse et du carcinome de Walker du rat ; de plus, ces auteurs au même titre que Loveless et Revell[16] puis Revell[19], considèrent qu'au contraire des photons X, dont l'influence est maximale à la fin de la période de repos du noyau ou au début de la prophase, $H.N_2$ agit essentiellement au début de l'intercinése c'est-à-dire au moment où la cellule se trouve en pleine phase métabolique préparatrice à la division. Pour ces derniers auteurs, $H.N_2$ et ses analogues ne sont nullement 'radiomimétiques', leur mode d'action étant vraisemblablement différent de celui des radiations ionisantes.

Une excellente mise au point de ce problème difficile a été publiée récemment par Koller[20] qui a notamment insisté sur le fait, signalé par Auerbach et Moser[21] que chez la Drosophile, l'abaissement de la tension d'oxygène diminue les effets génétiques des rayons X mais n'influence pas ceux de l'ypérite. Enfin, tout récemment Hicks[22], comparant au niveau des cellules nerveuses de l'embryon de sauterelle, les effets des R.X., des poisons radiomimétiques et de certaines hormones, a constaté que $H.N_2$, agissait sur ce substrat de manière nettement moins sélective que les radiations ionisantes.

L'action de l'ypérite et de ses dérivés sur les glandes génitales a été peu étudiée. Dans une revue générale des lésions provoquées par cette substance, dans l'espèce humaine, Sophie Spitz[23] n'apporte aucun élément concernant l'ovaire, mais signale un certain nombre d'altérations testiculaires, qui ont été décrites en détail par Landing et collaborateurs[24] ; ces lésions consistent essentiellement, dans les 24 premières heures, en déformations chromatiques

dans les noyaux au repos des spermatogonies et des spermatocytes I et dans une inhibition des mitoses reductionnelles ; plus tard, les tubules apparaissent désorganisées et ne montrent plus l'orientation classique des différentes couches, depuis les spermatogonies jusqu'aux spermatozoïdes achevés. Pour KINDRED[25], les éléments les plus sensibles de la lignée sont les spermatides.

Comme nous l'avons fait observer antérieurement, il existe, entre ces altérations testiculaires et les modifications que nous avons constatées dans l'ovaire traité par $H\!N_2$, une certaine analogie, en ce sens que la désintégration des parois tubulaires du testicule correspond à la désorganisation du mécanisme de l'évolution et de l'involution folliculaires.

Mais d'importantes différences sont à signaler entre les deux organes en ce qui concerne les types de cellules germinales présentant le maximum de sensibilité à $H\!N_2$. Dans le testicule, en effet, ce sont, d'après Kindred[25] les spermatides qui présentent ce caractère ; or ces éléments n'ont pas de correspondants dans l'ovaire de lapine normale, puisque l'ovotide (formée par élimination des deux globules polaires à la suite des deux mitoses réductionnelles) n'apparaît que dans la trompe après ovulation et fécondation. En fait, l'ovaire adulte ne renferme que des ovocytes de premier ordre qui vont s'accroître régulièrement, du stade folliculaire *P* à la fin du stade *D*, puis se préparer lentement du stade *E* au stade *G* à la prophase de la première mitose de maturation. Par conséquent, la sensibilité particulière, observée par nous, des follicules primordiaux et des follicules mûrissants doit tenir à un autre facteur que l'état nucléaire ou cytoplasmique de l'ovocyte ; et ce facteur nous croyons pouvoir le situer dans les cellules folliculeuses.

Lorsque un follicule *P*, faisant partie de la réserve corticale de ces élements est incité (pour une raison que nous cherchons depuis longtemps à situer de manière précise, mais que nous savons avec certitude être liée à l'ovaire lui-même—car elle continue à intervenir après hypophysectomie, thyroïdectomie, destruction chimique ou chirurgicale des attaches nerveuses de l'ovaire etc.) à prendre la voie qui le conduira au premier stade evolutif *A* et, s'il ne s'atrésie pas en cours de route, aux stades suivants jusqu'à la forme préovulatoire *G*, les minces cellules aplaties, en couche unique, de son enveloppe, deviennent cubiques, se multiplient et construisent en feuillets successifs la paroi interne granuleuse du follicule, secrètent le liquide folliculaire, produisent l'antrum et assurent par la corona radiata, la nutrition ainsi que le soutien de la cellule oeuf par la corona radiata.

Or il est bien connu (particulièrement depuis les expériences d'irradiation de l'ovaire) que la sensibilité maxima du follicule correspond aux moments où ses cellules folliculeuses traversent les deux périodes critiques qui se situent d'une part au passage du stade *P* au stade *A* et d'autre part, à la fin du stade *G*, lorsque la paroi, amenuisée par la distension antrale, est prête à la rupture.

C'est pourquoi, nous estimons, qu'en sus d'une possibilité de lésion directe de certains ovocytes fragilisés, l'atteinte préférentielle, par $H\!N_2$, des follicules *P* et des follicules *G*, est due, pour les premiers, à une lésion par ce toxique des minces cellules folliculeuses, prêtes à entrer en division pour constituer la future paroi multistratifiée du follicule *A*, et pour les seconds, à une destruction soit de certains éléments de la corona avec comme conséquence la mise à la paroi de l'ovocyte et l'entrée en atrésie méroxanthosomiale du

follicule, soit, plus rarement, de groupes granuleux sous thécaux, qui, en s'atrophiant, créent une zone de moindre résistance avec rupture intra-ovarienne.

Cette manière de voir nous conduit sans difficulté à l'une des deux interprétations qui peuvent être données de la genèse des formations polynuclées, pseudoplasmodiales, que nous avons observées dans le cortex ovarien, du quatrième au septième jour après HN_2 seul. Rappelons au préalable que des élements de structure comparable observés par Landing et collaborateurs[24] dans les testicules de souris traitées par diverses moutardes nitrogénées ont été tout spécialement étudiés par Kindred[25] dans les canaux séminifères du rat traité par le composé tris(β-chloroéthyl)amine ; ces groupes plurinucléés dérivent quelquefois de spermatocytes de premier ordre, mais le plus souvent ils ont pour origine soit les spermatocytes de deuxième ordre (d'après Landing et collaborateurs), soit les *spermatides*, qui de tous les élements de la lignée, sont d'après Kindred les plus sensibles aux effets de HN_3. Kindred a noté l'existence en abondance de ces cellules géantes chez quatre animaux sur 18 ; il en a rencontré mais en beaucoup plus petit nombre, chez certains témoins. Pour expliquer la genèse de ces éléments, cet auteur suppose que HN_3 (comme HN_2 d'ailleurs) qui est un alkyl soluble dans les graisses, provoque une modification de la tension superficielle et finalement la dégénérescence des barrières qui séparent les cytoplasmes des cellules séminales et entraîne ainsi leur confusion. Le fait qu'un certain nombre d'obstacles protéiques ou autres, peuvent s'opposer avec un succès, variable d'un animal à un autre, au passage du toxique du plasma sanguin vers les cellules testiculaires, serait, d'après Kindred, la raison des différences individuelles constatées quant au nombre présent de cellules géantes.

D'indiscutables analogies morphologiques existent entre ces groupements spéciaux des testicules de souris et de rats et les formations plasmodiales que nous avons vues dans les ovaires de lapine.

Notons cependant que dans ces derniers, l'élément en cause est et ne peut être que l'ovocyte I, alors que dans le testicule il s'agit de spermatocytes II ou de spermatides, n'ayant pas de correspondants dans la grande femelle.

En outre, les spermatocytes et les spermatides ne sont séparés les uns des autres que par leurs membranes cytoplasmiques, tandis que les ovocytes primordiaux sont déjà entourés d'un fin treillis protecteur de quelques cellules folliculeuses aplaties ; de sorte que, pour réaliser par un mécanisme analogue à celui admis par Kindred, le groupement des vésicules germinatives au sein d'une masse cytoplasmique commune, HN_2 devrait assurer la destruction, non pas d'une simple lame cytoplasmique condensée, mais d'une véritable barrière cellulaire ; or, nous avons vu plus haut que cette destruction de la granuleuse est responsable de la mort des follicules *P* isolés ; rien ne s'opposerait donc à ce que cette même lyse granuleuse—pour autant qu'elle soit limitée aux régions d'accolement d'un groupe de follicules *P* et respecte la région qui sépare ce groupe du stroma ovarien—soit à l'origine des groupes plasmodiaux.

Toutefois, la chronologie assez tardive de ce processus (qu'on ne voit clairement après HN_2 seul qu'à partir de la 96e heure) et l'intégrité remarquable de la plupart des ovocytes contenus dans les amas, nous ont amené

à reconsidérer son origine, dont l'essence dégénérative—probable dans le testicule—nous paraît ici moins évidente.

Nous avons en effet signalé que l'une des caractéristiques essentielles de l'ovaire ypérité est une hyperproduction très précoce (dès la douzième heure) de follicules jeunes *A* et *B* principalement, ce qui présuppose la mise en chantier rapide de nombreux follicules primordiaux ; cet effort a pour conséquence un hâtif 'départ en ligne' de groupes primordiaux dont les éléments n'ont pas le loisir d'organiser une paroi granuleuse individuelle ; seules, se divisent les cellules folliculeuses des éléments du groupe situés à la périphérie, tandis que les autres—qui devraient assurer la formation des 'septa' entre les différents follicules *P*, s'atrophient et réalisent ainsi la formation de pseudoplasmodes.

Quant à la raison pour laquelle ces groupes ne sont visibles qu'au delà de la 72° heure, alors que l'accroissement des éléments *A* et *B* est déjà très net à la douzième heure, nous la situons dans le système interprétatif suivant.

Lorsqu'à lieu une poussée folliculaire, le nombre des éléments *P* qui partent en évolution est conditionné par le nombre de follicules *A* qui se sont atrésiés lors du passage au stade suivant *B* ; si cette atrésie est excessive (et c'est le cas sous l'influence de $H\mathrm{N}_2$), les follicules *P* se dégagent de la réserve en beaucoup plus grand nombre, *mais à partir d'éléments déjà préparés à cet avenir*. Et ce n'est que plus tard que l'effort de réparation demandé au cortex ovarien met en jeu des formes primordiales non encore libérées les unes des autres et groupées en paquets dans les lacis conjonctifs qui occupent l'espace situé directement sous l'épithélium germinatif ; le départ de ces follicules primordiaux se fait hâtivement et en groupes compacts, dont les éléments peuvent aisement se confondre sous une enveloppe folliculeuse commune.

Vue de cette manière, la production de groupes primordiaux polynucléés serait donc d'essence purement régénératrice et traduirait simplement la hâte excessive que met l'ovaire à préparer ceux de ses éléments de réserve qui sont destinés à combler les vides créés par l'atrésie au sein des lignées de follicules évolutifs proprement dits.

Une troisième hypothèse—d'ailleurs séduisante—nous a été fort courtoisement soumise à Gênes par Aron[26] qui, frappé de l'analogie morphologique entre les amas plurinuclées décrits par nous et les cordons sous corticaux que montre l'ovaire prépubéral à la fin de l'ovogenèse, nous a demandé si nous ne devions pas considérer ces pseudoplasmodes comme la manifestation d'une néoformation d'ovocytes à partir de l'epithélium germinatif adulte.

Nous accepterions volontiers cette suggestion si, malheureusement, elle ne se heurtait à la très sérieuse objection que nous avons de tout temps faite à l'idée—déjà rejetée par de Winiwarter[27]—de la persistance de potentialités ovogenétiques chez la lapine adulte, à savoir l'impossibilité de mettre en évidence, dans cette espèce animale, les phases nucléaires successives qui conduisent du stade protobroque de l'ovogénie jeune au stade diplotène de l'ovocyte de premier ordre.

Bien que puissent être opposées à cette objection la notion probable de la fugacité de ces images transitionnelles et la démonstration par Gaillard[28] d'une ovogenèse indiscutable à partir de l'épithélium germinatif de fragments en culture d'ovaires de lapines adultes, nous croyons devoir nous

limiter—pour l'instant—à conférer aux formations polynuclées de l'ovaire ypérité une signification régénératrice, en attribuant leur production à l'accélération excessive de leur mise en chantier ; mais il est évident que la découverte de figures de passage entre l'épithélium germinal et ces groupes pseudoplasmodiaux modifierait immédiatement notre opinion actuelle au sujet de la genèse de ces interessantes formations.

Il n'est pas sans interêt de rappeler ici que dans le duodenum du rat, irradié (Engelstad[29], Warren et Friedman[30]) ou traité par $H.N_2$ (Desaive et Varetto-Denoel[6]) on peut voir, en doublure du fond des villosités, des formations pseudo-syncytiales analogues à celles observées dans le testicule et l'ovaire, et dont l'origine—à partir des cellules génératrices en multiplication accelérée—est également de signification réparatrice.

Nous avons déjà signalé que les ruptures folliculaires intra-ovariennes, exceptionnellement fréquentes après ypérite, ont pour cause une altération localisée de la paroi granuleuse des grands follicules mûrissants.

Quant aux éléments à ovocytes multiples, le tableau de leur distribution dans les ovaires ypérités qu'ils sont particulièrement nombreux tout au début de l'expérience, à la douzième heure, et à la fin de celle-ci, à la 96e heure. La première de ces deux poussées a vraisemblablement pour origine le brusque départ vers les stades *A* et suivants de follicules *P* préparés à cette évolution ; comme ce changement d'état affecte un nombre de ces éléments de loin supérieur à la normale, on conçoit ainsément que deux ou trois follicules, ou d'avantage puissent rester accolés pendant les premières phases de leur croissance, favorisant ainsi la production d'éléments multiovulaires, moyens ou grands. La seconde poussée trouve une explication dans les groupes primordiaux polynuclées, qui, comme nous l'avons décrit plus haut, apparaissent aux environs du troisième ou du quatrième jour.

Il nous reste à discuter le comportement des ovaires chez des lapines préparées par le bècaptan, avant d'être injectées de $H.N_2$.

Depuis la publication des recherches de Colter et Quastel[31], on connait l'effet protecteur qu'exerce *in vitro* la triméthylamine à l'égard de l'action inhibitrice d'$H.N_3$ sur certains enzymes. Pour notre part, nous avons précédemment observé que le bécaptan protège dans une certaine mesure, contre les effets de $H.N_2$, l'intestin grêle du rat, et nous venons de montrer qu'injecté avant $H.N_2$, le bécaptan abaisse, dans les premières heures, le pourcentage atrétique des follicules primordiaux et atténue par la suite le moindre passage, observé après $H.N_2$, des stades moyens aux stades préovulatoires *F* et *G*.

En d'autres termes, le bécaptan se comporte au niveau de l'ovaire comme un limitateur de l'atrésie ; et il est remarquable que ce même antagonisme s'observe à l'égard des radiations ionisantes puisque nous avons signalé antérieurement[32], que le bécaptan relève notablement le seuil de stérilisaion roentgenienne de l'ovaire.

Ces observations nous paraissent être en faveur d'une protection *directe* exercée par le bécaptan, à la fois vis à vis des ypérites azotées et des radiations ionisantes. En ce qui concerne l'ovaire, ce phénomène se situe soit dans l'ovocyte (bien que l'état de repos nucléaire de cette cellule ne plaide pas en faveur de cette hypothèse), soit plus vraisemblablement dans les cellules granuleuses qui, en constante multiplication à tous les étages de l'évolution

folliculaire, forment le feuillet trophique du follicule, et consiste probablement dans un processus de compétition entre le bécaptan et l'agent toxique employé, à l'égard des réactants cytoplasmiques ou nucléaires des cellules sensibles.

Par ailleurs, nous devons reconnaître que cette action directe du radioprotecteur n'aboutit qu'à des résultats protecteurs très limités ; de nombreuses expériences (que nous avons résumées et discutées dans notre travail précédent et sur lesquelles nous ne reviendrons pas ici) ont indiscutablement établi que la radioprotection se traduit d'avantage par une accentuation du processus de réparation ; au niveau de l'ovaire, nous avons vu plus haut que ces manifestations régénératrices se manifestent avant tout par l'apparition très précoce de groupements polynucléés sous corticaux ; et nous espérons que de prochaines recherches permettront d'établir de façon certaine les parts respectives que prennent, dans la couverture de l'organisme à l'égard des radiations ionisantes et des poisons radiomimétiques, l'intervention directe des substances protectrices à l'échelle de la cellule sensible, leur influence sur la genèse d'éventuels facteurs humoraux régénératifs ou encore leur participation à la libération d'agents hormonaux, stimulants de la réparation.

RÉSUMÉ

Les ovaires de lapines soumises à l'injection intraveineuse de 2 mmg de méthyl-bis-(2-chloroéthyl)amine présentent d'importantes altérations qui comprennent essentiellement, une part de manifestations atrétiques (trouble général du mécanisme de l'évolution et de l'involution folliculaires, accroissement considérable de l'atrésie des follicules primordiaux, diminution du nombre moyen des follicules mûrissants et atrésie atypique–méroxanthosomiale ou par rupture intra-ovarienne—de ces mêmes follicules) et une part de phénomènes régénérateurs (élévation dès la douzième heure du nombre de follicules jeunes, excès important de follicules pluriovulaires et apparition dans le cortex de groupements primordiaux plurinuclées).

La préparation des animaux par l'introduction intrapéritonéale de 50 mmg de β-mercaptoéthylamine (Bécaptan Labaz), trois minutes avant l'injection intraveineuse de 2 mmg de HN_2, se traduit par : un trouble plus discrèt du mécanisme complexe de l'évolution et de l'involution folliculaires, une moindre atrésie des follicules primordiaux dans les premières heures, le maintien à un taux moyen plus élevé des grands follicules préovulatoires, la surabondance de follicules multiovocytaires et l'extrême précocité d'apparition dans le cortex de groupements primordiaux polynuclées.

Les expériences ci-dessus relatées confirment donc les résultats que nous avons obtenus au niveau de l'ovaire au moyen de doses plus faibles de HN_2 ; bien que les ovaires lésés par les doses—non léthales—de HN_2 que nous avons employées soient capables de retrouver rapidement une apparente intégrité, il ne paraît pas douteux que les dérivés bi- ou tri-méthylés du gaz moutarde soient en mesure de provoquer chez la femme des altérations plus ou moins profondes de la fonction ovarienne dont la possibilité d'apparition doit être connue des cancérologues cliniciens.

La β-mercaptoéthylamine, introduite à la dose de 50 mmg dans la cavité intrapéritoneale quelques moments avant l'injection intraveineuse de 2 mmg

de HN_2, s'oppose dans une certaine mesure aux effets atrésiants de ce poison et accentue, en les hâtant, les processus folliculaires réactionnels à caractère réparateur.

Nous adressons tous nos remerciements à Messieurs les Docteurs A. Herve et J. Closon ainsi qu'à Madame Varetto-Denoel, Mademoiselle Y. Francis et Monsieur G. Thiry pour leur assistance technique.

REFERENCES

[1] Lacassagne, A. Étude physiologique et histologique des effets produits sur l'ovaire par les rayons X—Thèse de Lyon—1913.
[2] Gricouroff, G. *Radiophysiologie et radiothérapie*, 1931, **2** fasc. 1.
[3] Desaive, P. Contribution radiobiologique à l'étude de l'ovaire—Thèse 1940.
[4] Desaive, P. Communication—41e Réunion de l'Association des Anatomistes—Gênes, avril 1954, sous presse.
[5] Bacq, Z. M. et coll. *Science*, 1953, **117** 633.
[6] Desaive, P. et Varetto-Denoel, Jos. Communication—6e Congrès International de Lutte contre le Cancer, Sao Paulo, juillet 1954.
[7] Desaive, P. *Arch. Biol.*, Tome LVIII, fasc. 4, 1947, et Tome LIX, fasc. 1, 1948.
[8] Desaive, P. *Arch. Biol.*, Tome LX, fasc. 4, 1949.
[9] Auerbach, C. et Robson, J. Rev. Min. Suppl. no. W.3979, 1942. (Cités par Butler et Randall, *Progress in Biophysics*, Londres, 1954, vol. 4.).
[10] Koller, P. C. *Proc. Roy. Soc. (Edimbourg)*, 1943, **61** 398.
[11] Darlington, C. D. et Koller, P. C. *Heredity*, 1947, **1** 187.
[12] Gillette, R. et Bodenstein, D. *J. Exp. Zool.* 1946, **103** no. 1.
[13] Bodenstein, D. *J. Exp. Zool.* 1947, **104** 311, et 1948, **108** 93.
[14] Gilman, A. et Philips, F. S. *Science*, 1946, **103** 409.
[15] Ross, W. C. J. *Nature*, 1950, **165** 108.
[16] Loveless, A. et Revell, S. *Nature*, 1949, **164** 938.
[17] Ford, C. E. *Brit. J. Radiol.* 1952, **25** 189.
[18] Koller, P. C. et Casarini, A. *Brit. J. Surg.* 1952, **6** 173.
[19] Revell, S. *Heredity*, 1953, **6** suppl. 107.
[20] Koller, P. C. *In Progress in Biophysics*, 1954, **4** 195. London, Pergamon Press Ltd.
[21] Auerbach, C. et Moser, H. *Experientia*, 1951, **7** 341.
[22] Hicks, S. *Journ. of Cellular and Comparative Physiology*, 1954, **43** suppl. 1, 151.
[23] Spitz, S. *Cancer*, 1948, **1** 383.
[24] Landing, B. H., Goldin, A., Noe, H. E., Goldberg, B. et Shapiro, D. N. *Cancer*, 1949, **2** 1055.
[25] Kindred, J. *Arch. Pathol.* 1947, **43** 253.
[26] Aron, M. (Communication verbale.)
[27] De Winiwarter, H. *Arch. Biol.* 1910, **25** 169, 683.
[28] Gaillard, J. P. (Communication verbale.)
[29] Engelstad, R. B. *Amer. J. Röntgenol.* 1938, **40** 243.
[30] Warren, S. et Friedman, N. B. Cités par Friedman, *Arch. Pathol.* 1942, **34** 749.
[31] Colter, J. S. et Quastel, J. H. *Nature*, 1950, **166** 773.
[32] Desaive, P. *Acta Radiologica*, 1954, **41** 545.

HISTOLOGICAL CHANGES IN MAN AND RABBITS AFTER PARENTERAL THORIUM ADMINISTRATION

CHARLES JOHANSEN

Finsen Institute, Copenhagen

THORIUM has a half-life of $1{\cdot}4 \times 10^{10}$ years, the decay products have an appreciably shorter life, and the activity of 4 g of thorium is fairly equivalent to that of 1 μg of radium. 90 per cent of the activity of thorium is due to the alpha activity, 9 per cent to the beta and 1 per cent to the gamma activity. The alpha radiation is thus completely dominant. Thorium is generally used as a 20 per cent colloidal suspension of thorium dioxide, that is to say 20 ml of this suspension are equivalent to about 1 μg of radium.

It is well known from the literature that thorium is practically not excreted from the organism and, in the case of intravenous and intra-arterial administration, is deposited in the cells of the reticulo-endothelial system, and therefore especially in the spleen, the liver and the active bone marrow ; furthermore, it is known that in and around the thorium deposits, degenerative changes and fibrosis are found.

When thorium is injected outside the blood-stream, the greater part remains at the site of injection and may here produce more distinctive changes, as will be shown here in the vascular wall at the site of injection, in paravascular deposits and in the articular capsule when it has been used for arthrography.

It has been discussed in the literature whether the effects of thorium are so detrimental that its use is dangerous. A few cases of aplastic anaemias have thus been mentioned (SPIER and co-workers[1], SCHMIDT and co-workers[2]), and the occurrence of a few tumours has been reported (MACMAHON and co-workers[3], LÜDIN[4], HORTA[5], ZOLLINGER[6], ABRAHAMSON and O'CONNOR[7]), and animal experiments on rats have shown an indubitable carcinogenic effect of thorium. Other authors (for instance LIMA[8]) consider that they have seen no harmful sequelae of the use of thorium, while THOMAS[9] and co-workers adopt a view between these two extremes ; on the basis of a series of 4,300 patients they consider that they have seen no severe sequelae but, owing to a tendency to fibrosis of the liver and the spleen and to cicatricial changes, they nevertheless think that thorium should only be used when the indications weigh much in favour of such treatment.

Our series comprises about 250 patients who received doses equivalent to from 0·5 to 5 μg of radium. Four of the patients died of diseases of the blood.

These were :

(*1*) A man, aged 40, who 11 years earlier had been given doses of thorium equivalent to about 1 μg of radium. He now had a severe, aregenerative anaemia with a

Hgb. percentage of about 30. In the course of 2 years he received about 65 blood transfusions, but then died of agranulocytosis with sepsis and severe haemosiderosis.

(*2*) A woman, aged 37, who 7 years earlier had received an injection of a quantity of thorium equivalent to 1 to 2 μg of radium. She died of typical myelosclerosis.

(*3*) A man, aged 60, who 10 years earlier had had a dose of thorium deposited which was equivalent to 1 μg of radium. He died of chronic myelogenic leukaemia.

(*4*) A man, aged about 40, who died of stem-cell leukaemia 14 years after he had been contaminated with a thorium dose equivalent to 2 μg of radium.

On the other hand, our series showed no increased incidence of cancer and contained no cases of malignant tumours whose occurrence could be reasonably attributed to the thorium contamination.

In experiments on rabbits I have given intravenous injections of doses of thorium equivalent to from 0·005 to 0·125 μg of radium per kg. Hitherto, none of these animals have presented leukaemic changes, but so far the cause of death in 8 animals has been a disseminated, malignant tumour, a reticulo-endothelio-sarcoma. These animals had all been given doses of thorium equivalent to 0·07 μg of radium per kg, or more, and they died between 2 and 3 years after the injection. Presumably a much greater number of animals in the groups will present this form of tumour, since, so far, all animals which received these doses and died more than 2 years after the injection had this tumour as cause of death. None of the controls has presented tumours of any kind. With regard to the malignancy of the tumour, there can be no doubt, as it can be transplanted. After intramuscular injection of tumour tissue, cortisone-treated animals showed tumour-cell emboli in the pulmonary capillaries a few days later, and a few animals have had tumours in the lungs.

Exactly the same form of tumour has been found in human beings in three cases, all following injection of thorium into the blood-stream. Horta's[5] and Lüdin's[4] patients thus died 3 and 14 years respectively after injections of thorium doses equivalent to about 1 μg of radium, and MacMahon's[3] patient died 14 years after injection of a dose corresponding to about 4 μg of radium. This last-mentioned patient died of intraperitoneal haemorrhage after spontaneous rupture of the tumour, and I may mention that 2 of my rabbits showed the same cause of death. This form of tumour may thus occur in both man and rabbits, and is presumably one that is specific of thorium deposition in the parenchymatous organs. It may therefore seem strange that we have not observed one case of this tumour in our series of about 250 persons. In my opinion this is just a question of time. Our series exclusively comprises persons who were contaminated with thorium from 4 to 16 years ago, and we know nothing definitely about the length of the period that will pass before radiation-induced tumours in man manifest themselves clinically.

Several scientists, however, suggest that radiation-induced cancers will appear from 10–30 years after the contamination with an optimum time of 16 years. If we acknowledge this assumption the following calculations may give an idea of the cancer problem for our patients. In *Figure 1* the block diagram to the left gives absolute numbers of patients as ordinates and years after contamination as abscissa ; it is first noted that only 3 of the patients have passed the 16-years limit. To the right a Gaussian-like curve

for assumed cancer incidence has been drawn ; the optimum is at 16 years and the ordinate is arbitrary as nothing is known about the percentage incidence ; for the uranium mine workers the cancer incidence was about

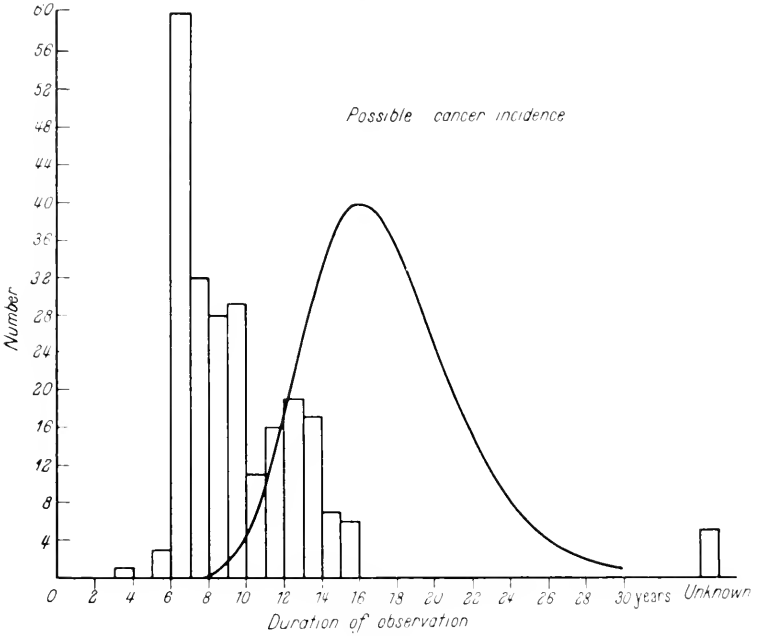


Figure 1

50 per cent, and for the New Jersey patients the cancer incidence has been about 10 per cent so far in a non-selected material*. It is evident from *Figure 1* that we must wait about 10 years before we can answer the cancer question and about 20 years before it can be fully cartographed.

REFERENCES

[1] Spier, J., Cluff, L. E. and Urry, W. D. *J. Lab. Clin. Med.* 1947, **32** 147.
[2] Schmidt, W., Schultz, A. and Lapp, H. *Strahlentherapie*, 1950, **81** 93.
[3] MacMahon, H. E., Murphy, A. S. and Bates, M. I. *Amer. J. Path.* 1947, **23** 585.
[4] Lüdin, M. *Schweiz. Z. Allg. Path.* 1953, **16** 987.
[5] Horta da Silva, J. *Chirurg.* 1953, 218.
[6] Zollinger, H. V. *Schweiz. Med. Wsch.* 1949, **52** 1266.
[7] Abrahamson, L., O'Connor, M. H. *Irish J. med. Sci.* 1950, **6** 229.
[8] Lima, P. A. *Cerebral Angiography*, London, 1950.
[9] Thomas. Cited Loeb, V., Jr., Seamann, W. B. and Moore, C. V. *Blood*, 1952, **7** 915.

DISCUSSION

M. A. Gerebtzoff : Errera has asked me to present a document showing the long persistence of thorium in the human organism. It is an autoradiography of a liver preparation taken from a man who had received an injection of thorotrast some 20 years previously. One still sees thorium granules from which escape α-particles.

* Aub, Evans, Hempelmann and Martland (*Medicine* **31**, Sept. 1952) describe a cancer-incidence of 33 per cent in their selected material of patients with deposits of 0·7–7 μg C Ra± MsTh.

INDEX TO CONTRIBUTORS

Bold figures refer to papers read; ordinary figures indicate contribution to discussion