HEALTH EFFECTS OF PROJECT SHAD CHEMICAL AGENT:

BETAPROPIOLACTONE
[BETA-PROPIOLACTONE]

[CAS # 57-57-8]

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Health Effects of Betapropiolactone [Beta-propiolactone]
This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced personally from actual or perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person also possessing a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers. Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.
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I. EXECUTIVE SUMMARY

Betapropiolactone (beta-propiolactone; BPL) bears the chemical formula C₃H₄O₂ and is identified by the Chemical Abstract Service’s Registry Number 57-57-8. It normally appears as a colorless liquid with a pungent irritating odor. Betapropiolactone is soluble in water and miscible with acetone, chloroform, and ethanol.

Betapropiolactone has been used as a disinfectant. Capable of sporicidal action, it has been employed in the making of vaccines and in the sterilization of surgical instruments and tissue grafts. Other medical sterilization uses have included the sterilization of blood plasma, water, nutrient broth, and milk. Betapropiolactone has also served as a versatile intermediate in organic synthesis (acrylic acid and esters). In Project SHAD, it was used as a decontaminant.

Betapropiolactone is quickly hydrolyzed, metabolized, and excreted by mammals. The hydrolysis products excrete rapidly as well. The main metabolite of betapropiolactone is lactic acid; its main hydrolysis product is hydracrylic acid. The alkylating action of betapropiolactone reacts with polynucleotides and DNA to form carboxylethyl derivatives and this process is regarded as responsible for the genotoxicity characteristic of the compound.

Betapropiolactone is a significant irritant to several systems, and has shown permanent effects on the eye, liver, and kidney. Since the 1960s awareness has grown of the compound's high tumorigenic, genotoxic, and carcinogenic toxicity in animals, which have been observed to occur even from single dose administration. Human epidemiological, case-study, and in vivo experimental reports have not been found, however, except for reports of a series of Henry Ford Hospital volunteer experiments in the 1950s using beta-propiolactone's as an anti-hepatitis blood plasma disinfectant and the testing in 1968 of beta-propiolactone as a disinfectant in reaginic sera administered for allergy studies. The Henry Ford studies reported that human acute and chronic risks from intravenous administration are negligible; the reaginic sera study found minor irritations, displayed vesicles, discoloration, and papules in the areas of human skin inoculation. Related animal studies at Henry Ford did find chronic cumulative toxicity in animals, manifested as weight loss and necrosis of kidney tubules and the liver.

In acute administration in animals, beta-propiolactone has proven an irritant to skin, eyes, the respiratory and digestive systems. Dermal contact can elicit blisters and burns. Scarring, erythema, and hair loss have been found on mouse skin after 1-6 administrations of 0.8-100 mg of beta-propiolactone.

Ocular administration in rabbits has resulted in pain, miosis, and corneal opacity, which can become permanent. Respiratory exposure is associated with inflammation of the respiratory tract. Oral ingestion can cause stomach and mouth burns. Acute intravenous administration has resulted in liver necrosis and kidney tubular damage. Systemic
absorption may result in twitching and gasping, with convulsion and death at higher
doses. Frequent urination, dysuria, and hematuria may also attend higher systemic doses.

Degradation products from the hydrolysis of beta-propiolactone have been tested. They
have been found to be significantly less toxic than beta-propiolactone. A comparison of
their LD\textsubscript{50}s shows toxicity levels of the degradation products to be as much as 5-10 times
less toxic than beta-propiolactone.

Beta-propiolactone is rated a confirmed animal carcinogen with unknown relevance to
humans (Group A3) by the ACGIH (American Conference of Governmental Industrial
Hygienists). The Threshold Limit Value (TLV) recommended by the ACGIH is 0.5 ppm
(1.5 mg/m\textsuperscript{3}). The \textit{NIOSH Pocket Guide to Chemical Hazards} considers beta-
propiolactone to be a potential occupational carcinogen. The International Agency for
Research on Cancer (IARC) regards beta-propiolactone as a possible human carcinogen
(Group 2B) and cautions that a single-dose exposure is enough to pose a significant risk
of cancer

Probably as a result of the fact that beta-propiolactone degrades rapidly in water and
plasma, its tumorigenic effects appear to occur primarily around the initial site of
exposure. Thus, in tested animals, benign and malignant skin tumors (papillomas,
squamous cell carcinomas, keratocanithomas, melanomas; subcutaneous injection-site
sarcomas, fibrosarcomas, adenocarcinomas, squamous cell carcinomas), nasal tumors,
and forestomach tumors (squamous cell carcinomas) are the observed effects, related to
dermal/subcutaneous, inhalational, and oral administration respectively. Meanwhile,
beta-propiolactone has been ruled out as an agent causing central nervous system cancer
in rats.

Single dose administration has resulted in cancer induction in experimental animals.
After single dose administration of 100 mg beta-propiolactone on suckling mice 9-11
days after birth, lymphomas and hepatomas were induced. Single dose exposures also
have been genotoxic.

The genotoxicity of beta-propiolactone has been well studied. Genotoxicity testing
indicates a wide range of effects, both \textit{in vivo} and \textit{in vitro}. Cell transformation and gene
mutations have been observed in human cells \textit{in vitro}. Bacterial testing has induced gene
conversion, aneuploidy, and mutations. In \textit{Drosophila}, beta-propiolactone produced
translocations and sex-linked recessive lethal mutations. \textit{In vivo}, gene mutations in the
stomach and liver of mice, and DNA strand breaks in rat bone marrow cells have been
reported, along with covalent binding to mouse skin DNA and RNA.

The treatment for acute exposure to beta-propiolactone is the standard emergency
treatment for a highly irritant chemical, including avoiding emesis and diluting the
chemical in the stomach after oral consumption. A possibility for chemoprevention of
cancer effects is sodium thiosulfate, which may inhibit beta-propiolactone’s capacity for
stomach tumorigenesis.
Psychogenic effects of exposure specifically to beta-propiolactone were not found in the literature. General psychogenic effects of perceived exposure to agents involved in chemical and biological warfare are examined in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”

An online “glossary” of Project SHAD agents suggests that beta-propiolactone’s carcinogenicity is subject to question due to the absence of adequate controls in experiments. That appears to derive from a comment by the National Toxicology Program (2002) referring to one prior study’s finding of beta-propiolactone induction of keratocanthomas and melanoma in one species. Controls, however, are reported in many studies, and the studies have been generally evaluated as adequate; beta-propiolactone’s animal carcinogenicity is regarded as confirmed by the IARC; the chemical is regularly used to induce animal cancer in controlled tests.
II. BACKGROUND DATA

Chemistry

Chemical Formula: C₃-H₄-O₂

Chemical Structure:

![Chemical Structure](image)

Project SHAD Chemical Agent Name: Betapropiolactone

Chemical Abstracts Service (CAS) Registry Number: 57-57-8

Other Commonly Appearing Names: Beta-propiolactone, β−-propiolactone, b-propiolactone, beta-lactone, propanolide, NSC-21626, Betaprone, 3-propiolactone, 3-propanolide, lactone, 2-Oxetanone.

Abbreviations: BPL, BP (Project SHAD)

Molecular Weight: 72.06

Density/Specific gravity: 1.146 @ 20 °C

Vapor Pressure: 3.4 mm Hg @ 25 °C

Boiling Point: 162 °C

Melting Point: -33.4 °C


Because the compound is more commonly and readily found as "beta-propiolactone" in medical and academic literature, that rendering is used throughout this review.

Beta-propiolactone appears as a colorless liquid with a pungent irritating odor. It is soluble in water and miscible with acetone, chloroform, and ethanol (HSDB 2004; NTP 2002; Pohanish 1996).
Beta-propiolactone hydrolyzes readily into hydracrylic acid. Other hydrolysis products include sodium beta-chloropropionate and lactic acid. Lactic acid is the major metabolite of beta-propiolactone (Kelly 1957).

Use & Manufacture

Beta-propiolactone was first commercialized in 1958, though it was tested for many years prior. Its most common use was as a versatile intermediate in organic synthesis (acrylic acid and esters). This use has diminished in recent years due to more efficient methods of synthesis. One of beta-propiolactone’s other significant uses has been as a disinfectant agent (HSDB 2004; NTP 2002; IARC 1999). Capable of virucidal action, it has been employed in the making of vaccines, the sterilization of surgical instruments, tissue grafts, blood plasma, water, nutrient broth, and milk (HSDB 2004; NTP 2002; IARC 1999) In Project SHAD, it was used as a decontaminant (Project SHAD Glossary 2004).

Mechanistic Considerations

Beta-propiolactone is quickly hydrolyzed, metabolized, and excreted. The hydrolysis products excrete rapidly as well (Kelly 1957).

The genotoxicity engendered by beta-propiolactone appears to derive from its alkylating action, which reacts with polynucleotides and DNA to form carboxylethyl derivatives. Beta-propiolactone reacts with polynucleotides and DNA at the N7 of guanine and N1 of adenine. It also engages in adduct formation with N3 of cytosine and thymine (IARC 1999; NTP 2002).
III. TOXICITY/HEALTH EFFECTS

General

Beta-propiolactone is a significant irritant to several systems; it can produce permanent effects on the eye, liver, and kidney (NTP 2002). Additionally, and of greater significance, awareness has grown since the 1960s of the compound's high (arising possibly from even a single dose) tumorigenic, genotoxic and carcinogenic toxicity (Palmes 1962; Parish 1966a; Parish 1966b; IARC 1999; NTP 2002; HSDB 2004). These endpoints of concern have been determined from animal studies.

Human epidemiological, case-study, and in vivo experimental reports have not been found, except for Henry Ford Hospital plasma transfusion studies of the 1950s, and tests in the late 1960s of human volunteers on the use of beta-propiolactone as a disinfectant in reaginic sera for passive transfer of allergy (Kelly 1957; LoGrippo 1964; Perlman 1969). The Henry Ford studies found no human acute or chronic risks from intravenous administration. The reaginic antibody study reported only transitory local injection-site irritation and discoloration.

Degradation products have been tested. They have been found to be less toxic than beta-propiolactone. A comparison of their LD$_{50}$'s shows the degradation products to be as much as 5-10 times less toxic than beta-propiolactone (Kelly 1957).

Further discussion of the acute and chronic effects of beta-propiolactone follows. The material related to carcinogenicity and related toxicity (tumorigenicity and genotoxicity) is treated separately further below because of its scope and importance.

Acute (Non-Carcinogenic) Administration/Effects

Acute administration has both immediate and long-term effects.

Acute LD$_{50}$/LC$_{50}$ values (HSDB 2004; Kelly 1957; Rees 1974):

Intravenous (IV):

Mouse ------ 345 mg/kg
Rabbit ------ 91 mg/kg
Guinea pig - 155 mg/kg
Dog --------- 90 mg/kg
Rat --------- 170 mg/kg

Inhalation:

Rat -------- 250 ppm/30 min.
25 ppm/6h
In acute administration, beta-propiolactone has proven an irritant to skin, eyes, the respiratory and digestive systems (HSDB 2004; NTP 2002).

Dermal contact can elicit blisters and burns. Scarring, erythema, and hair loss have been seen on mouse skin after 1-6 administrations of 0.8-100 mg. Guinea pigs showed less response (HSDB 2004; NTP 2002). Minor transitory irritations, displayed vesicles, discoloration, and papules around the sites of inoculation with beta-propiolactone as a sterilizing agent in reaginic sera used in allergy assays have been observed (Perlman 1969).

Ocular administration in rabbits has resulted in pain, miosis, and a corneal opacity which can become permanent (HSDB 2004; NTP 2002).

Respiratory exposure is associated with inflammation of the respiratory tract. Oral contact can cause stomach and mouth burns. Acute intravenous administration has resulted in liver necrosis and kidney tubular damage. Systemic absorption may result in twitching, gasping, convulsion and death. Frequent urination, dysuria, and hematuria may attend higher doses (HSDB 2004; NTP 2002; Kelly 1957).

Human testing or case reports of oral or respiratory acute exposure were not found. Nonetheless, the Henry Ford Hospital ran a series of clinical tests with Beta-propiolactone as an anti-hepatitis disinfectant agent in plasma for intravenous transfusion (LoGrippo 1964; Kelly 1957). They reported no acute toxicity over 10 years and 2563 transfusions (LoGrippo 1964; Kelly 1957). As noted above, acute administration of beta-propiolactone as a sterilizing agent in reaginic sera has induced signs of inoculation-area human skin irritation (Perlman 1969).

Chronic (Non-Carcinogenic) Administration/Effects

The Henry Ford Hospital transfusion tests reported absolutely no chronic toxic effects from their infusion of beta-propiolactone-treated plasma into human volunteers. On average, patients received under 2-3 liters total and the vast majority received less than 3 administrations. A relative few did receive up to 70 transfusions, but only 6 received more than 20 administrations. Patient follow-up ran from 6 months to 5 years. 995 different recipients were administered beta-propiolactone (or beta-propiolactone and ultraviolet radiation)-treated plasma over a 10 year period. Marked cumulative toxicity in the form of weight loss and necrosis of the liver and kidney tubules was nevertheless observed in related animal experiments. (LoGrippo 1964; Kelly 1957).

Tumorigenicity/Carcinogenicity

In the practice of experimental toxicology, beta-propiolactone is generally accepted to be a carcinogen. Beta-propiolactone is used regularly to induce cancer in experimental animals. (NTP 2002; IARC 1999; Snyder 1986; Peristianis 1988; Atchison 1982; Stewart 1981; see generally the Bibliography with Abstracts section below, passim).
Beta-propiolactone has also proven to be highly genotoxic (see subsection Genotoxicity, below).

Beta-propiolactone is rated a confirmed animal carcinogen with unknown relevance to humans (Group A3) by the ACGIH (American Conference of Governmental Industrial Hygienists). The Threshold Limit Value (TLV) recommended by the ACGIH is 0.5 ppm (1.5 mg/m$^3$) (HSDB 2004; NTP 2002). The IARC regards beta-propiolactone as a possible human carcinogen (Group 2B) and cautions that a single-dose exposure is enough to pose a significant risk of cancer (IARC 1999). The NIOSH Pocket Guide to Chemical Hazards considers beta-propiolactone to be a potential occupational carcinogen (NIOSH 2004).

Although animal tests have repeatedly elicited tumors and malignancies and (as further discussed below) various genotoxicity tests (including human tissue) have confirmed extensive beta-propiolactone genotoxicity, in vivo human data have not been systematically evaluated for cancer, nor have selected human case studies been found (NTP 2002; IARC 1999). As noted, however, the Henry Ford Hospital testing of the 1950s cited above in the subsection on non-carcinogenic chronic effects found no human toxic effects of any kind from the human exposure to beta-propiolactone during tests of plasma infusions pre-treated with the chemical (and also with ultraviolet radiation in combination) as a proposed anti-hepatitis agent. (LoGrippo 1964; Kelly 1957).

Probably as a result of the fact that beta-propiolactone degrades rapidly in water and plasma, its tumorigenic effects appear to occur primarily around the initial site of exposure (HSDB 2004; NTP 2002; Kortselius. 1979). Thus, in tested animals, benign and malignant skin tumors (papillomas, squamous cell carcinomas, keratocanthomas, melanomas, subcutaneous injection-site sarcomas, fibrosarcomas, adenocarcinomas, squamous cell carcinomas), nasal tumors, and forestomach tumors (squamous cell carcinomas) are the observed effects, related to dermal/subcutaneous, inhalational, and oral administration respectively (NTP 2002; IARC 1999). Beta-propiolactone has been ruled out, however, as an agent causing central nervous system cancer in rats (Robbiano 1987).

Single dose administration has resulted in cancer in experimental animals (NTP 2002; Chernozemski et al. 1970). After single dose intraperitoneal administration of 100 mg beta-propiolactone on suckling mice 9-11 days after birth, lymphomas and hepatomas were induced (IARC 1999).

The carcinogenicity of beta-propiolactone appears to be enhanced on mouse skin by co-action with catechol (Van Duuren 1986).

**Genotoxicity**

Genotoxicity studies indicate a wide range of effects, both in vivo and in vitro. Cell transformation and gene mutations have been observed in human cells in vitro. Bacterial testing has induced gene conversion, aneuploidy, and mutations. In *Drosophila*
**melanogaster**, beta-propiolactone produced translocations and sex-linked recessive lethal mutations. *In vivo*, gene mutations in the stomach and liver of mice, and DNA strand breaks in rat bone marrow cells have been observed, along with covalent binding to mouse skin DNA and RNA (IARC 1999; Kortselius 1979).

Single dose exposures have been genotoxic (Brault 1999; Brault 1996; Stewart 1981).

The following is a summary of genotoxic studies reported by the GENE-TOX subset of the TOXNET database of the National Library of Medicine which illustrates the breath of study affirming the genotoxic action potential of beta-propiolactone exposure.

### GENE-TOX Evaluation B (post-1980):

**Species/Cell Type:** Chinese hamster ovary (CHO) cells  
**Assay Type:** Forward gene mutation at the HPRT locus  
**Assay Code:** CHO+  
**Results:** Positive  
**Panel Report:** EMICBACK/71517; MUTAT RES 196:17-36,1988  
**Reference:** EMICBACK/45725; MUTAT RES 94:449-466,1982

### GENE-TOX Evaluation A (pre-1980):

**Species/Cell Type:** Syrian hamster embryo (SHE) cells  
**Assay Type:** Cell transformation, focus assay  
**Assay Code:** CTF+  
**Results:** Positive  
**Panel Report:** EMICBACK/50076; MUTAT RES 114:283-385,1983

**Species/Cell Type:** Syrian hamster embryo (SA7/SHE) cells  
**Assay Type:** Cell transformation, viral enhanced  
**Assay Code:** CT7+  
**Results:** Positive  
**Panel Report:** EMICBACK/50076; MUTAT RES 114:283-385,1983
Species/Cell Type: Salmonella typhimurium
Assay Type: Forward and reverse gene mutation, host-mediated assay
Assay Code: HMA+
Results: Positive
Reference: EMICBACK/31455; JNCI J NATL CANCER INST 62:911-918,1979

Species/Cell Type: Mouse lymphoma (L5178Y) cells
Assay Type: Forward gene mutation at the thymidine kinase (TK) locus; chromosome aberrations
Assay Code: L51+
Results: Positive

Species/Cell Type: Mammalian polychromatic erythrocytes
Assay Type: Micronucleus test, chromosome aberrations
Assay Code: MNTT
Results: No conclusion

Species/Cell Type: Neurospora crassa
Assay Type: Forward gene mutation
Assay Code: NEF+
Results: Positive
Panel Report: EMICBACK/52327; MUTAT RES 133:87-134,1984

Species/Cell Type: Neurospora crassa
Assay Type: Reverse gene mutation  
Assay Code: NER+  
Results: Positive  
Panel Report: EMICBACK/52327; MUTAT RES 133:87-134,1984

Species/Cell Type: Allium cepa  
Assay Type: Chromosome aberrations  
Assay Code: ALC+  
Results: Positive  

Species/Cell Type: Vicia faba  
Assay Type: Chromosome aberrations  
Assay Code: VIC+  
Results: Positive  

Species/Cell Type: Escherichia coli polA (W3119 vs P3478)  
Assay Type: Rec-assay, DNA effects (bacterial DNA repair)  
Assay Code: RE1+  
Results: Positive  

Species/Cell Type: Nonhuman  
Assay Type: Sister-chromatid exchange (SCE) in vitro  
Assay Code: SC2+D  
Results: Positive  
Dose Response: With dose response  
Species/Cell Type: Mouse
Sex: Male
Assay Type: Sperm morphology
Assay Code: SPI-
Results: Negative

Species/Cell Type: Drosophila melanogaster
Assay Type: Sex-linked recessive lethal gene mutation
Assay Code: SRL+
Results: Positive

Species/Cell Type: Human diploid fibroblasts
Assay Type: Unscheduled DNA synthesis (UDS) in vitro, DNA effects
Assay Code: UDH+
Results: Positive

Species/Cell Type: Saccharomyces cerevisiae
Assay Type: Mitotic recombination or gene conversion
Assay Code: YEC+
Results: Positive
Panel Report: EMICBACK/52546; MUTAT RES 133:199-244, 1984

Species/Cell Type: Saccharomyces cerevisiae
Assay Type: Mitotic recombination or gene conversion
Assay Code: YEH+
Results: Positive
Panel Report: EMICBACK/52546; MUTAT RES 133:199-244,1984

Species/Cell Type: Saccharomyces cerevisiae
Assay Type: Reverse gene mutation
Assay Code: YER+
Results: Positive
Panel Report: EMICBACK/52546; MUTAT RES 133:199-244,1984

Species/Cell Type: Drosophila melanogaster
Sex: Male
Assay Type: Heritable translocation test, chromosome aberrations
Assay Code: DHT+
Results: Positive

Species/Cell Type: Nonhuman
Assay Type: In vivo carcinogenicity studies
Assay Code: CCG+
Results: Positive

Species/Cell Type: Salmonella typhimurium (one or more of the five standard strains: TA98, TA100, TA1535, TA1537, and TA1538)
Assay Type: Histidine reverse gene mutation, Ames assay
Assay Code: SAL+
Results: Positive
Reference: EMICBACK/21338; PROC NATL ACAD SCI USA 72:5135-5139, 1975
IV. PSYCHOGENIC EFFECTS

Psychogenic effects of exposure specifically to beta-propiolactone are not found in the literature. General psychogenic effects of perceived exposure to agents involved in chemical and biological warfare are examined in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”
V. TREATMENT & PREVENTION

Industrial safety instructions authorize the use of protective gear, including goggles, for any contact with beta-propiolactone (Spectrum Laboratories 2004; NIOSH 2004; Pohanish 1996). An exposure monitoring badge was tested for use in the 1970s (Segal 1976). Direct contact of any kind is strongly discouraged (Spectrum Laboratories 2004; NIOSH 2004; Pohanish 1996). The treatment for acute exposure is the standard emergency treatment for a highly irritant chemical, including avoiding emesis and diluting the chemical in the stomach after oral consumption (HSDB 2004).

A possible agent for chemoprevention of cancer is sodium thiosulfate which may inhibit beta-propiolactone’s capacity for stomach tumorigenesis (Wattenberg 1987).
VI. SECONDARY SOURCE COMMENT

An online “glossary” of Project SHAD agents suggests that beta-propiolactone’s carcinogenicity is subject to question due to the absence of adequate controls in experiments (Project 112 Glossary 2004). This appears to be a paraphrase of a comment by the National Toxicology Program referring to one study’s finding of beta-propiolactone induction of keratocanthsomas and one melanoma in one species (presumably but not clearly identified as Parish 1966b) (NTP 2002). Controls, however, are reported in many studies, and the studies have been generally evaluated as adequate; beta-propiolactone’s animal carcinogenicity is regarded as confirmed by the IARC and it is regularly used to induce animal cancer in controlled tests (NTP 2002; IARC 1999; Snyder 1986; Peristianis 1988).
VII. BIBLIOGRAPHY WITH ABSTRACTS

{Unless otherwise noted, the abstracts for the following references are rendered verbatim as provided by the original publication or as made available in a standard print or electronic catalogue, or database. Errors, omissions, or other defects of language, style, or substance are strictly those of the original source or its transmission.}


Reactions after booster injections of human diploid cell rabies vaccine (HDCV) were investigated to determine the possibility of IgE-type antibody involvement. Although normal manufacture of HDCV involves the inactivation of the virus with beta-propiolactone (BPL), the effect of BPL on nonviral vaccine components, such as host cell components or stabilizing proteins, may be typical of the haptenic action of small molecular weight chemicals. Specific IgE to commercial HDCV preparations, BPL-treated preparations of noninfected host MRC5 cell sonicate (BPL-MRC5), and a human albumin (HA) (BPL-HA) used as a stabilizing agent were detected in sera from five individuals who reported reactions after booster doses of HDCV. However, these patients had no detectable IgE to normal HA. Sera from nonvaccinated individuals, vaccinated individuals who reported no reaction after HDCV booster, and pollen-allergic individuals had no detectable IgE to HDCV, BPL-MRC5, or BPL-HA. Changes in the ratios of pre- to postbooster serum levels of specific IgE to HDCV and BPL-HA were significantly different in a group of 19 individuals who reported reactions to HDCV boosters; these changes in pre- to postbooster IgE levels in nonreactive vaccinees were not significant. Prebooster serum IgE RAST ratios to HDCV or BPL-HA were not predictive of potential reactions to HDCV. A number of experimental BPL-HA reaction mixtures were assayed to examine the effect of variable concentrations of BPL to HA. Increasing relative molar concentrations of BPL to HA resulted in increased electrophoretic mobility, whereas the highest relative specific IgE binding was detected in BPL-HA molar reaction mixtures of approximately 12.5:1. (ABSTRACT TRUNCATED AT 250 WORDS)


The quantitative study of adsorption, elution, and penetration of oncogenic polyoma virus (PV) labeled with 3H-thymidine under the influence of beta-propiolactone-inactivated Sendai virus (SV) was carried out in a permissive culture of mouse embryo fibroblasts and in two nonpermissive cultures of human embryo and chick embryo fibroblasts. In
adsorption of PV on permissive and nonpermissive cells SV was found to reduce the amount of the adsorbed virus if used before the adsorption and to have no effect on adsorption upon reverse order of treatment of the cells with the two viruses. SV facilitates elution with 5 mM EDTA of PV adsorbed on both permissive and nonpermissive cells. Under the influence of SV, PV could be eluted from the surface of the permissive cells within 1 hour after adsorption and from the surface of the nonpermissive cells within 5-6 hours. Three hours after PV adsorption on permissive cells, both treated and untreated with SV, most of PV DNA (approximately 60%) was found in the nuclear fraction. At the same time, in the nuclear fraction of nonpermissive cells, treated or untreated with SV, the amount of PV DNA was insignificant (about 13%). The fraction containing large granules isolated from the cells which had been in contact with SV was found to contain approximately 50% of 3H-thymidine label.


The mouse skin cocarcinogens fluoranthene, pyrene, and undecane were used with the indirect-acting carcinogen, benzo(a)pyrene (BP), and the direct-acting alkylating carcinogen, beta-propiolactone (BPL), in an in vitro transformation assay. Dose response, cytotoxicity, and transformation studies with these compounds were performed with a subclone (A31-1-1) of the Balb/3T3 cell line. Transformation frequencies were found to increase with increasing concentrations of BP used up to 1.0 micrograms/ml or when BPL was used up to 4.0 micrograms/ml. A significant increase (P less than 0.05) in the transformation frequency over that seen with carcinogen alone was observed when cells were exposed to a combination of fluoranthene (4.0 micrograms/ml) and BP (0.063 micrograms/ml) or pyrene (5.0 micrograms/ml) and BP (0.063 micrograms/ml). Thus, the transformation frequency obtained with BP + fluoranthene was $3.8 \times 10^{-4}$ compared to $1.2 \times 10^{-4}$ when BP was tested alone. Similarly, the transformation frequency using BP + pyrene was $2.8 \times 10^{-4}$ vs. $1.2 \times 10^{-4}$ when BP was tested alone. Undecane did not exert any cocarcinogenic effect with BP in the dose range tested. In this in vitro assay, no cocarcinogenic effect was observed when BPL was used with any of the above mouse skin cocarcinogens. All cells isolated from transformed foci showed characteristics of transformed cells including anchorage-independent growth.


This study was performed for the detection of cocarcinogens by transformation in vitro with the use of a subclone of a Balb/3T3 cell line. Dose response, cytotoxicity, and transformation studies were done with the use of an indirect-acting carcinogen, benzo[a]pyrene (B[a]P), a direct-acting alkylating carcinogen, beta-propiolactone (BPL), and the mouse skin cocarcinogen catechol. The rate of transformation was notably higher in groups treated with B[a]P and catechol or BPL and catechol than in groups treated with either B[a]P or BPL. Catechol alone did not induce any transformation. All the cells isolated from the transformed foci showed characteristics of malignantly transformed cells, such as anchorage-independent growth. Thus chemical cocarcinogenesis was accomplished in vitro similar to that accomplished in in vivo studies reported earlier on mouse skin.

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Health Effects of Betapropiolactone [Beta-propiolactone]

SCE induction in Chinese hamster Don (lung) cells was compared with that in CHO (ovary) cells exposed under identical conditions to 14 known mutagens. Test protocols used for comparison were selected following a study of Don and CHO cell responses to aflatoxin B1 and benzo[a]pyrene. In the absence of added metabolizing enzymes 9-aminoacridine, 4-nitroquinoline 1-oxide, N-methyl-N-nitrosourea, dimethylcarbamoyl chloride, beta-propiolactone, daunomycin, aflatoxin B1 and 2-aminoanthracene were directly active in both cell lines; every substance positive in CHO cells was also positive in Don cells. However, the latter detected cyclophosphamide, hydrazine sulphate, benz[c]acridine, 3-methylcholanthrene and benzo[a]pyrene without addition of S9. CHO cells did not respond equivalently to these mutagens, either in the presence or absence of S9. Other differences between the cell lines depended on chemical exposure time, S9 pre-incubation or co-incubation conditions. For example, the ability of CHO cells to detect SCEs due to 2-aminoanthracene was acutely dependent on exposure time. In addition, Don cells exhibited lower background SCE values which were less variable than those of CHO cells under the same culture conditions. Although incapable of detecting 4-dimethylaminoazobenzene (butter yellow) and not as sensitive to cyclophosphamide as certain cell lines of liver origin, the pseudodiploid Don cell line possesses other desirable characteristics required for in vitro SCE assays, particularly with regard to intrinsic metabolic activation of polycyclic aromatic hydrocarbons and related substances.


The quantitative relationship between carcinogenicity in rodents and mutagenicity in Salmonella typhimurium was examined, by using 10 monofunctional alkylating agents, including N-nitrosamides, alkyl methanesulfonates, epoxides, beta-propiolactone and 1,3-propane sultone. The compounds were assayed for mutagenicity in two *S. typhimurium* strains (TA1535 and TA100) and in plate and liquid assays. The mutagenic activity of the agents was compared with their alkylating activity towards 4-(4'-nitrobenzyl)pyridine and with their half-lives (solvolysis constants) in an aqueous medium. No correlations between these variables were found, nor was mutagenic activity correlated with estimates of carcinogenicity in rodents. There was a positive relationship between carcinogenicity and the initial ratios of 7-O6-alkylguanine formed or expected after their reaction with double-stranded DNA in vitro. The results suggest that alkylation of guanine at position O6 (or at other O atoms of DNA bases) may be a critical DNA-base modification that determines the overall carcinogenicity of these alkylating agents in rodents.


Pyrene was found to act as a cocarcinogen in the induction of transformation of cultured Balb/c3T3 cells by three different types of carcinogens: a direct acting chemical carcinogen, beta-propiolactone, a chemical carcinogen requiring metabolic activation,
benz[a]pyrene, and a physical carcinogen (60Co) gamma radiation. Since pyrene enhanced transformation in vitro by approximately the same amount for all the carcinogens tested, these results suggest that the carcinogenic action of pyrene is not related to carcinogen metabolism or uptake in vitro. An extract of soybeans containing the Bowman-Birk protease inhibitor was shown to reduce transformation induced by beta-propiolactone, benz[a]pyrene and gamma-rays, both with and without the cocarcinogenic effect of pyrene, to background levels; the magnitude of the reduction in transformation by the protease inhibitor preparation was unrelated to the concentration of carcinogen. Neither the mechanism for the cocarcinogenic action of pyrene nor the anticarcinogenic effect of the soybean extract is known, but several hypotheses are discussed.

The ability of u.v.-A light (320-400 nm) to induce cellular transformation in vitro and to modify chemical carcinogen-induced cellular transformation was investigated in BALB/c 3T3 cell cultures. When administered as a series of nontoxic exposures, u.v.-A alone was found to induce cellular transformation as a linear function of the numbers of u.v.-A exposures. Possible interactions of u.v.-A with environmentally encountered chemical carcinogens were studied by examining the effects of u.v.-A light exposures on cellular transformation in cells exposed to the direct acting carcinogen, beta-propiolactone (BPL), an alkylating agent, with a standard initiation/promotion protocol. Twenty-four hours after a single treatment with 2.5 micrograms/ml of beta-propiolactone, cells were exposed to 3.0 kJ/m2 of u.v.-A light. U.v.-A exposures were repeated weekly for up to 5 weeks, after which cells were fixed, stained and dishes were scored for type III transformed foci. Weekly exposures to u.v.-A alone for 5 weeks induced approximately 3 foci/dish. Treatment with BPL alone induced approximately 1 focus/dish (background was 0.17 foci/dish). A combination of the two treatments resulted in a marked increase in the yield of transformed foci/dish, with the u.v.-A enhancement increasing with increasing numbers of exposures (approximately 10 foci/dish after a single exposure to BPL and five u.v.-A exposures). These results suggest a synergistic interaction between BPL and subsequent u.v.-A exposures in the induction of in vitro neoplastic transformation.

Numerous chemicals to which humans are exposed either therapeutically or as a result of living in an industrial environment constitute a potential threat as carcinogens, mutagens, and/or tumor promoters and cocarcinogens. Anthralin, and antipsoriatic agent, acts as a tumor promoter for Balb/c-3T3 mouse embryo cell cultures that were previously exposed to a low dose of either benzo-a-pyrene (BaP), an indirect-acting carcinogen needing metabolic conversion for its carcinogenic action, or beta-propiolactone (BPL), a direct-acting carcinogen which needs no metabolic conversion. As a cocarcinogen, i.e., when exposure of cells to anthralin was simultaneous with exposure to the carcinogen, anthralin enhanced neoplastic transformation only when the carcinogen was BaP. Several explanations are explored. The possibility that cocarcinogens and tumor promotion occur by separate mechanisms is suggested.
In order to validate the in vivo micronucleus test in mouse splenocytes using the cytokinesis block method, 14 compounds with various mechanisms of action were tested: three direct alkylating agents (mitomycin C, ethylnitrosourea, beta-propiolactone), seven indirect alkylating agents (cyclophosphamide, benzo[a]pyrene, diethylnitrosamine, dimethylnitrosamine, 4-aminophenol, 4-aminobiphenyl, 1,1-dimethylhydrazine), two intercalating agents (acridine orange, ethidium bromide) and two spindle poisons (vincristine, colchicine). Male mice were dosed once with the compound, and spleen samples were taken 2 or 14 days after treatment. A significant increase in the binucleated micronucleated splenocyte rate was observed with all the alkylating and intercalating agents at at least one sampling time. In contrast, no increase in the binucleated micronucleated splenocyte rate was observed with the spindle poisons. In conclusion, under these experimental conditions, this in vivo test seems appropriate for the detection of clastogenic compounds including compounds that cannot be detected in the bone marrow micronucleus test. The limit of this test, as expected, is the lack of detection of aneugenic compounds.

We used the positive selection Muta Mouse model to detect organ-specific activity of N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) and beta-propiolactone (BPL), two highly reactive alkylating agents known to induce genetic damage and tumors in rodent stomach when administered orally. Seven days after a single oral administration of MNNG (100 mg/kg) or BPL (150 mg/kg), the mutation frequency in the Muta Mouse stomach increased significantly by 6.4-fold and 8.8-fold, respectively. A slight (1.8-fold) but significant increase in mutation frequency was also observed in the livers of BPL-treated mice, but not in the livers of MNNG-treated mice or the bone marrow of MNNG- and BPL-treated animals. These data indicate that the Muta Mouse model can be used to predict the gastric specificity of genotoxic carcinogens.

Beta-Propiolactone (BPL) and N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) are two direct alkylating agents that induce multiple genetic lesions and tumors in the rodent stomach. We measured the kinetics of the induction of DNA damage by using the single-cell gel electrophoresis assay (SCGE) and the induction of gene mutations by using the MutaMouse model in the glandular stomach mucosa of mice exposed to a single oral administration of BPL or MNNG. The aims were to determine the optimal sampling time and to investigate the cause-effect relationship between DNA damage and gene mutations. The induction of comets, evaluated in individual cells with the tail moment, was analyzed 1, 2, 4, 24, and 72 hr after a single oral administration of 25 mg/kg BPL or 20 mg/kg MNNG. The effects of both compounds were most intense at the earlier
sampling times (1-2 hr), tailing off 4 hr after treatment and becoming undetectable at 72 hr. The lacZ mutant frequency (MF) was measured 3, 7, 14, 28, and 50 days after a single oral administration of 150 mg/kg BPL or 100 mg/kg MNNG, and 3 and 14 days after a single administration of 25 mg/kg BPL or 20 mg/kg MNNG. The MF was strongly enhanced at the highest doses and all sampling times, the most marked effects being observed 14 days (11.1-fold) and 28 days (19.0-fold) after BPL and MNNG administration, respectively. At the lowest doses, only a small increase in MF (approximately 2.5- to 3.5-fold) was found at both sampling times. Primary DNA damage detected with SCGE shortly after treatment (1-2 hr) was rapidly (3 days) transformed into stable gene mutations that remained detectable for 50 days. These results illustrate the ability and complementarity of the SCGE and MutaMouse models to assess the genotoxicity of direct alkylating agents in the mouse gastric mucosa in vivo.


Tumor induction data in the mouse skin initiation-promotion system were found to be consistent with a quadratic function where the coefficient of the linear term depended on the dose of the promoter. The model implies that the existence of promoters may be more important at low doses of the carcinogen than at high doses where most testing is performed. Experiments are described showing that the initiating effect of carcinogenic chemicals, such as benzo(a)pyrene, 7,12-dimethyl-benz(a)anthracene, nitroquinoline oxide and beta-propiolactone, accumulates in a linear, irreversible manner at low doses. Even when 7,12-dimethylbenz(a)anthracene was applied intragastrically to pregnant females, initiating activity was found in the skins of exposed offspring about in proportion to dose applied and number of cells at risk. The initiated cells essentially represent a potential for cancer that has a high probability for expression in the presence of a promoter. Risk then can be interpreted in terms of the accumulated dose of initiator which alone presents a small risk of cancer. However, a promoter may substantially expand the overall risk, possibly by clonally expanding the initiated cells. Promotion needs to be sustained since there is a reduction of cancer risk if promotion is ended early. Some tissues, such as mouse bladder, may be intrinsically promoted more than others so that comparisons between tissues and between species are best made when the combination of intrinsic promotion and response to extrinsic promotion are comparable.


Treatment of Syrian hamster embryo cells with diverse classes of chemical carcinogens enhanced transformation by a carcinogetic simian adenovirus, SA7. Optimal enhancement was a function of time of chemical addition in relation to time of virus addition and cell transfer. Aflatoxin B1 (AFB1) and the polycyclic hydrocarbons, benzo(a)pyrene (B(a)P), 3-methylcholanthrene (MCA), and 7,12-dimethylbenz(a)anthracene (DMBA) enhanced SA7 transformation when added prior to virus, but inhibited transformation when added after virus absorption and cell transfer. The enhancement of SA7 transformation was maximal when cytosine arabinoside, caffeine and 6-acetoxy-benzo(a)pyrene (6-ac-B(a)P) were added after virus, but minimal when added before virus. A third class of chemicals, including beta-propiolactone (beta-PL), methyl methanesulfonate (MMS), N-acetoxy-2-acetylaminofluorene (Ac-AAF), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and methylazoxymethanol acetate (MAM-ac), enhanced SA7 transformation added before, or after, virus inoculation and cell transfer. All chemicals which induced changes in DNA sedimentation in alkaline sucrose gradients and unscheduled DNA (repair) synthesis in hamster cells, increased the frequency of SA7 transformation. However, several chemicals such as dibenz(a,h)anthracene (DB(a,h)A), benzo(e)pyrene (B(e)P), cytosine arabinoside, and caffeine enhanced SA7 transformation but did not induce DNA sedimentation changes or repair. Chemicals that cause DNA damage, which can be repaired by hamster cells, may enhance viral transformation by providing additional sites for integration of viral DNA during the repair process. Chemicals that apparently do not induce DNA repair synthesis may enhance viral transformation by incorporation of viral DNA into gaps in cell DNA at sites of unrepaired damage during scheduled DNA synthesis.


The effect of ozonation on the mutagenicity of selected chemicals in water was determined. The use of the Salmonella-microsome assay for mutagensis allowed kinetic studies to be performed on the ozonation of all chemicals tested. The results indicate that the mutagenicity of certain pesticides, including captan and Dexon, was inactivated by short periods of ozonation. The mutagenicity of certain alkylating agents including bis(2-chloroethyl)amine and sodium azide was rapidly inactivated by ozonation while other alkylating agents such as beta-propiolactone, propanesultone, and N-methyl-N'-nitro-N-nitrosoguanidine were unaffected by treatment with ozone. The mutagenicity of aflatoxin B1 was rapidly inactivated by treatment with ozone. Three chemicals were shown to be converted to direct mutagens by ozone treatment. Under certain conditions, dimethylhydrazine could be converted to a mutagen that was stable for 3 weeks. A similar chemical, 2-hydroxyethylhydrazine, was converted to an unstable mutagen that was inactive after 24 hr at room temperature. When benzidine was treated with ozone,
there was a transient increase in mutagenicity which was lost after longer treatment with ozone.

**Chen et al. 1975.** Mutagenicity testing with Salmonella typhimurium strains. II. The effect of unusual phenotypes on the mutagenic response. *Mutat.Res.* Vol. 28(1): 31-35. The enhanced sensitivity of some Salmonella typhimurium strains to the mutagenic action of a number of chemicals appears to be due to the defect in the uvrB gene product and not to an inability to produce H-2-S or to the absence of formic acid hydrogenlyase which also characterizes these strains.

**Chen et al. 1981.** The reaction of beta-propiolactone with derivatives of adenine and with DNA. *Carcinogenesis.* Vol. 2(2): 73-80. The reaction of deoxyadenosine with beta-propiolactone produces two derivatives. One is 1-(2-carboxyethyl)-2'-deoxyadenosine (CEDA) first described by Mate et al. The proposed structure for the other is 3-(beta-D-2-deoxyribosyl)-7,8-dihydropyrimido-[2,l-i]purine-9-one (dDPP). Spectral characteristics of both compounds are presented. These include u.v. spectra of each in acidic, neutral and alkaline solutions, i.r. spectra, fluorescence spectra, and n.m.r. spectra. The extinction coefficient for CEDA is 12,900 M--1cm--1 at 258 nm and that for dDPP is 12,400 M--1cm--1 at 305 nm. The dDPP can be converted to CEDA by mild acid hydrolysis, and the CEDA can be converted to dDPP by reaction with a carbodiimide derivative. When poly A was reacted with beta-propiolactone, the yield of dDPP in the polymer was 7-9%. When double-stranded DNA was alkylated by [3H]beta-propiolactone at relatively high concentrations and then acid hydrolyzed to separate 1-(2-carboxyethyl)adenine (CEA) and 7-(2-carboxyethyl)guanine (CEG), a CEA to CEG ratio of up to 0.62 was obtained. With relatively low concentrations of [3H]beta-propiolactone, the yield of CEA was low with double-stranded DNA but was 5-6 fold greater with single-stranded DNA.


**Cliet et al. 1989.** In vivo micronucleus test using mouse hepatocytes. *Mutat.Res.* Vol. 216(6): 321-326. The bone-marrow micronucleus (BMM) test is highly specific for clastogenic effects but its sensitivity is determined to a great extent by the substances tested, particularly by their metabolism. Some compounds, such as unstable mutagens or those which generate short-lived metabolites, are not detected in this test because the metabolites produced in the liver do not reach the bone marrow. In an attempt to provide qualitative and quantitative assessments of chromosomal mutations produced in vivo by genotoxic agents not detected in the mouse BMM test, a mouse-liver micronucleus test, adapted from Tates model, was developed. The animals were treated twice, with an interval of 24 h between treatments, and then subjected to partial hepatectomy (PH) 24 h after the second treatment in order to induce mitotic stimulation. The incidence of micronucleated hepatocytes was determined 96 h after PH. The test was evaluated with 5 procarcinogens, each with a complex metabolic pattern: dimethylnitrosamine (DMN), diethylnitrosamine

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(DEN), 1,1-dimethylhydrazine (1,1-DMH), 4-aminophenol (4-APOL), 4-aminobiphenyl (4-ABPYL) and one direct unstable mutagen, beta-propiolactone (BPL). All these compounds are negative in the mouse BMM test but caused a major increase in the incidence of micronuclei in mouse hepatocytes. This test is simple and can be readily compared with the BMM test. Furthermore, it offers a better assessment of the impact of a compound at the chromosomal level in a metabolically competent cell and can therefore be used for the evaluation of the genotoxic activity of compounds with complex metabolic pathways.

**Cliet et al. 1993.** Lack of predictivity of bone marrow micronucleus test versus testis micronucleus test: comparison with four carcinogens. *Mutat.Res.* Vol. 292(2): 105-111. In vivo somatic chromosome mutation tests are usually carried out using the bone marrow micronucleus test in the mouse. This test is also considered predictive for the study of clastogenic effects in germ cells. However, it has been reported that the sensitivity of the bone marrow micronucleus test is insufficient to detect unstable compounds or short-lived metabolites and the use of target cells with metabolic activity (hepatocytes) has been questioned. In order to analyze in vivo micronucleus induction in cells with metabolic enzyme activity, we compared the sensitivity of somatic and germ cells to four carcinogens in the bone marrow and spermatid micronucleus test in the mouse. Three procarcinogens with a complex metabolic pattern (dimethylnitrosamine, diethylnitrosamine and 1,1-dimethylhydrazine) and one direct unstable mutagen (beta-propiolactone) were tested. All four carcinogens were not detected by the bone marrow micronucleus test but were detected in the mouse spermatid micronucleus test in which they induced clear clastogenic effects, as was the case in a previous study in liver micronucleus test. In conclusion, this study demonstrates that the bone marrow micronucleus test is not sufficient for the prediction of a clastogenic hazard in germ cells. In addition to a second in vivo test in an organ with metabolic enzymes, i.e., the liver, the spermatid micronucleus test can be performed when a specific risk to the testis is likely.


**Colburn et al. 1968.** The binding of beta-propiolactone and some related alkylating agents to DNA, RNA, and protein of mouse skin; relation between tumor-initiating power of alkylating agents and their binding to DNA. *Cancer Res.* Vol. 28(4): 653-660.


A delivery system for vanadium was developed using poly(beta-propiolactone) (PbetaPL) films. The release kinetics of a complex of vanadium (IV) with aspirin (VOAspi) was evaluated with films prepared from polymers of different molecular weights, as well as with variable drug load. A sustained release of vanadium over 7 days was achieved. The drug release kinetics depends on contributions from two factors: (a) diffusion of the drug; and (b) erosion of the PbetaPL film. The experimental data at an early stage of release were fitted with a diffusion model, which allowed determination of the diffusion coefficient of the drug. VOAspi does not show strong interaction with the polymer, as demonstrated by the low apparent partition coefficient (approximately 10(-2)). UMR106 osteosarcoma cells were used as a model to evaluate the anticarcinogenic effects of the VOAspi released from the PbetaPPL film. VOAspi-PbetaPL film inhibited cell proliferation in a dose-response manner and induced formation of approximately half of the thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, compared to that with free VOAspi in solution. The unloaded PbetaPL film did not generate cytotoxicity, as evaluated by cell growth and TBARS. Thus, the polymer-embedded VOAspi retained the antiproliferative effects showing lower cytotoxicity than the free drug. Results with VOAspi-PbetaPL films suggest that this delivery system may have promising biomedical and therapeutic applications.


A new procedure (the spiral test) has been set up and validated for the distribution of chemicals in bacterial mutagenesis assays. This method involves the use of a special instrument (spiral plater), which dispenses, along a spiral track, decreasing volumes of liquid samples, from the near centre to the periphery of a rotating agar plate. A gradient of concentration of a compound up to about 1500:1 is thus formed on a single plate. The activity of 18 mutagens of various potencies and chemical classes was checked in the Salmonella/microsome test by dispensing their solutions either on the surface of top agar (method A) or of the minimal-glucose agar medium, before the addition of molten top agar incorporating bacteria and eventually S9 mix (method B). Compared with the spot test, the gradient of concentration of a compound produced by the spiral diluter was much wider and more gradual. Even nondiffusible chemicals (e.g. benzo[a]pyrene and benz[a]anthracene) were efficiently detected in the spiral test, as well as very weak (e.g. mebanazine and trimethylphosphate) or borderline (e.g. perylene, 1,1-dimethylhydrazine and procarbazine) mutagens, which were negative in the spot test. Method B was at least as sensitive as the plate-incorporation test, such a goal being achieved in a single plate instead of in serial plates. Technical problems made method A less sensitive, but it was more efficient in detecting unstable mutagens (e.g. beta-propiolactone). Like the plate test, the spiral test appeared to be suitable for a semi-quantitative assessment of mutagenicity data, and was efficient in demonstrating both the activation of promutagens and the deactivation of some directly acting mutagens. Preliminary assays were also carried out with repair-proficient (WP2) or -deficient (TM1080: lexA-/polA-/R391, and CM871: lexA-/uvrA-/recA-) trp- strains of E. coli.

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The pathologic consequences of infection of newborn mice and rats with MuLV (Scripps leukemia virus--SLV) were observed. Serum MuLV p30 concentrations of most strains were elevated 20 to 100 times that of controls while MuLV gp70 levels were elevated only 1.1 to 14.8 times, probably reflecting in part the higher concentrations of gp70 in control sera but also indicating the lack of parallelism in regulation of synthesis of these two viral antigens. Infected mice of most strains developed immunologic diseases, including antinuclear antibody and glomerulonephritis, but not Coombs' antibodies. The nature and severity of the immunologic disease varied considerably, depending upon the genetic character of the host. Most infected animals developed lymphatic leukemias, but four strains showed partial to complete resistance to SLV oncogenesis: BALB/c (nude); C57 Bl/6; (NZB times NZW) F1, and (NZW times BALB/c) F1.


The carcinogenic effects of limited and repeated skin applications of propane sultone were investigated in three strains of mice, CF1, C3H and CBah (a hairless strain). Propane sultone was shown to be carcinogenic when given as a single application of a 25% w/v solution in toluene and also following twice weekly application of a 2.5% w/v solution for up to 58 weeks. More limited exposure to 2.5% w/v solutions of propane sultone resulted in a few skin tumours, although the incidences were not statistically significant. Most neoplasms were papillomas or carcinomas, although a small number of mesenchymal tumours of dermal origin also developed. No skin neoplasms were found in any control mice. The skin application of propane sultone was associated with a statistically significant increase in the incidence of systematic neoplasia in CF1 and C3H mice. The exposed CF1 mice had a higher incidence of neoplasms of lymphoreticular and lung origin, while female C3H mice showed a higher incidence of mammary gland and uterine tumours. In mice exposed to beta-propiolactone as a positive control, neoplasms developed at the site of application but, there was no evidence of increased systemic neoplasia in contrast to the findings with propane sultone.


The number of apurinic/apyrimidinic (AP) sites in supercoiled SV40 deoxyribonucleic acid (DNA) after treatment with several electrophilic mutagens was quantitated by electrophoretic analysis of the DNA after cleavage of the phosphodiester bonds adjacent to AP sites by a specific endonuclease. The compounds studied, in order of increasing yields of AP sites obtained on incubation with the DNA for 5 h at 37 degrees C, were dimethylcarbamoyl chloride, ethyl methanesulfonate, N-ethyl-N-nitrosourea, 2-(N-
acetoxyacetylamino)fluorene, beta-propiolactone, N-methyl-N-nitrosourea, methyl methanesulfonate, 1'-acetoxyestragole, 4-(N-acetoxyacetylamino)stilbene, (+/-)-7 beta, 8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, N-(benzoyloxy)-N-methyl-4-aminoazobenzene, and 1-pyrenyloxirane. After a 5-h incubation at 37 degrees C and extraction of unreacted compound, further incubation at 70 degrees C generally increased the yield of AP sites; an exception was N-(benzoyloxy)-N-methyl-4-aminoazobenzene-reacted DNA. Except for DNA treated with N-ethyl-N-nitrosourea and N-methyl-N-nitrosourea, which are known to bind to a significant extent to DNA phosphates, the number of alkali-labile lesions in the treated DNA was similar to the number of AP sites. For the compounds studied there was no direct correlation between the number of AP sites produced and missense mutagenic activity, as measured in Salmonella typhimurium strain TA100.

Duker et al. 1976. Detection of different types of damage in alkylated DNA by means of human corrective endonuclease (correndonuclease). Proc. Natl. Acad. Sci. U.S.A. Vol. 73(8): 2629-2633. Corrective endonuclease (correndunclease) activity of HeLa cells was assayed with alkylated DNA. Double-stranded, covalently closed DNA from phage PM II was treated with methyl methanesulfonate, N-methyl-N-nitrosourea, beta-propiolactone, or diepoxybutane to introduce alkylated bases and alkali-labile sites into the DNA. The damaged DNA was incubated with an extract of HeLa cells that catalyzes the formation of breaks at apurinic sites in double-stranded DNA. Methylated DNA was broken at every alkali-labile site by the HeLa correndonuclease, which indicated that these sites are similar to the apurinic sites produced by heating at acid pH. DNA alkylated with beta-propiolactone or diepoxybutane containing the same number of alkali-labile sites was broken to a far lesser extent. This indicates the presence of a second type of alkali-labile damage that is correndonuclease-insensitive.

Dunkelberg. 1982. Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. Br. J. Cancer. Vol. 46(6): 924-933. Ethylene oxide and 1,2-propylene oxide were each administered intragastrically by gavage at 2 dosages (30 and 7.5 mg/kg body wt; 60 and 15 mg/kg body wt respectively) to groups of 50 female Sprague-Dawley rats twice weekly for a period of nearly 3 years using salad oil as the solvent. Both compounds induced local tumours, mainly squamous-cell carcinomas of the forestomach, dependent on the dosage. The first tumour occurred in the 79th week both in the group treated with ethylene oxide and in that treated with 1,2-propylene oxide. The following tumour rates resulted: ethylene oxide 62 and 16%; 1,2-propylene oxide 40 and 4%. In addition carcinomata in situ, papillomas and reactive changes of the squamous epithelium of the forestomach were observed in other animals, but neither ethylene oxide nor 1,2-propylene oxide induced tumours at sites away from the point of administration.

el-Karamany. 1987. Production in Vero cells of an inactivated rabies vaccine from strain FRV/K for animal and human use. Acta Virol. Vol. 31(4): 321-328. A new concentrated and purified rabies vaccine was produced in Vero cells. Two rabies virus strains, the fixed rabies virus Pasteur (FRV) and Pittman Moore (PM) were adapted
to Vero cells by 20 cycles of alternating passages in the brain of weaning mice.
Intracerebral (i.c.) inoculation of weaning mice was followed then by 17 and 20 serial
passages in Vero cells of RFV and PM strains, respectively. The adapted strains
designated as FRV/K and PM/K gave titres of 10(6) +/- 1.5 log (LD50/ml for i.c.
inoculated mice) in several harvests taken from one infected cell culture. Pooled harvests
were concentrated 20-fold by ultrafiltration and were tested as animal vaccine after
inactivation with beta-propiolactone (BPL). Another vaccine preparation destined for
human use, in addition to concentration and inactivation, was also purified by gel
filtration. Control tests revealed that the antigenic content of different strain FRV/K
harvests was very high in comparison with that of strain PM/K and the reference tissue
culture vaccine (RIV, Netherland). In sheep the antibody response induced by the FRV/K
strain was very high; serum neutralizing index (NI) higher than 4 was reached 40 days
after the second vaccine dose, whereas the vaccine preparation from strain PM/K gave NI
of 2.3 and the reference vaccine NI of 3.8, respectively. Safety tests in rabbits and guinea
pigs showed neither pyrogenicity nor toxicity.

EPA. 1999. beta-Propiolactone. Technology Transfer Network Air Toxics Website.
[http://www.epa.gov/ttn/atw/hlthef/propiola.html]

Freeman et al. 1983. Inhibition of unscheduled DNA synthesis in human lymphocytes
Freshly isolated human peripheral lymphocytes were treated with an alkylating agent
immediately after collection and subsequently treated with UV radiation. This system
was used because it represents a method for assaying damage in cells immediately after
their removal from the host. The amount of UV-induced repair was measured as
unscheduled DNA synthesis (UDS) by incorporation of [3H]deoxythymidine into the
cellular DNA. The alkylating agents beta-propiolactone (BPL) and methyl methane-
sulfonate (MMS) inhibited UDS at concentrations of 0.08 mM and 0.6 mM, respectively.
Lower concentrations had no effect. Lymphocytes allowed to remain in culture medium
after treatment with the alkylating agents did not recover the ability to perform UV-
induced UDS even when cells were irradiated 48 h after carcinogen treatment. The
decrease in UV-induced UDS resulting from alkylating agent treatment could not be
attributed to cell death.

Fujii et al. 1994. Target cells of cytotoxic T lymphocytes directed to the individual
Target cells of cytotoxic T lymphocytes (CTL) directed to the individual structural
proteins (except for the large polymerase (L) protein) of rabies virus were established by
expressing only the respective protein in murine neuroblastoma (NA) and murine
macrophage (J774-1) cell lines. Mice infected with the ERA strain of rabies virus
developed CTL responses to all of these rabies virus proteins. The cytotoxic activity was
abrogated by pretreatment of the effector cells with anti-CD8 monoclonal antibody
(MAb) and complement but not with anti-CD4 MAb. Cell lysis by CTL was blocked in
the presence of anti-major histocompatibility complex (MHC) class 1 antibodies in J774-
1 cell lines. Rabies virus-infected cells express these proteins at the surface, which can be
recognized and lysed by the respective CTL. Mice immunized with beta-propiolactone-
inactivated virus induced a CTL response against glycoprotein but not against internal viral components. This assay system might be useful for further analysis of the possible contribution of these proteins in the cell-mediated immune protection against rabies.


Techniques of molecular biology have been used to determine the relationship of cellular oncogenes to mechanisms of experimental carcinogenesis. Model systems involving three direct-acting alkylating carcinogens, two organ sites, and two species have been employed to elucidate the relationships between carcinogenic etiology, tissue specificity, and activation of known and novel oncogenes. Dimethylcarbamyl chloride, a reactive acylating agent, induces tumors of both the rat nasal mucosa and mouse skin whose DNA is devoid of NIH 3T3 transforming activity. Beta-propiolactone-induced rat nasal carcinomas contain a novel oncogene, 6 to 9 kb in size, whereas a mouse skin carcinoma induced by this agent possesses an H-ras oncogene activated by a 61st codon A to T transversion mutation. The novel oncogene activated in rat nasal tumors induced by beta-propiolactone is distinct from one found in methylmethane sulfonate-induced tumors. The implications of these findings for understanding how oncogenes fit into general mechanism of carcinogenesis are discussed.


DNAs from rat nasal and mouse skin carcinomas and fibrosarcomas induced by the alkylating agents methylmethane sulfonate (MMS), beta-propiolactone (BPL), and dimethylcarbamyl chloride (DMCC) were tested for their ability to transform NIH3T3 cells by DNA transfection. Each of eight MMS-induced rat nasal carcinomas and two of five BPL-induced mouse skin tumors were positive in the transfection assay while all of four fibrosarcomas and six carcinomas induced by DMCC were negative. Anchorage independent growth, tumorigenicity in nude mice, and secondary transfection confirmed the transformed phenotype of the positive transfectants. The transfectants from MMS-induced tumor DNAs did not contain restriction fragments homologous to rat H-, K- or N-ras oncogenes although exogenous (rat) tumor-derived DNA sequences were detected in transfectant genomes by Southern analysis. In contrast a BPL-induced mouse skin tumor showed evidence of containing activated H-ras. These results suggest specificity among causal chemical carcinogens for activation of transforming genes in experimental tumors.


The bacterial SOS chromotest with Escherichia coli PQ37 was used for the assessment of genotoxicity of combined xenobiotic treatments. The modulation of test compound genotoxicity by dimethyl sulfoxide (DMSO), a common solvent for test compounds, was assessed as well. It was shown that DMSO modulated SOS chromotest genotoxicity of several xenobiotics: in comparison to test compound dissolution in water, the commonly used addition of 3.2% (v/v) DMSO as solvent lead to a significant increase in the genotoxicity of K(2)RhCl(5) and beta-propiolactone (BPL). However, the effects of
cisplatin decreased significantly when DMSO was added. Thus, albeit DMSO is not genotoxic in this test itself, it can interfere with SOS chromotest responses. Further experiments were performed in the absence of DMSO. BPL and cisplatin in combination showed an over-additive synergism in SOS genotoxicity as well as K(2)RhCl(5) and cisplatin did. Addition of Pd(NH(3))(4)Cl(2) and NaAsO(2), which are non-genotoxic in the SOS chromotest, did not enhance the K(2)RhCl(5)- or BPL-mediated SOS sfiA induction. Nevertheless, at the highest subcytotoxic dose of NaAsO(2) tested (200 microM), a slight yet significant suppression of BPL-mediated SOS genotoxicity was observed. These results confirm that the SOS chromotest is a useful tool for the rapid evaluation of the combined genotoxicity of compound mixtures. However, the use of DMSO as test solvent has to be taken with caution.


In a randomised study the efficacy of a cytomegalic hyperimmune globulin preparation (CMV-HIGP) which had been treated with beta-propiolactone was analysed. The study included 85 patients with acute lymphoblastic leukemia (ALL) and Non-B-Non-Hodgkin-lymphoma (NHL) who were treated initially or underwent a relapse therapy. During the intense chemotherapeutical period within leukemia treatment the patients were passively immunised by the intravenous route with CMV-HIGP (1 ml per kilogram of body weight) every two to three weeks at the latest. In the initial stages the basic immunisation protection was achieved by the application of double dose CMV-HIGP. The Frankfurt patients were recruited from the BFM-ALL- and the NHL-study since october 1982. When they were admitted their CMV serostatus was determined by means of the ELA-ELISA or IFA-method. Seronegative patients were given the passive immunisation immediately or 48 hours after the first blood transfusions at the latest. The patients who had become CMV-IgG-positive by passive immunisation were randomised when reaching long-term therapy according to the protocol. Because of a 30% cytomegaly disease incidence rate in our patient population a randomisation was unwarrantable at the beginning of leukemia treatment. During randomisation one group of patients were immunised by the intravenous route with CMV-HIGP (2 ml per kg body weight one time in four weeks), the second group was a control group.(ABSTRACT TRUNCATED AT 250 WORDS)


A novel test system for the detection of mutagenic and recombinogenic activity of chemicals is described in detail. Drosophila melanogaster larvae trans-heterozygous for the mutations multiple wing hairs (mwh) and flare (flr) are exposed to the test compounds for various periods of time ranging from 96 hr to 1 hr. Induced mutations are detected as single mosaic spots on the wing blade of surviving adults that show either the multiple wing hairs or flare phenotype. Induced recombination leads to mwh and flr twin spots.
and also to a certain extent, to mwh single spots. Recording of the frequency and the size of the different spots allows for a quantitative determination of the mutagenic and recombinogenic effects. This and earlier studies with a small set of well-known mutagens indicate that the test detects monofunctional and polyfunctional alkylating agents (ethyl methanesulfonate, diepoxybutane, mitomycin C, Trenimon), mutagens forming large adducts (aflatoxin B1), DNA breaking agents (bleomycin), intercalating agents (5-aminooacridine, ICR-170), spindle poisons (vinblastine), and antimetabolites (methotrexate). In addition, the test detects mutagens unstable in aqueous solution (beta-propiolactone), gaseous mutagens (1,2-dibromoethane), as well as promutagens needing various pathways of metabolic activation (aflatoxin B1, diethylnitrosamine, dimethylnitrosamine, mitomycin C, and procarbazine). The rapidity and ease of performance as well as the low costs of the test necessitate a high priority for validation of this promising Drosophila short-term test.

**Gupta et al. 1982.** Mutagenic responses of five independent genetic loci in CHO cells to a variety of mutagens. Development and characteristics of a mutagen screening system based on selection for multiple drug-resistant markers. *Mutat.Res.* Vol. 94(2): 449-466. With the aim of developing a sensitive mutagen screening system, the responses of 15 different chemical mutagens at 5 independent genetic loci in Chinese hamster ovary (CHO) cells have been determined. The genetic markers which have been employed include resistance to thioguanine (Thgr), ouabain (OuaR), the protein synthesis inhibitor emetine (Emtr), the polyamine synthesis inhibitor methylglyoxal bisguanylhydrazone (Mbgr) and the nucleoside analog 5,6-dichlororibofuranosyl benzimidazole (DrbR). The optimal selection conditions for all of these genetic markers in CHO cells have been described. The chemicals whose response was investigated in these studies include direct-acting alkylating agents (ethyl methane-sulfonate, methyl methanesulfonate, betapropiolactone, ethyleneimine, N-nitrosomethylurea and 4-nitroquinoline-N-oxide), DNA intercalating and cross-linking agents (ICR-170, acridine orange, ethidium bromide, mitomycin C and actinomycin D), polycyclic hydrocarbons (benzo[a]pyrene (B(a)P) and 7,12-dimethylbenz[a]anthracene (DMBA)) and aromatic amines (benzidine and betanaphthylamine). Simultaneous examination of the response of the set of genetic markers to these chemicals revealed that although all of these chemicals caused a dose-dependent increase in the frequency of mutations at many of the above genetic loci, the magnitude of the mutagenic response at different genetic loci varied greatly depending upon the chemical. Of the genetic loci examined, no one single locus showed higher response to all of the above chemicals, instead, depending upon the chemical, specific loci were found to be more responsive than others. The polycyclic hydrocarbons and aromatic amines were weakly mutagenic in this system at several genetic loci even without any exogenous microsomal activation, although in the presence of a rat liver S9 fraction similar toxic and mutagenic effects of B(a)P and DMBA were observed at 5-20-fold lower concentrations. These results indicate that CHO cells may possess significant capacity for the metabolic activation of many procarcinogens, and also underscore the merits of measuring the mutagenic response at multiple genetic loci in mutagen screening studies.


An endonuclease which is active with regard to depurinated, alkylated, arylated, and arylamidated DNA has been purified 500-fold from Micrococcus luteus. In this purification, separation from the pyrimidine-dimer-specific ultraviolet-endonuclease has been achieved. The enzyme has a molecular weight of 30000 on the basis of gel filtration; its activity is not absolutely dependent upon the presence of Mg2+, but 5--30 mM Mg2+ produces a five-fold stimulation. Potassium chloride concentrations of less than 100 mM are optimal, while concentrations exceeding 100 mM inhibit. The enzyme has no effect on native DNA, but introduces single-strand breaks into DNA containing apurinic/apyrimidinic sites produced by heating at an acidic pH. DNA treated with such carcinogens as N-alkyl-N-nitrosoureas, alkyl methanesulfonates, alkyl sulfates, nitrogen mustard, beta-propiolactone, 7-bromomethyl-benz[a]anthracene, N-acetoxy-2-acetylaminofluorene, and 7,12-dimethyl-benz[a]anthracene-5,6-oxide also becomes susceptible to enzymic action. The activity of the enzyme has been detected by making use of the difference in mobility between supercoiled closed-circular DNA of Pseudomonas phage PM2 and its nicked form in agarose gel electrophoresis. Even depurinated or carcinogen-modified supercoiled PM2 DNA migrated faster than the respective relaxed nicked forms. A comparison of the number of enzyme-catalyzed single-strand breaks with the number of alkali-labile (i.e. apurinic) sites in carcinogen-modified PM2 DNA showed that the enzyme preparation introduced approximately twice as many breaks into the substrates as the number of apurinic sites present. We conclude that the enzyme preparation either recognizes both apurinic sites and DNA bases carrying carcinogenic residues or contains DNA glycosidase activity in addition to the endonuclease activity. Exposure of ultraviolet-irradiated PM2 DNA to the endonuclease preparation showed that pyrimidine dimers were not substrates. The yield of enzyme-catalyzed single-strand breaks found in ultraviolet-irradiated DNA was five times the number of alkali-labile sites present suggesting that minor photoproducts, possibly 5,6-saturated pyrimidine residues, were recognized in addition to apurinic sites.

**Hennings et al. 1968.** Inhibition by actinomycin D of DNA and RNA synthesis and of skin carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene or beta-propiolactone. *Cancer Res.* Vol. 28(3): 543-552.


Studies have been initiated to find compounds that can trap direct-acting carcinogens within the lumen of the gastrointestinal tract and thus prevent these carcinogens from attacking tissues of the host. Sodium 4-mercaptobenzene sulfonate (4-MBSNa) is a...
potent nucleophile and was found to react rapidly in vitro with the direct-acting carcinogen beta-propiolactone (BPL). In further investigations 4-MBSNa was shown to inhibit mutagenesis resulting from exposure of Salmonella typhimurium strain TA-100 to BPL and a second direct-acting carcinogen, N-methyl-N’-nitro-N-nitrosoguanidine. Subsequent experiments were performed to determine if 4-MBSNa would inhibit BPL-induced carcinogenesis in vivo. In the first of these, 4-MBSNa was administered by p.o. intubation to female A/J mice 5 min before p.o. administration of BPL. Under these conditions inhibition of carcinogenesis of the forestomach occurred. In a second experiment, 4-MBSNa was given by rectal intubation 5 min before BPL also administered intrarectally. Administration of BPL intrarectally produced adenomatous polyps of the large intestine. The occurrence of these neoplasms was inhibited by the prior administration of 4-MBSNa. The data presented show that 4-MBSNa has the capacity to trap direct-acting carcinogens and to inhibit the occurrence of BPL-induced neoplasia.

A mouse skin squamous cell carcinoma induced by topical application of the direct-acting alkylating agent beta-propiolactone contains an activated H-ras oncogene with an A----T transversion at the second nucleotide of codon 61. The mutation was detected in NIH3T3 transfectant and original tumor DNA by an XbaI restriction enzyme polymorphism and confirmed by oligonucleotide “;mismatch”; hybridization. The mutation was not seen in the liver of the same animal. The activated oncogene also exhibited several restriction enzyme polymorphisms in transfectant DNA due to a reciprocal translocation 3' to the coding region of the gene, which occurred during transfection. The activating mutation was found in only 1 of 6 beta-propiolactone induced mouse skin tumors examined, the only tumor with a transforming H-ras oncogene. This is a much lower frequency of activation than that previously reported for the same tumor type induced by polycyclic aromatic hydrocarbons. The A----T transversion mutation is consistent with a potentially direct mutagenic effect of a specific beta-propiolactone-DNA adduct.

Rat nasal squamous cell carcinomas induced by inhalation of three direct-acting alkylating agents yielded DNA containing activated oncgenes with no homology to any member of the ras family. The novel NIH 3T3 transforming oncogenes from tumors induced by beta-propiolactone and methylmethane sulfonate are distinct from each other based on restriction analysis. The gene isolated from beta-propiolactone-induced tumors is between 6 and 9 kb in size. None of the tumors induced by dimethylcarbamyl chloride contained positive DNA in the NIH 3T3 focus assay or in the nude mouse cotransfection assay. The rat nasal tumor model is apparently ideally suited for analysis of the roles of carcinogen and tissue specificity in oncogene activation, especially related to novel (non-ras) transforming oncogenes.


Iversen et al. 1981. Circadian variation in the susceptibility of mouse epidermis to chemical carcinogens. *Int.J.Chronobiol.* Vol. 8(1): 49-62. Groups of hairless mice were given a single topical application of each of two highly-reactive carcinogens (methylmethanesulphonate and beta-propiolactone) at 12(00) and 00(00). The tumor yield was as follows: for methylmethanesulphonate there was a slightly higher yield of skin tumours in the animals painted at 12(00); for animals painted with beta-propiolactone, there was a significantly higher skin tumour yield in those painted at 12(00). When the groups painted with the two carcinogens are considered together, there was a significantly higher number of animals with all types of tumours in the group painted at 12(00). Hence, there seems to be a circadian variation in the sensitivity of a tissue to a single contact with a chemical carcinogen. This may be related to the proliferative state of the tissue involved: at 12(00), DNA and cell division takes place at a more rapid rate than at 00(00).


The Escherichia coli gpt gene coding for xanthine-guanine phosphoribosyl transferase has been stably transfected into HPRT- Chinese hamster V79 cells. Several gpt- cell lines have been established, which retain the sequence(s) even after long-term culture without selection for gpt. Each cell line exhibits a characteristic spontaneous mutation frequency (10(-5) to 10(-2)) in 6-thioguanine (6TG) selection. While spontaneous mutagenesis to gpt- occurs rather frequently for most cell lines, it cannot be correlated with either the number of plasmid integration sites or deletion of the plasmid sequence(s). One transgenic cell line (g12), which continuously maintains a low spontaneous mutation frequency (approximately 3 x 10(-5)), was used in comparative mutagenesis studies with wild-type V79 cells (gpt vs. hprt). Alkylation agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and beta-propiolactone (BPL) are shown to be equally toxic and mutagenic in both g12 and V79 cells. UV and X-rays are also equally toxic to both cell lines. The gpt locus of the g12 transfectants, however, is two to three times more...
sensitive to UV and 2.5-4.5 times more sensitive to X-ray mutagenesis than the endogenous hprt of wild-type V79 cells. The data presented here suggests that g12 cells may be useful to study mammalian mutagenesis by agents which yield limited response at the hprt locus. Future studies with these transgenic cells and other transgenic lines are planned to compare the mutability and repair of the same gene (gpt) at different integration sites in mammalian cells.


Beta-propiolactone (BPL) was tested for the induction of sex-linked recessive lethals and autosomal translocations in Drosophila melanogaster. The compound was administered to adult males either by oral application or by abdominal injection. When injected, BPL was a potent inducer of sex-linked recessive lethals. When BPL was given by feeding, its mutagenic activity was detectable only when the flies were starved and when the BPL-containing solutions were renewed several times. Nevertheless, the recessive-lethal frequency was one order of magnitude higher with injection. This difference in effects is attributed to (1) rapid decomposition of the compound in aqueous feeding solutions, and to (2) rapid degradation in vivo which restricts the activity of BPL mainly to the site of application. These data are compared with other studies in which both routes of application were applied. BPL induced translocations in stored spermatozoa when injected, but not when fed. This finding seems a logical consequence of (1) the difference in effectiveness of the two routes of application for BPL, and (2) the existence of different LECs for mutation induction (recessive lethals) and for chromosome breakage (translocations). In Drosophila, the breakage capacity of BPL was one order of magnitude lower than that of MMS, when a comparison was made on the basis of equal recessive-lethal frequencies.


Brief exposure to beta-propiolactone (BPL) increases the sedimentation rate of purified Escherichia coli DNA in neutral and alkaline sucrose gradients. However, when electrophoresed in polyacrylamide-agarose gels, this BPL-treated DNA moves ahead of the control. Longer incubation with BPL gives rise to two new fractions, the first one sedimenting as a heterogeneous material of 6-8S, and the second one of very high sedimentation velocity. In acrylamide-agarose gels, the first fraction is again recovered in the 6-8S area, while the second fraction does not enter the gel at all. The DNA at this stage is hyperchromic in ultraviolet light suggesting that as much as 20% may be denatured. Coliphage lambda DNA treated briefly with BPL and spread in a protein monolayer appears under the electron microscope as a rigid, extended molecule, up to 15% longer than the control DNA, and usually in compact, folded configurations suggesting intramolecular linking. After longer exposure, localized denaturation
associated with single-strand breaks is observed. The single-stranded "whiskers"; then interact with other DNA molecules, creating highly complex branched networks of single- and multi-stranded DNA. The possible relevance of these observations to the mechanisms involved in carcinogenesis and mutagenesis is considered.


Transforming DNA was exposed to either beta-propiolactone or 1,3-propane sultone and then used for transformation of competent bacteria to nutritional independence from tyrosine and tryptophan (linked markers) and leucine (an unlinked marker). The ability of transform was progressively lost by the DNA during incubation with either of these two chemicals. For all three markers the inactivation curve was biphasic, with a short period of rapid inactivation followed by one characterized by a much slower rate. The overall rate of inactivation was different for all three markers and presumably was related to the size of the marker. The decrease in the transforming activity was in part due to the slower rate of penetration of alkylated DNA through the cellular membrane and its inability to enter the recipient bacteria. This decrease in the rate of cellular uptake, even for DNA eventually destined to enter the cell, began almost immediately after its exposure to the chemical and ended up with an almost complete lack of recognition of the heavily alkylated DNA by the specific surface receptors of competent cells. Such DNA attached to sites on the surface of competent bacteria which were different from receptors specific for the untreated nucleic acid. This attachment was not followed by uptake of the altered DNA. Presence of albumin during the incubation with a carcinogen further increased the degree of inactivation, indicating that the artificial nucleoproteins produced under such conditions were less efficient in the transformation assay than was the naked DNA. Cotransformation of close markers progressively decreased, beginning immediately after the start of incubation of DNA with the chemicals. Extensively alkylated DNA fractionated by sedimentation through sucrose density gradients showed a peculiar distribution of cotransforming activity for such markers; namely, molecules larger than the bulk of DNA ("megamolecules") showed less ability to transform the second marker than did some of the apparently smaller molecules which sedimented more slowly through the gradient. An increase in cotransformation of distant markers was evident in DNA molecules after a short exposure to an alkylating agent, but cotransformation of such markers was absent in DNA treated for longer periods. The observed changes in the transforming and cotransforming activities of the alkylated DNA can be explained by what is known about the physicochemistry of such DNA and in particular about the propensity of the alkylated and broken molecules to form complexes with themselves and with other macromolecules.

**Lagrange et al. 1978.** Delayed-type hypersensitivity to rabies virus in mice: assay of active or passive sensitization by the footpad test. *Infect. Immun.* Vol. 21(3): 931-939.

With a purified beta-propiolactone-inactivated rabies virus, a significant increase in footpad swelling was elicited in normal or in BCG-pretreated mice after immunization with varying doses of rabies vaccine. These footpad reactions were shown to peak at 24 h and to be associated with an infiltration of newly formed blood monocytes demonstrated.
by histology and [125I]deoxyuridine labeling. A relationship between the lymphoproliferation and the degree of sensitization is described, and the susceptibility to cyclophosphamide treatment is also examined. Adoptive transfer of specific reactivity to normal recipient mice with immune lymphoid cells, but not with immune serum, was demonstrated, and the results represent another argument for a cell-mediated immunological mechanism.

The DNA of bacteriophage SPO2c12 was treated with methylmethane sulfonate (MMS), beta-propiolactone (BPL), 2-anthramine (AA) or benzo[a]pyrene (BP) and then exposed to 254-nm radiation. Competent Bacillus subtilis host cells were transfected with DNA subjected to the carcinogen-UV treatment or with DNA treated with carcinogen only. Survival curves were obtained for loss of plaque-forming ability as a function of UV dose. The UV sensitivity of DNA treated with MMS, BPL or AA was not significantly different from that of untreated DNA. The results indicate that in competent B. subtilis the pathways for repair of alkylating agent damage and for repair of UV damage are probably different.


The studies indicating the importance of TNF alpha in dengue virus infection have led us to determine whether monocyte-like cells produce TNF alpha exposure after dengue virus. The supernatant fluids of mosquito cells (AP61) infected with dengue virus (DV) type 1 and DV type 3 were harvested 7 days post-infection and clarified. DV inactivation was performed in the presence of betapropiolactone that preserves antigenicity of viruses. We used the monocytic-like cell line THP-1 that is a model system of TNF alpha production. Polymyxin B (50 micrograms/ml) was added to block untoward effects resulting from possible LPS contamination of media or cultures. THP-1 cells were primed with a phorbol ester (PMA) for 24 h, then they were cultured for 4 and 24 h in the presence of inactivated culture supernatant of dengue infected AP61 cells or control preparations. The concentrations of TNF alpha in the culture supernatants were measured by using an immunoenzymatic assay. PMA-treated THP-1 cells rapidly secreted TNF alpha in response to inactivated culture supernatant of DV-infected cells. We found high levels of TNF alpha with cells exposed to DV1 and DV3 preparations compared with controls (mean values; 465 and 829 vs. 70 pg/ml, respectively, at 24 h post exposure, n = 4). We obtained a substantial inhibition of the enhancing activity of DV1 and DV3 infected supernatants in the presence of dengue hyperimmune mouse ascitic fluids. Our results demonstrate that exposure of monocytes/macrophages to DV particles or virus proteins derived from DV may be responsible for the enhanced production of TNF alpha in DV-infected patients.

Establishment of selective immunity, local or systemic, made it possible to evaluate the pathogenesis of Newcastle disease virus (NDV) in the respiratory tract of chickens that were previously immunized with beta-propiolactone-inactivated antigen. NDV was inoculated intranasally or intramuscularly to chickens in different states of immunity (local or systemic). Humoral antibodies protected chickens against intranasal as well as intramuscular infection. Local antibodies, on the other hand, conferred immunity only against intranasal challenge. The respiratory tract supported multiplication of the virus, producing a self-limited subclinical infection. Replication of the virus in this system was negligible, playing only a minor role in the pathogenesis of the disease.


The vaccine produced by the Institut Pasteur of Algeria consists of a 5% suspension of brain matter of young goats inoculated with the Louis Pasteur Saigon strain. The suspension is BPL-inactivated, supplemented with a protective agent and lyophilized to a volume of 2 ml. The vaccine loses all specific and non-specific toxicity when injected into newborn mice, adult mice and rabbits. The stability of this vaccine, studied over a period of 24 months at room temperature and 6 months at 37 degrees C, is very satisfactory. The average protective capacity for some twenty batches is 1.60 as determined by the NIH test and 700,000 protective units according to the Habel test. The immunogenicity of this vaccine is evaluated by measuring the neutralizing antibodies in individuals who have been vaccinated for preventive purposes as well as in individuals who have been treated after a possibly contaminating contact. The possibility of reducing the number of injections to 7 + 3 boosters rather than 14 + 2 boosters is discussed in the light of the results obtained.


In vitro reaction of beta-propiolactone (BPL) with calf thymus DNA and mouse liver DNA followed by acid (HCL) hydrolyses of the BPL-reacted DNA's resulted in the isolation of a new compound, 1-(2-carboxyethyl)-adenine (1-CEA). The structure was assigned on the basis of ultraviolet spectra at acidic, alkaline and neutral pH and electron impact and chemical ionization mass spectra as well as chemical synthesis of 1-CEA from BPL and 2'-adenosine-5'-monophosphoric acid. The only other compound previously isolated from the in vitro and in vivo reactions of BPL and DNA was 7-(2-carboxyethyl)guanine (7-CEG) which we also identified as a product of our in vitro reaction. Under the conditions used the main product of alkylation was 1-CEA and the ratios of the concentrations of 1-CEA to 7-CEG was approx 3 : 1. The possible effect of
the formation of 1-CEA on the structure of DNA and its role in chemical carcinogenesis is discussed.

Milo et al. 1978. Chemical carcinogen alteration of SV40 virus induced transformation of normal human cell populations in vitro. Chem.Biol.Interact. Vol. 22(2-3): 185-197. The frequency of simian papovirus 40 (SV40) induced transformation of human cells was enhanced after pretreatment with either naphthylamine-2,N-methyl-N'-nitrosoguanidine (MNNG), N-acetyl-2-fluorenylacetamide (N-Ac-AAF), benzo[a]pyrene (BP), aflatoxin B1, propane sulfone (PS), beta-propiolactone, 4-nitroquinoline oxide (4-NQO), methylmethane sulfonate (MMS) or diethyl nitrosamine (DEN). Posttreatment with 4-NQO, MMS, MNNG or DEN inhibited transformation; while posttreatment with either aflatoxin B1, beta-propiolactone or naphthylamine-2 did not alter transformation similar to the action of N-Ac-AAF and BP. All carcinogens that altered transformation after pretreatment damaged cellular DNA. Pretreatment or posttreatment with carcinogens 3-methylcholanthrene (3-MCA) or 7,12-dimethylbenzanthrene (7,12-DMBA), that did not damage cellular DNA also did not enhance transformation. Moreover, pre- or posttreatment with other weak or non-carcinogens that did not damage cellular DNA did not alter virus induced transformation. All foci formed in the co-carcinogen treated cultures whether the carcinogen inhibited or enhanced transformation were virus directed. While a similar pattern of response existed for carcinogens that either enhance or inhibit transformation, each of the carcinogens that enhanced or inhibited foci formation damaged cellular DNA. Moreover, those carcinogens that enhanced focus formation, compared to the carcinogens that inhibited focus formation, exhibited similar DNA damage profiles.

Milo et al. 1981. Neoplastic transformation of human epithelial cells in vitro after exposure to chemical carcinogens. Cancer Res. Vol. 41(12 Pt 1): 5096-5102. Human foreskin epithelial cells were transformed to an anchorage-independent state of growth (in soft agar) and neoplasia (invasion of chick embryonic skin in vitro). Aflatoxin B1, N-methyl-N'-nitro-N-nitrosoguanidine, propanesulfone, beta-propiolactone, or ultraviolet absorbance at 254 nm were used successfully as carcinogens. These foreskin epithelial cells, like human foreskin fibroblasts, were readily transformed when treated in S phase but, unlike the transformed fibroblasts, expression of cellular neoplasia did not require an extended period of time in culture. The invasive features of the transformed human epithelial cells in chick embryonic skin in vitro simulated squamous cell carcinoma.

Mondorf et al. 1981. A clinical study of the tolerance and safety of a beta-propiolactone-treated immunoglobulin. Arzneimittelforschung. Vol. 31(11): 1928-1930. In an open clinical study, 31 healthy volunteers were each given three i.v. infusions of 50 ml of a immunoglobulin preparation (Intraglobin) from three different production batches. During a 35-week observation period every three weeks blood specimens were drawn from the participants and analysed for serological (HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe) and clinico-chemical parameters of hepatitis (SGOT, SGPT, gamma-GT and bilirubin levels). In no case evidence of the development of hepatitis arose, nor did the participants suffer from any side-effects during or after the infusions.
Ten out of the 31 subjects then received another 150 ml infusion followed by an intradermal application of the new immunoglobulin in a 1:10 dilution four weeks later. No signs of immediate or delayed hypersensitivity reactions could be observed in the volunteers.


A method which allows growth of normal human tissue to be studied in vitro is used to investigate possible interactive effects of radiation and environmentally important carcinogens on oesophageal and urothelial cell growth. Carcinogens chosen were selected for their known or suspected effect on the oesophageal mucosa or urothelium in vivo. The results indicate that with carcinogens alone concentrations can be identified that result in increased proliferation of cells. With radiation alone inhibition of cell proliferation occurs at all dose points examined. However, at precise combinations of radiation and carcinogen, greatly enhanced cell proliferation could be detected, suggesting a synergistic interaction between the two agents. The results may have implications for the design and interpretation of experiments aimed at elucidating early or premalignant changes in epithelial tissues and may indicate hitherto unsuspected interactions between radiation and environmentally important carcinogens.


High resolution 1H-NMR spectroscopy has been used to study the infection of chicken embryo fibroblasts by influenza virus. Marked changes in the NMR spectrum occur when infectious influenza virus is introduced into the fibroblasts and these changes appear to depend upon the presence of active neuraminidase (EC 3.2.1.18). A crude preparation of neuraminidase from *Vibrio cholerae* is able to effect similar changes. Only minor spectral changes are observed in the absence of culture medium or when the viral genome material is inactivated by beta-propiolactone. Similarly, little change is seen in the NMR spectrum when amantadine, which is thought to inhibit uncoating of the virus inside the cell, or actinomycin D, which inhibits cellular nucleic acid metabolism, are incubated with fibroblasts prior to the addition of virus. The results suggest that neuraminidase, in co-operation with a factor in the infectious process, initiates a cellular event which can be monitored by NMR. The nature of this cellular mechanism is unknown, but further studies are under way to determine its importance in viral infection.


The induction of sister chromatid exchanges (SCE) and mutation at the hypoxanthine-guanine phosphoribosyl transferase locus and toxicities of 40 different chemical and physical agents were examined on Chinese hamster V79 cells. These agents included mono-, di-, tri-, and polyfunctional alkylating agents, intercalators, gamma-rays, and UV light irradiation. Mutation was measured as resistance to 6-thioguanine and toxicity as loss of cell-plating efficiency. SCE were examined 29 hr after treatment. With the agents examined, a highly positive correlation ($r = 0.89$) existed between SCE-inducing and mutagenic potencies, when expressed as increase in the number per a unit dose over the control values. But the great difference of the ratios of mutagenic potencies versus SCE-inducing potencies among agents was observed, the maximal difference in the ratios being about 200-fold. The agents that showed the higher values of the ratio (agents producing more mutations than SCE) were bleomycin, cobalt-60 gamma-rays, all ethylating agents (N-ethyl-N-nitrosourea, N-ethyl-N'-nitro-N-nitrosoguanidine, ethyl methanesulfonate, and diethylsulfate), N-propyl-N-nitrosourea, N-butyl-N-nitrosourea, isopropyl methanesulfonate, intercalating acridine compounds (2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl)aminopropylamino]acridine X 2HCl and 2-methoxy-6-chloro-9-[3-(chloroethyl)-aminopropylamino]acridine 2HCl) and UV light at 254 nm. The agents that showed the lower values (agents producing more SCE than mutations) were platinum compounds (cis-diamminedichloro-platinum and trans-diamminedichloroplatinum), epoxides (epichlorohydrin, styrene oxide, and diepoxbutane) and aziridines (mitomycin C, decarbamoyl mitomycin C, tris(1-aziridinyl)phosphine sulfide, triethylenemelamine, and carboquone). The agents that showed the intermediate values included all methylating agents (N-methyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine, methyl methanesulfonate, and dimethyl sulfate), N-(2-hydroxyethyl)ethylamine, beta-propiolactone, treatment of 8-methoxypsoralen plus near-UV light irradiation at 352 nm, 4-nitroquinoline-1-oxide, quinacrine mustard, sodium sorbate, cigarette tar, and diesel tar. For most agents that induced SCE, the toxicity dependency of induced SCE was rather biphasic; increase in SCE was steep at low to moderate toxicity and less at moderate to high toxicity. At equitoxic doses, the agents showed great difference in induction of SCE.

**Noskov et al. 1990.** Genetic analysis of spontaneous and 6-N-hydroxylaminopurine and propiolactone induced Adp+ mutants in Saccharomyces yeasts. *Genetika.* Vol. 26(7):
652 spontaneous and 6-N-hydroxylaminopurine and propiolactone-induced mutants were obtained in yeast. 598 of them were LYS2 mutants. Detailed genetic analysis of the mutants was performed, including analysis of growth pattern on lysineless medium, suppressibility by nonsense suppressors of three types and localization on the recombination map of the LYS2 gene. Mutants induced by different agents were different for all these criteria, except for distribution among the map regions.

Concentrated murine leukemia virus (MuLV) or MuLV producing cells induce XC cell fusion within an hour leading to syncytia formation. While MuLV inactivated by UV irradiation, beta-propiolactone or hydroxylamine treatment still caused cell fusion, Bromelin- or trypsin treated MuLV was no longer able to fuse XC cells. Though sonicated MuLV induced no XC cell fusion, it interfered with cell fusion as caused by untreated MuLV. XC cells infected by diluted MuLV of a titer lower than 1 X 10(5) PFU/ml formed no syncytia although they produced MuLV. The cell fusion mechanism is discussed.

The standard method of the C3H/10T1/2 cell transformation assay cannot adequately detect alkylating agents. A modification of the standard procedure as described by Bertram and Heidelberger using a large number of synchronized cells and high levels of toxicity was evaluated for transformation using several alkylating agents. By using this method, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), beta-propiolactone (BPL), methyl methanesulfonate (MMS), methylnitrosourea (MNU) and 1,3-propane sultone (PS) transformed these cells. However, methyl iodide (MI) failed to induce any transformed foci.

Extensive studies have shown that chemical carcinogenesis involves an initiation-promotion pattern. A gene amplification model of carcinogenesis predicts that initiation involves induction of a genetic tandem duplication. We use a system developed by Anderson and Roth to select for tandem duplication of the histidine operon of Salmonella typhimurium by selection for resistance to 3-amino-1,2,4-triazole. Evidence reported here shows that, consistent with prediction, 10 carcinogens are all active in inducing tandem duplications. Two toxic noncarcinogens show little or no activity under the conditions used in inducing tandem duplication but azide, a mutagenic noncarcinogen, did show some activity. 9 types of evidence now support the gene amplification initiation-promotion model of carcinogenesis.


Chemical mutagens 6-N-hydroxylaminopurine (HAP) and propiolactone (PRO) induce Lys2 mutants with high frequency in diploid yeast Saccharomyces cerevisiae. HAP induces such mutants even in tetraploid strains. The genetic analysis of mutants was performed. It is shown that PRO induces mutants by means of “mutation-mitotic segregation” mechanism, while HAP induces mutants through novel mechanism “both allele mutation”. Manifestation of such mechanism is the null fertility after meiosis of diploid mutants induced by HAP.


The toxic and mutagenic effects of the alkylating agents N-methyl-N-nitrosourethane (MNUT) and beta-propiolactone (BPL) were quantitatively measured in human lymphoblasts and Salmonella typhimurium. Forward mutation to 6-thioguanine resistance was measured in the human lymphoblasts, and forward mutation to 8-azaguanine resistance was measured in the bacterial cells after equigenerational (1.5 doubling times) exposures. In both systems, the induced mutant fraction rose linearly as a function of concentration for BPL and was biphasic for MNUT. The responses of the two assay systems to eight alkylating agents were compared. The exposure of the cells to each alkylating agent was calculated as exposure concentration multiplied by the time of exposure, and allowance was made for the decomposition of the alkylating agents during exposure (integral exposure). Human cells were 2.5–13 times more sensitive than was S. typhimurium to the alkylating agents methyl methanesulfonate, ethyl methanesulfonate, propyl methanesulfonate, N-methyl-N’-nitro-N-nitrosoguanidine, methylnitrosourea, and MNUT. S. typhimurium cells were three times more sensitive to butyl methanesulfonate and 25 times more sensitive to BPL than were human cells.


A comparison was performed of the results reported in the literature of chemicals tested in the rat liver foci assay and/or in the strain A lung tumor assay to the results of the chemicals tested in long-term carcinogenicity bioassays. The rat liver foci assay was Contract No. IOM-2794-04-001 Health Effects of Betapropiolactone [Beta-propiolactone]
sensitive to 69% of 54 compounds found to be carcinogenic in long-term bioassays and the strain A lung tumor assay to 54% of 93 carcinogens. None of 10 compounds found to be noncarcinogenic in long-term bioassays were active in the rat liver foci assay, while 7 of 23 noncarcinogens (30%) were active in the lung tumor assay. Ten of the 17 carcinogens negative in the rat liver foci assay are believed to exhibit tumor-promoting activity; 3 are direct-acting alkylating agents (dimethylsulfate, epichlorohydrin, and beta-propiolactone); and the remaining 3 are azobenzene, 1,2-dibromoethane, and thioacetamide. Thirty-two of the 43 carcinogens negative in the lung tumor assay were active in either (1) the mouse liver only, (2) the rat and not in the mouse, or (3) in both the rat and mouse liver but not in other organs of the mouse. It is proposed that additional investigations be undertaken to further evaluate the rat liver foci assay and the strain A mouse lung tumor assay as short-term in vivo tests for the demonstration of the carcinogenic potential of genotoxic (mutagenic) chemicals and environmental samples of complex mixtures.


The cutaneous and systemic carcinogenic potentials of pure and two technical diglycidyl ethers of bisphenol A, DGEBA, EPON 828 and EPIKOTE 828, respectively, have been investigated in six groups each of 50 male and 50 female CF1 mice. Twice weekly over a period of 2 yr, 0.2 ml of a 1 or 10% (w/v) solution of one of the epoxy resins in acetone was applied to the dorsal skin. A group of 50 male and 50 female CF1 mice was similarly treated with 2% (w/v) beta-propiolactone in acetone (the positive control) while a group of 100 male and 100 female mice was treated with acetone alone (negative control). Survival of the CF1 mice to 2 yr was unaffected by cutaneous exposure to each epoxy resin. The compounds proved to be mildly irritant to murine skin, the response in males being greater than in females. There was a very low incidence of benign and malignant tumours of the skin and subcutis after exposure to any of these compounds. The number of systemic lymphoreticular/haematopoietic tumours was increased only in females treated with EPIKOTE 828 or DGEBA. In male mice, treated with 10% EPON 828, there was a slight increase in the number of renal tumours. The incidence of other systemic tumours in either sex was not increased following cutaneous application of the purified or the two technical DGEBA resins (1 to 10% in acetone). The significance of all these findings is fully discussed.


During the production of Intraglobin, an i.v. tolerable IgG-immunoglobulin preparation, fraction Cohn II is treated with beta-propiolactone. Thereby the IgG-molecules are chemically modified thus preventing an increase in secondary aggregates which could give rise to adverse reactions in the patients treated with the IgG-preparation. The safety tests described here neither showed any evidence for the presence of unhydrolysed
mutagenic-beta-propiolactone in the final product nor that neoantigenic determinants had been created by the reaction of beta-propiolactone with the protein molecules.


Covalent adducts formed by the reaction of DNA with chemical carcinogens and mutagens may be detected by a 32P-labeling test. DNA preparations exposed to chemicals known to bind covalently to DNA [N-methyl-N-nitrosourea, dimethyl sulfate, formaldehyde, beta-propiolactone, propylene oxide, streptozotocin, nitrogen mustard, and 1,3-bis(2-chloroethyl)-1-nitrosourea] were digested to a mixture of deoxynucleoside 3’-monophosphates by incubation with micrococcal endonuclease (EC 3.1.31.1) and spleen exonuclease (EC 3.1.16.1). The digests were treated with [gamma-32P]ATP and T4 polynucleotide kinase (ATP:5’-dephosphopolynucleotide 5’-phosphotransferase, EC 2.7.1.78) to convert the monophosphates to 5’-32P-labeled deoxynucleoside 3’,5’-bis-phosphates. These compounds were then separated on polyethyleneimine-cellulose thin layers in ammonium formate and ammonium sulfate solutions. Autoradiograms of the chromatograms obtained by this high-resolution procedure showed the presence of nucleotides derived from chemically altered, as well as normal, DNA constituents. Maps from DNA exposed to any of the chemicals used exhibited a spot pattern typical for the particular chemical. This method detected a single adduct in 10(5) DNA nucleotides without requiring that the compound under investigation be radioactive and thus provides a useful test to screen chemicals for their capacity to damage DNA by covalent binding.


Scored at 24 hours, the LD-50 of a solution of beta-propiolactone administered intravenously to young rats was 225 +/- 55 mg/kg. Twenty-four hours after a single intravenous injection (100 mg/kg = 1.4 m mole/kg) of beta-propiolactone into male and female rats of both the Long-Evans and Sprague-Dawley strains, the incidence of breaks found in the chromosomes of metaphase marrow cells was low (8.8 percent vs. 5.0 percent in controls). The s5 chromosomes were preferentially damaged. A 200 mg/kg dose increased the incidence modestly to 11.3 percent. In comparison, a single intravenous dose of benzo(a)pyrene (40 mg/kg = 0.16 m mole/kg) produced a break
incidence of 19 percent. In long-term experiments multiple (five) intravenous injections (100 mg/kg each) of beta-propiolactone given in a 6 week period elicited only two neoplasms (a chloro-leukemia and a mammary fibroadenoma) among 37 animals during the following 12-13 months. In contrast, four injections of benzo(a)pyrene (40 mg/kg) produced a 14-times greater mammary tumor incidence in the Sprague-Dawley female rat than did beta-propiolactone. Marrow cell chromosome examination indicated no significant chromosomal changes due to the earlier beta-propiolactone treatment except for one animal with a consistent 43-chromosome karyotype resulting from S1 trisomy; no neoplasm was evident in that animal. Earlier treatment with benzo(a)pyrene produced a persistent and significant elevation in break incidence. Both the carcinogenic and clastogenic effects of intravenous beta-propiolactone are low in rats and are not comparable in magnitude to those produced by benzo(a)pyrene.


The alkaline elution technique has been used to evaluate DNA damage in brain of rats treated with a single equimolar dose of 14 carcinogens of different chemical structure. A clear-cut increase of DNA elution rate, which is considered indicative of DNA fragmentation, was produced by 10 compounds known to induce the development of tumors in the rat central nervous system: N-nitroso-N-methylurea, N-nitroso-N-ethylurea, N-nitroso-N-butylurea, N-nitroso-N-methylurethane, methyl methanesulfonate, ethyl methanesulfonate, dimethyl sulfate, diethyl sulfate, 1,3-propansultone, and procarbazine. Similar amounts of DNA fragmentation were produced by both potent and weak brain carcinogens. In contrast, any significant increase of DNA elution rate was absent in rats treated with N-methyl-N'-nitro-N-nitrosoguanidine, N-nitrosodimethylamine, N-nitrosodiethylamine, and beta-propiolactone, all of which are devoid of carcinogenic activity for the rat central nervous system. These results suggest that the described in vivo brain DNA damage/alkaline elution assay deserves further studies on a wide number of carcinogens and noncarcinogens aimed to establish its possible usefulness for a qualitative preliminary assessment of the ability of a compound to induce neurogenic tumors.


RTECS. 2004. 2-Oxetanone. Registry of Toxic Effects of Chemical Substances. [Database] [http://www.cdc.gov/niosh/rtecs/rq7d80e8.html]


Vaccine strains of influenza A virus inhibited the growth of ascitic tumour cells and outbred rats or inbred mice. The infected tumour bearers had an enhanced immune response to viral and specific tumour antigens. These phenomena are apparently due to
the formation of complexes of both antigens on cell membranes and increased immunogenicity of such complexes.


beta-Propiolactone (beta PL) has been tested on preimplantation mouse embryos for possible genotoxic effects. Tests were performed at different stages of meiosis (late prophase I, diakinesis/metaphase I, anaphase I, telophase I/prophase II and metaphase II) by injecting females at various times after the induction of superovulation. Male and female derived chromosome complements from first-cleavage embryos were analysed before syngamy for cytogenetic abnormalities. A higher proportion of diploid oocytes, produced by the non-extrusion of the first or second polar body, was found after fertilization when the compound was administered immediately before metaphase I or II. No obvious effect was detected at any other time of beta PL exposure. Based on these results, several possible modes of action for beta PL are postulated.


The effect of modifying phi chi 174 viral DNA by the chemical carcinogens beta-propiolactone, N-acetoxyacetylaminofluorene and anti-benzo[a]pyrene diol-epoxide was investigated by transfecting the modified DNA into Escherichia coli spheroplasts. Modification of the DNA in vitro by each of these agents was mutagenic for the phi chi 174 amber mutants am3 and am86. Mutagenicity depended on the induction of the “;SOS”; response in the host spheroplasts. Heating beta-propiolactone-treated DNA at neutral pH caused strong inactivation such that the number of lethal hits was increased 4-fold. Sucrose gradient analysis showed the induction of alkali-labile sites in the heated DNA. The “;nicked circle assay”; with double-stranded phi chi 174 DNA showed greater than 70% of these sites to be apurinic sites. Concomitantly with the production of these new sites, a strong increase in the mutation frequency was observed. This mutagenesis also depended upon the induction of the error-prone SOS response in the spheroplasts, as was previously shown to be the case for mutagenesis at putative apurinic sites induced directly by acid-heat treatment. These results suggest that depurination may be of importance to the mechanism of mutagenesis by beta-propiolactone and other carcinogens.


We had previously reported that the carcinogen, beta-propiolactone (BPL) reacted in

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vitro with histones in whole mouse skin chromatin and that among the histone classes BPL was preferentially bound to the lysine-rich histones H1 and H1 degrees. In order to determine if in vitro reaction of BPL with calf thymus histones resulted in binding of BPL to L-lysine, we synthesized the model compounds XI-N-(3-hydroxypropionyl)lysine (HPL) and xi-N-(1-carboxyethyl)lysine (CEL) from BPL and L-lysine. The alpha-amino group of L-lysine was protected from reaction with BPL by the formation of a copper chelate. Structures were assigned on the basis of infrared spectra, pKa values and chemical analyses. BPL was reacted in vitro with calf thymus histones and the BPL-reacted calf thymus histones and control calf thymus histones were digested with trypsin followed by pronase. The respective digests were each chromatographed on a column of AA-15 cation-exchange resin. The elution profiles of the two digests were very similar except for the appearance of a new ninhydrin-positive peak (NNPP) in the eluate of the trypsin-pronase digest of BPL-reacted calf thymus histones. When compounds HPL and CEL were added to the trypsin-pronase digest of control calf thymus histones and the mixture chromatographed on AA-15, both compounds were resolved from the other peptide (or amino acid) peaks. HPL was eluted in the same fractions as NNPP, HPL and NNPP exhibited identical RF values on silica gel TLC with acidic, alkaline and neutral solvents. CEL was not identified as a product of the reaction between BPL and calf thymus histones.

Segal et al. 1978. A new personal monitoring device for the detection of beta-propiolactone and other alkylating agents. Arch. Environ. Health. Vol. 33(1): 33-35. A personal monitoring badge has been developed for the detection of the direct-acting, alkylating carcinogen beta-propiolactone at atmospheric concentrations as low as 6 ppb for 24-hour and 0.6 ppm for 0.25-hour exposure. The method employs the trapping reagent p-nitrobenzyl pyridine (p-NBP) absorbed on a cellulose thin-layer chromatography (TLC) strip. Deoxyguanosine can be used in place of p-NBP, but its lower limit of detection is 60 ppb for 24-hour exposure. The authors also obtained positive results with the carcinogens bis (chloromethyl) ether, chloromethyl methyl ether, diepoxybutane, dimethylcarbamoyl chloride, ethyleneimine, and glycinaldehyde. In practice, the TLC strip is positioned in a film badge holder. The TLC strip monitoring badges are easy to prepare; they should encounter no resistance of personnel to their use since they are not cumbersome. Monitoring at the end of an exposure is simple and requires no expensive equipment or specialized personnel.

Segal et al. 1979. In vitro Dimroth rearrangement of 1-(2-carboxyethyl) adenine to N6-(2-carboxyethyl) adenine in single-stranded calf thymus DNA. Chem. Biol. Interact. Vol. 28(2-3): 333-344. The new adduct N6-(2-carboxyethyl) adenine (N6-CEA) was prepared from 1-(2-carboxyethyl) adenine (1-CEA) by base catalyzed (Dimroth) rearrangement of 1-CEA. The structure of N6-CEA was assigned on the basis of UV spectra and electron impact and isobutane chemical ionization mass spectra. When the carcinogen beta-propiolactone was reacted in vitro with calf thymus DNA, 1-CEA but not N6-CEA was detected on paper chromatograms following acid hydrolysis of the DNA. When BPL-reacted single-stranded DNA was incubated at pH 11.7 (37 degrees C, 18 h) prior to acid hydrolysis, it was found that 1-CEA was completely converted to N6-CEA in DNA by Dimroth.
rarrangement, whereas no conversion occurred at pH 7.5. The extent of Dimroth rearrangement at various pHs and temperatures was determined for 1-CEA, 1-methyladenine (1-MeA), 1-(2-carboxyethyl)-deoxyadenosine-5'-monophosphoric acid (1-CEdAdo5'P) and the phosphodiester 5'-O-(2-carboxyethyl)phosophono-1-(2-carboxyethyl)deoxyadenosine (1-CE-Ado-5'-P-CE).


The new adduct 3-(2-carboxyethyl)cytosine (3-CEC) was isolated following in vitro reaction of the carcinogen beta-propiolactone (BPL) with calf thymus DNA. The structure of 3-CEC was confirmed by synthesis from BPL and dCyd. Reaction of BPL with cCyd (pH 7.0-7.5, 37 degrees C) gave 3-(2-carboxyethyl)deoxyctydine (3-CEdCyd) (9% yield) and 3,N4-bis(2-carboxyethyl)deoxycytidine (3,N4-BCEdCyd) (0.6% yield). 3-CEdCyd and 3,N4-BCEdCyd were hydrolyzed (1.5 N HCl, 100 degrees C, 2 h) to 3-CEC and 3,N4-bis(2-carboxyethyl)cytosine (3,N4-BCEC), respectively. The structure of 3-CEC was assigned on the basis of UV and NMR spectra and the electron impact (EI) mass spectra of 3-CEC and a tri-trimethylsilyl (TMS) derivative of 3 CEC as well as deuterated (d27) tri-TMS derivative of 3-CEC. The structure of 3,N4-BCEC was assigned on the basis of UV spectra and the EI mass spectra of a tri-TMS derivative. Ei and isobutane chemical ionization mass spectra of 3-methylcytosine (3-MeCyt) and a di-TMs derivative of 3-MeCyt were obtained and were helpful in deducing the structures of 3-CEC and 3,N4-BCEC. This is the first report of the alkylation by BPL of an exocyclic atom on a base in DNA. Compound 3,N4-BCEC was not detected in BPL-reacted calf thymus DNA.


Reaction of acrylic acid (AA) at pH 7.0 and 37 degrees C for 40 days with 2'-deoxyadenosine (dAdo), 2'-deoxycytidine (dCyd), 2'-deoxyguanosine (dGuo) and thymidine (dTd) resulted in the formation of 2-carboxyethyl (CE) adducts via Michael addition. The alkylated 2'-deoxynucleoside adducts isolated (percent yield after 40 days) were 1-CE-dAdo (5%), N6-CE-dAdo (11%) (via Dimroth rearrangement of 1-CE-dAdo), 3-CE-dCyd (7.5%), 7-CE-Gua (4%), 7,9-bis-CE-Gua (0.9%) (formed by reaction of AA with depurinated 7-CE-Gua during the course of the reaction) and 3-CE-dTdh (0.5%). The products isolated following in vitro reaction of AA with calf thymus DNA at pH 7.0 and 37 degrees C for 40 days were (nmol/mg DNA) 1-CE-Ade (9.9), N6-CE-Ade (8.2), 7-CE-Gua (7.2) and 3-CE-Thy (1.9). Compound 3-CE-Cyt was not detected. Thus the adducts formed following in vitro reaction of AA with DNA are identical to those formed by in vitro reaction of the carcinogen beta-propiolactone (BPL) with DNA as reported in...
an earlier paper. Structures were assigned on the basis of identical UV spectra, Rf values on paper chromatograms and Rt values on HPLC as marker compounds prepared from reactions of BPL with 2'-deoxynucleosides and 2'-deoxynucleotides-5'-monophosphoric acids. AA was assayed for carcinogenic activity by s.c. injection (20 mumol, once a week for 52 weeks) in female Hsd: (ICR)Br mice. Two mice with sarcomas at the site of application were observed out of 30 mice. Malignancies were not observed in solvent and no-treatment controls. The bioassay results reported in this paper and elsewhere in the same strain of mice suggest that AA is a weak carcinogen in female Hsd:(ICR)Br mice.

The goal of these experiments in female Hsd:(ICR)Br mice was to determine whether the direct-acting SN1 alkylating carcinogen isopropyl methanesulfonate (IMS) is carcinogenic and to compare its effects with those of the direct-acting SN2 methyl homologue, methyl methanesulfonate (MMS). The compounds were administered by topical application and s.c. injection. Analysis at the 288th day of mice receiving s.c. injections of IMS and MMS was the subject of a previous report (A. Segal et al., Proc. Soc. Exp. Biol. Med., 183: 132-135, 1986). The s.c. and topical application experiments were terminated at the 450th day and the final results are reported in this paper. In mice treated by s.c. injection with IMS, thymic lymphomas were observed in at least 20 of 32 mice, the first at the 40th day, and neoplasms were not observed at the injection site. Of the 30 MMS-treated mice, 11 developed sarcomas at the injection site and one thymic lymphoma was observed. In mice treated topically with IMS, thymic lymphomas were observed in 20 of 30 treated mice, the first at the 102nd day, and squamous cell carcinomas at the injection site were observed in 9 mice. Neither squamous cell carcinomas nor thymic lymphomas were observed in 30 mice following topical application of MMS. The direct-acting SN2 alkylating carcinogen beta-propiolactone was also administered by topical application. At the 450th day, at the same dose used for MMS (40 mumol/application), papillomas of the skin were observed in 25 of 30 treated mice, squamous cell carcinomas of the skin were seen in 17 mice, and one thymic lymphoma was observed. The results suggest that the rapid induction of thymomas by IMS may be related to its ability to alkylate exocyclic oxygen atoms in DNA of hemopoietic cells and also to a sensitivity of these cells to such lesions.

A series of earlier studies showed that inhalation exposures of rats to three water-reactive electrophilic compounds produced brisk yields of nasal cancer even when the animals were exposed for only 30 days (6 hr/day X 5 day/wk). In addition, carcinogenic potencies of the compounds appeared to relate to their chemical reactivities as measured by hydrolysis rates. For a further study of this phenomenon, inhalation exposures were conducted with five additional water-reactive compounds: beta-propiolactone [(BPL) CAS: 57-57-8], methylmethane sulfonate [(MMS) CAS: 66-27-3], ethylchloroformate
[(ECF) CAS: 541-41-3], dichloroacetyl chloride [(DCAC) CAS: 79-36-7], and propylene oxide [(PO) CAS: 75-56-9] on male Sprague-Dawley rats. The hydrolysis rates of these compounds span 6 orders of magnitude. The compounds were administered for 30 days (6 hr/day X 5 days/wk) with the use of exposure concentrations that were inversely proportional to the respective hydrolysis rates. With this protocol, all compounds except PO (the slowest reacting compound) produced nasal cancer in rats. The concentrations of MMS and BPL employed in the studies produced similar nasal cancer yields, indicating that the carcinogenic potencies of these compounds in rat nasal mucosa were proportional to their hydrolysis rates. The nasal cancer yields of DCAC and ECF were less than expected. DCAC hydrolyzes so rapidly at in vivo temperatures (half-life much less than 0.01 min) that it may not reach target DNA in reactive form. Why the exposures to ECF produced yields of nasal cancer not predicted by its reactivity is currently under investigation. These results combined with our earlier results demonstrate that the carcinogenic potencies of some inhaled reactive electrophilic compounds are related to their hydrolysis rates.


Previous studies have indicated that the alkylating agent, 2-methoxy-6-chloro-9-(3-[ethyl-2-chloroethyl]aminopropylamino)acridine dihydrochloride (ICR-170), induces much more killing and mutation in conidia of Neurospora crassa treated in an atmosphere of N2 than in an atmosphere of O2. It was desirable to determine if a similar effect--more killing and mutation in N2 than in O2--could be observed with two other known alkylating agents, beta-propiolactone (BPL) and ethyl methanesulfonate (EMS), in the same test system. Conidia of a heterokaryotic strain of N. crassa were bubbled with N2 or O2 during treatment with BPL or EMS. Forward-mutation was measured in the ad-3 region by a direct method. The results indicate that N2 or O2 do not influence the lethal and mutagenic activities of BPL or EMS during treatment of conidia. Hence the influence of N2 or O2 on the lethal and mutagenic activities of ICR-170 is different from the influence of these gases on BPL or EMS using the ad-3 test system in N. crassa.


Increasing concentrations of malonaldehyde and beta-propiolactone were increasingly mutagenic with 7 mutants of Salmonella typhimurium, 5 of which mutated by a frameshift mechanism and 2 of which mutated through base-pair substitution. The antioxidants vitamin C, vitamin E, selenium and butylated hydroxytoluene (BHT) at 3 logarithmic concentrations markedly reduced mutagenesis in those strains which mutated by frameshift mechanism.


The in vitro accuracy of DNA replication has been investigated through the measurement of the frequency with which noncomplementary nucleotides were incorporated during polynucleotide replication. The effect of beta-propiolactone treatment of deoxynucleotide templates, ribopolynucleotide templates, and the DNA polymerase from avian
myeloblastosis virus was determined. Treatment of the deoxynucleotide template, poly(dA) (see article) oligo(dT) 12-18, by beta-propiolactone resulted in an increased frequency of noncomplementary nucleotide incorporation during DNA polymerization. Carcinogen treatment of the ribonucleotide templates, poly(rA) (see article) oligo(dT) 12-18, and poly(rC) (see article) oligo(dG) 12-18, and carcinogen treatment of avian myeloblastosis virus DNA polymerase did not alter the frequency of noncomplementary nucleotide incorporation. This suggested that carcinogen-induced error incorporation during DNA synthesis was restricted solely to the treatment of a deoxynucleotide template.


Immunization of BALB/c mice with measles virus inactivated with beta-propiolactone and mixed with 100 micrograms of the cationic surface-active lipid dimethyl dioctadecyl ammonium bromide (DDA) primes for a strong virus-specific delayed-type hypersensitivity (DTH) response that peaks 1 week later. Optimal immunization and challenge doses were found to be 8 and 4 micrograms/mouse, respectively, and pretreatment with 200 mg of cyclophosphamide/kg 2 days prior to immunization significantly enhanced the DTH response. When compared to Freund's complete and incomplete adjuvants, DDA was superior for induction of DTH to inactivated purified measles virus. As DDA could be administered to animals at a site different from the measles virus antigens, or 1 day previously, and still significantly enhance the DTH response, DDA is probably acting more as an immune modulator than as a simple adjuvant. The conditions for an optimal DTH response to measles virus were also shown to be applicable to other enveloped viruses, for example, a strong DTH response was similarly generated to inactivated purified influenza PR8 virus and to herpes simplex virus type I antigens present in plasma membranes isolated from infected Vero cells.


Three direct-acting carcinogens, beta-propiolactone (BPL), methylmethane sulfonate (MMS), and dimethylcarbamyl chloride (DMCC), were evaluated for their carcinogenic potencies in the nasal mucosa of rats and for their abilities to bind in vivo to rat nasal mucosal DNA. The relative carcinogenic potencies of BPL and MMS corresponded well with their overall levels of binding to nasal mucosal DNA. DMCC, however, the most potent carcinogen of the three compounds, produced the lowest level of binding to nasal mucosal DNA. These results indicate that the DNA adducts formed by DMCC in rat nasal mucosa DNA are more readily expressed as cancer than those formed by BPL or MMS.

Reaction of the rodent carcinogen acrylonitrile (AN) at pH 5.0 and/or pH 7.0 for 10 and/or 40 days with 2'-deoxyadenosine (dAdo), 2'-deoxycytidine (dCyd), 2'-deoxyguanosine (dGuo), 2'-deoxyinosine (dIno), N6-methyl-2'-deoxyadenosine (N6-Me-dAdo) and thymidine (dThd) resulted in the formation of cyanoethyl and carboxyethyl adducts. Adducts were not detected after 4 h. The adducts isolated were 1-(2-carboxyethyl)-dAdo (1-CE-dAdo), N6-CE-dAdo, 3-CE-dCyd, 7-(2-cyanoethyl)-Gua (7-CNE-Gua), 7,9-bis-CNE-Gua, imidazole ring-opened 7,9-bis-CNE-Gua, 1-CNE-dIno, 1-CE-N6-Me-dAdo and 3-CNE-dThd. Structures were assigned on the basis of UV spectra and electron impact (EI), chemical ionization (CI), desorption chemical ionization (DCI) and Californium-252 fission fragment ionization mass spectra. Evidence is presented which strongly suggests that N6-CE-dAdo was formed by Dimroth rearrangement of 1-CE-dAdo during the reaction between AN and dAdo. The carboxyethyl adducts resulted from initial cyanooethylation (by Michael addition) at a ring nitrogen adjacent to an exocyclic nitrogen atom followed by rapid hydrolysis of the nitrile moiety to a carboxylic acid. It was postulated that the facile hydrolysis is an autocatalyzed reaction resulting from the formation of a cyclic intermediate between nitrile carbon and exocyclic nitrogen. AN was reacted with calf thymus DNA (pH 7.0, 37 degrees C, 40 days) and the relative amounts of adducts isolated were 1-CE-Ade (26%), N6-CE-Ade (8%), 3-CE-Cyt (1%), 7-CNE-Gua (26%), 7,9-bis-CNE-Gua (4%), imidazole ring-opened 7,9-bis-CNE-Gua (19%) and 3-CNE-Thy (16%). Thus a carcinogen once adducted to a base in DNA was shown to be subsequently modified resulting in a mixed pattern of cyanoethylated and carboxyethylated AN-DNA adducts. Three of the adducts (1-CE-Ade, N6-CE-Ade and 3-CE-Cyt) were identical to adducts previously reported by us to be formed following in vitro reaction of the carcinogen beta-propiolactone (BPL) and calf thymus DNA. The results demonstrate that AN can directly alkylate DNA in vitro at a physiological pH and temperature.


Mouse embryo fibroblast cultures were pretreated with a variety of chemicals found in the environment. After chemical treatment, polyriboinosinic-polyriboctidylic acid was added to the cultures to induce alpha/beta interferon. Pretreatment of the cell cultures with the chemical carcinogens chloroform and beta-propiolactone severely inhibited the production of alpha/beta interferon, while pretreatment of the cell cultures with their poorly or noncarcinogenic analogs 1,1,1-trichloroethane and gamma-butyrolactone had no effect on interferon induction. Pretreatment of the cell cultures with the possible carcinogen diethylstilbestrol had no effect on alpha/beta interferon induction. Pretreatment of the cells with the poor or noncarcinogens pyrene and ascorbic acid did not affect interferon induction; in fact, treatment with ascorbic acid may have enhanced interferon production. These results augment previous findings that most potent carcinogens can inhibit the induction of alpha/beta interferon.

Data were collected following the administration of various compounds to meiotic yeast cultures to investigate their effects on disomic or diploid induction. Epi chlorohydrin, actinomycin D, and 9-aminoacridine were unable to induce either diploidy or disomy. Ethyl methanesulphonate was active in inducing diploids, while hycanthone induced disomics. beta-Propiolactone, N-methyl-N'-nitro-N-nitro-soguanidine, and beta-naphtylamine were able to increase both disomy and diploidy.


When mice are injected intravenously with a large dose of vaccinia virus, prepared in the Ehrlich ascites carcinoma of the mouse, there is a precipitous loss of plasma fibrinogen and blood platelets. Death occurs usually within 24 hours. A specific role of the virus in this toxic syndrome can be demonstrated when heparin is employed to circumvent intravascular coagulation and fibrinogen loss. Heparin does not prevent a profound thrombocytopenia from occurring, but it modifies the rate of platelet loss. Toxicity is prevented when heparinized virus preparations are pretreated with beta-propiolactone or specific antibody, although a mild thrombocytopenia occurs. Thrombocytopenia does not occur in mice injected with heparinized material prepared from uninfecte tumors. These studies indicate that the basic mechanism of vaccinia virus toxicity is an early interaction between infectious virus and blood platelets, with marked thrombocytopenia and consequential pathophysiologic changes.


The purpose of these studies was to examine an early carcinogen-induced change in primary human epithelial cell cultures and to attempt to reverse this change with retinoic acid. Primary cultures of human foreskin keratinocytes were prepared and exposed to the carcinogen, propane sultone. After each passage, a portion of cells were plated into medium containing increasing amounts of calcium. In a series of experiments it became evident that carcinogen exposed cells continued to grow in the presence of added calcium. Solvent control cell growth was decreased under such conditions. This new phenotype became apparent after the third subculture, but was pronounced after the fourth subculture. The addition of retinoic acid to the culture medium at each medium change reduced this effect and the keratinocytes grew more slowly, similar to control cells, in the presence of added calcium. The results suggest that carcinogen-exposed human keratinocytes acquire a resistance to calcium-induced differentiation or growth cessation and that retinoic acid can ameliorate this process. Although the mechanism of retinoic acid's inhibition remains unclear, these studies do provide a human cell model...
system which can be used to screen potential chemopreventive agents and for further mechanistic research.


A combined treatment of plasma or plasma derivatives by beta-propiolactone (beta-PL)/UV irradiation is in use at Biotest for the preparation of the virus-safe, stabilized serum (Biseko) and coagulation factor concentrates. The efficacy of this sterilization procedure has been demonstrated for HAV (greater than 8.2 log10), HBV (6.9 log10), NANBH (greater than 4.5 log10) and HIV (greater than 6.0 log10). The methods used in these studies (titration in chimpanzees or cell cultures) are not applicable in routine monitoring of sterilization processes. We therefore developed a test system using four types of bacteriophages: phi X174, phi e, Kappa and f2. Using these bacteriophages in 88 single tests, sterilization efficacy was regularly monitored during the period from 1981 to 1986. The four types of bacteriophages showed, on average, an inactivation rate of 6.7 log10, independent of size or genome structure. This inactivation is in the range of the inactivation of the relevant pathogenic virus, HBV, by beta-PL/UV. It was shown that under the production conditions of Intraglobin and the other Biotest immunoglobulin preparations, beta-PL (without UV) is as virucidal as the combination of beta-PL/UV in plasma or cryo-poor plasma.


Chromatographic separation of native DNA from DNA containing single-stranded regions has been used to determine the relative concentrations of structural intermediates generated during chemically induced DNA repair. Single doses of each of ten compounds were administered to rats. After periods ranging from 90 min to 13 days, hepatic DNA was isolated and analyzed by stepwise elution from benzyloated diethylaminoethyl cellulose with 1.0 M NaCl followed by caffeine solution. The compounds used were benzo(a)pyrene, carbon tetrachloride, diethylnitrosamine, dimethylnitrosamine, ethyl methanesulfonate, galactosamine, N-hydroxy-2-acetylaminofluorene, methyl methanesulfonate, nitrosomorpholine, and beta-propiolactone. Doses of the various agents and/or treatment times were restricted such that hepatic necrosis did not occur. No increase in the amount of caffeine-eluted DNA occurred after administration of carbon tetrachloride or galactosamine. All the remaining chemicals caused a dose-dependent
increase in the proportion of hepatic DNA eluted from benzoylated diethylaminoethyl cellulose with caffeine. In most cases, the varying times required to produce maximal increase in the proportion of caffeine-eluted DNA could be related to the rate of metabolism of the carcinogens. A distinction could be made according to whether repair intermediates were detected only within 24 hr of administration (ethyl methanesulfonate, methyl methanesulfonate, and beta-propiolactone) or were present for at least 3 days after treatment (diethylnitrosamine, dimethylnitrosamine, benzo(a)pyrene, N-hydroxy-2-acetylaminofluorene, and nitrosomorpholine). The data, considered with reference to previously ascribed modes of DNA repair for the respective adducts, suggest that base excision repair is immediately operative and rapidly completed in rat liver. However, reactions involved in the completion of nucleotide excision repair may be rate limiting, resulting in persistent structural damage to DNA. Implications of these findings for the use of benzoylated diethylaminoethyl cellulose chromatography as a carcinogen bioassay are considered.

This study was undertaken to determine the ability of a series of 19 compounds representing different chemical classes of carcinogens to induce lung tumors in strain A/J mice after either ip or po administration. Aflatoxin B1, dibutylnitrosamine, 1,2-dimethylhydrazine, and methylnitrosourea induced a significant increase in the lung tumor response in both sexes after ip and po administration. Azaserine was active in both sexes only after ip administration. Benzene, 1,2-dibromoethane, and epichlorohydrin, following ip administration, produced significant increases in the tumor response in at least one sex. Aflatoxin B1, azaserine, benzene, 1,2-dibromoethane, dibutylnitrosamine, and epichlorohydrin were more active when given ip than after po administration. In contrast, dimethylhydrazine and methylnitrosourea were more active (in females only) when given po. 2-Acetylamino fluorene, azobenzene, chloroform, 1,4-dioxane, FANFT (N-[4-(5-nitro-2-furyl)-2-thiazoyl]formamide), lead subacetate, methylmethanesulfonate, beta-naphthylamine, beta-propiolactone, safrole, and 2,4,6-tri-chlorophenol did not induce lung tumors in strain A/J mice. These data confirm previous observations on the importance of the route of chemical administration on the lung tumor response in strain A mice, and on the inability of the lung tumor bioassay to detect certain liver and bladder carcinogens and unstable alkylating agents.

**Sukeepaisarncharoen et al. 2001.** Long-term follow-up of HIV-1-infected Thai patients immunized with Remune monotherapy. *HIV.Clin.Trials.* Vol. 2(5): 391-398. PURPOSE: The purpose of this 2-year follow-up study was to investigate the long-term effect of Remune as monotherapy for HIV-1 infection. BACKGROUND: Participants previously enrolled in the phase II double-blind, randomized, adjuvant-controlled study of the HIV-1 Immunogen (Remune) were followed for 2 years. Open-label immunization with Remune monotherapy was given to each participant every 12 weeks. Remune, a gp120-depleted HIV-1 that was inactivated in beta-propiolactone and irradiation, was emulsified with mineral oil (incomplete Freund's adjuvant). METHOD: In Study 2101B, the effect of four doses of Remune given every 12 weeks over 40 weeks was compared to placebo in 297 asymptomatic type E HIV-infected patients [Churdboonchart et al., 2000].
A group of 17 volunteers were separated into a subset study and another 57 were excluded from analysis due to discontinuation or addition of other treatments. This 2-year follow-up study continued with open-label dosing of HIV-1 Immunogen every 12 weeks for the remaining 223 patients. Changes in CD4+ cells, CD8+ cells, and body weight were monitored at each patient visit. **RESULTS:** Overall, immunizations were safe; common adverse events were tolerable injection site reactions. CD4+ T-cell counts remained stable over the 132-week observation period for this cohort with a slight increase of 36.01 cells/microL. CD8+ T-cell counts showed an increase from baseline during the follow-up period (415.21 cells/microL). Furthermore, we also observed an increase in body weight from baseline (1.08 kg) at week 132. In addition, baseline CD4 count appeared to predict CD4 count at week 132 (slope = 0.31, p <.0001).

**CONCLUSION:** These results suggest that long-term treatment of HIV-1 infection with Remune monotherapy is safe and results in a stabilization of CD4+ counts. Furthermore, it is likely that HIV-1 therapeutic immunization may show its greatest clinical benefit in participants with higher CD4+ cell counts. Such an approach may have important ramifications in developing countries where access to antiviral drugs is limited and also in early chronic HIV-1 infection when CD4+ cells are still over 300 cells/microL in order to limit the cost and toxicity.


The resistance of Micrococcus radiodurans to the lethal and mutagenic action of ultraviolet (UV) light, ionising (gamma) radiation, mitomycin C (MTC), nitrous acid (NA), hydroxylamine (HA), N-methyl-N'-nitro-N-nitrosoguanidine (NG), ethylmethanesulphonate (EMS) and beta-propiolactone (betaPL) has been compared with that of Escherichia coli B/r. M. radiodurans was much more resistant than E. coli B/r to the lethal effects of UV light (by a factor of 33), gamma-radiation (55), NG (15) and NA (62), showed intermediate resistance to MTC (4) and HA (7), but was sensitive to EMS (1) and betaPL (2). M. radiodurans was very resistant to mutagens producing damage which can be repaired by a recombination system, indicating that it possesses an extremely efficient recombination repair mechanism. Both species were equally sensitive to mutation to trimethoprim resistance by NG, but M. radiodurans was more resistant than E. coli B/R to the other mutagens tests, being non-mutable by UV light, gamma-radiation, MTC and HA, and only slightly sensitive to mutation by NA, EMS and betaPL.

The resistance of M. radiodurans to mutation by UV-light, gamma-radiation and MTC is consistent with an hypothesis that recombination repair in M. radiodurans is accurate since these mutagens may depend on an “error-prone” recombination system for their mutagenic effect in E. coli B/r. However, because M. radiodurans is also resistant to mutagens such as HA and EMS, which are mutagenic in E. coli in the absence of an “error-prone” system, we propose that all the mutagens tested may have a common mode of action in E. coli B/r, but that this mutagenic pathway is missing in M. radiodurans.


The RD-114 virus rapidly induced syncytia in the human transformed cell lines, RSa,
RSb and IFr. Treatment of the virus with heat or ultrasonic vibration completely eliminated the syncytium-forming activity. Irradiation with u.v.-light or treatment with beta-propiolactone (BPL) reduced but did not completely destroy the activity. Pre-treatment of the cells for 16 h with 25 to 500 units/ml of human leucocyte interferon (Le-IF) or fibroblast interferon (F-IF) significantly reduced formation of syncytia by active virus or inactivated (u.v. or BPL) virus. This activity of interferon was inhibited by treatment of the cells with cycloheximide. Interferon did not increase the binding of 3H-uridine-labelled RD-114 virus to the cells. It is postulated that interferon treatment altered the plasma membrane of the cells and thus reduced their capacity to fuse.


The enhancement of the carcinogenicity of benzo(a)pyrene (B[a]P) and beta-propiolactone (BPL) by the mouse skin cocarcinogens phorbol myristate acetate (PMA) and catechol were examined in female SENCAR mice, 30 per group. The carcinogen and cocarcinogen were applied simultaneously, three times weekly for 490-560 days. B(a)P and BPL were used at constant doses of 5 and 50 micrograms, respectively, in all experiments. PMA was used at three doses, 2.5, 1.0, and 0.5 micrograms per application, and catechol was used at one dose, 2 mg per application. Control groups included animals that received carcinogen only, cocarcinogen only, acetone only, and no treatment. The carcinogenicity of B(a)P and BPL were enhanced by the cocarcinogens, particularly in terms of tumor multiplicity. For both carcinogens, the most marked cocarcinogenic effects were observed at the lowest dose of PMA used (0.5 microgram per application). This observation applied for days to first tumor, animals with tumors, tumor multiplicity, and incidence of malignant skin tumors. Catechol applied alone did not induce any tumors; with PMA alone there were significant incidences of benign and malignant tumors, e.g., at a dose of only 0.5 microgram per application, 15 of 30 animals had 28 tumors, 5 of which were squamous carcinomas. In two-stage carcinogenesis experiments with 7,12-dimethylbenz(a)anthracene (DMBA) as initiator and PMA as promoter, SENCAR mice showed a greater susceptibility to tumor induction when compared to ICR/Ha mice used in earlier work. This susceptibility was most notable in terms of rate of tumor appearance and tumor multiplicity.


Ten patients developing a serum sickness-like hypersensitivity reaction to human diploid cell rabies vaccine were studied and compared with control subjects matched for previous vaccination history and level of rabies virus-specific IgG response to immunization. The clinical reaction consisted of delayed onset, generalized urticaria, and angioedema, with some arthralgias. Skin biopsy specimens demonstrated a leukocytoclastic vasculitis. Individuals reacting to the vaccine possessed IgE antibodies to human diploid cell rabies vaccine, to mock vaccine lacking viral antigen, and to fetal calf serum (FCS), a vaccine trace contaminant. Increased levels of IgG antibodies to FCS, mock vaccine, and beta-propiolactone-modified FCS, and human serum albumin were also found. Such humoral
responses to vaccine components other than rabies virus might be responsible for the hypersensitivity reactions developing after rabies vaccination.

Previously, p-methoxyphenol fed in the diet was found to be the most potent inhibitor of benzo(a)pyrene-induced neoplasia of the mouse forestomach of 18 phenols investigated. In the present study, the effects of p-methoxyphenol on the direct-acting carcinogen, beta-propiolactone (BPL), were determined. p-Methoxyphenol administered at 1 or 4 hr prior to BPL or fed in the diet markedly inhibited BPL-induced neoplasia of the mouse forestomach. Of 10 phenols tested by p.o. intubation, it was the only one that exerted a significant inhibitory activity. Thus far, p-methoxyphenol appears to be an effective inhibitor only when given prior to carcinogen administration. During these studies, it was found that the nature of the diet markedly altered the neoplastic response of the mouse forestomach to BPL but not to benzo(a)pyrene.

Studies have been initiated to find compounds that can trap direct-acting carcinogens within the stomach. Sodium thiosulfate (STS) is a potent nucleophile and in initial experiments was found to inhibit mutagenesis resulting from exposure of Salmonella typhimurium strain TA100 to the direct-acting carcinogens beta-propiolactone and styrene oxide. In in vitro experiments STS was shown to maintain its nucleophilicity in the acid pH range. It reacted with beta-propiolactone as rapidly at pH 2 as at pH 7.4. Thus STS has the prerequisite attributes to inhibit the carcinogenic effects of electrophiles in the stomach. Experiments were performed in which STS was administered by p.o. intubation to female A/J mice 5 min before p.o. administration of beta-propiolactone. Under these conditions, inhibition of formation of the forestomach tumors occurred. The data obtained suggest that use of nucleophiles to protect against direct-acting carcinogens is a potential strategy for chemoprevention.

Mice exposed to live or beta-propiolactone-inactivated rabies virus generated a strong, specific cell-mediated cytotoxic response which was generally maximal 6 days after inoculation. Release of 51Cr was apparently a function of immune thymus-derived lymphocytes (T cells) because it was abrogated by prior incubation of spleen cells with anti-thymus antisera and complement but was undiminished by passage of spleen cells through nylon-wool columns. Cytotoxicity was always maximal for interactions in which thymus-derived cells and targets shared H-2 genes but, unlike the situation found in other assays of this type, considerable lysis of allogeneic, virus-infected target cells may also occur. Perhaps the most significant finding from these experiments is that an inactivated virus has been shown to stimulate a potent cytotoxic thymus-derived cell response. Manipulation of this experimental model may allow analysis of the antigens required for stimulation of cell-mediated immunity. A more practical consequence may be the development of more rational protocols for postexposure vaccination against rabies.
treatment of mice with antirabies antibody severely depressed the generation of cell-mediated immunity.


As part of the validation phase of the Drosophila melanogaster segment of the National Toxicology Program, a comparison has been made of positive and negative controls for sex-linked recessive lethal mutations and reciprocal translocations from three laboratories. This comparison involves approximately 700,000 spontaneous recessive lethal mutation tests, 70,000 spontaneous translocation tests, and screens for genetic damage induced by N-nitrosodimethylamine and beta-propiolactone. Spontaneous frequencies for lethal mutations and translocations were homogeneous in the laboratories regardless of solvent or broods sampled. Inhomogeneity was observed in induced frequencies among laboratories, but the variation was no greater than that found within a laboratory.


Using a two-step carcinogenesis protocol, SENCAR mice were initiated with 25 micrograms 7,12-dimethylbenz[a]anthracene (DMBA) and were then treated twice weekly with either (a) 0.5 mg beta-propiolactone (BPL) or (b) 1 microgram fluocinolone acetonide (FA) followed in 30 min by 0.5 mg BPL. The tumor incidence for the group receiving FA prior to BPL was significantly greater than for BPL alone (P less than 0.0005). Under these experimental conditions, BPL alone showed neither promoting activity nor complete carcinogenic activity. These results were not anticipated, but the reasons for their occurrence are being explored.


Agents of transmissible spongiform encephalopathy (prion) are known to be extremely resistant to physicochemical inactivation procedures such as heat, radiation, chemical disinfectants such as detergents, alcohols, glutaraldehyde, formalin, and so on. Because of its remarkable resistance, it is difficult to inactivate prion. Chemical inactivation seems to be a practical method because it is applicable to large or fixed surfaces and complicated equipment. Here, three epoxides: beta-propiolactone, propylene oxide, and glycidol (GLD) were examined of their inactivation ability against scrapie-mouse prion protein (PrP(Sc)) under various conditions of chemical concentration, incubation time, and temperature. Among these chemicals, GLD worked most effectively and degraded PrP into small fragments. As a result of the bioassay, treatment with 3% GLD for 5 hr
and 5% GLD for 2, 5 hr or 12 hr at room temperature prolonged the mean incubation time by 44, 30, 110 and 73 days, respectively. From dose-incubation time standard curve, the decrease in infectivity titers were estimated as 10(3) or more. Therefore, degradation of PrP(Sc) by GLD decreased the scrapie infectivity. It is also suggested that pH and salt concentrations influence the effect of GLD. Although further study is necessary to determine the optimal condition, GLD may be a potential prion disinfectant.

Yendle et al. 1997. The genetic toxicity of time: importance of DNA-unwinding time to the outcome of single-cell gel electrophoresis assays. Mutat. Res. Vol. 375(2): 125-136. Single-cell gel electrophoresis assays (comet assays) are described in which DNA damage is assessed in mouse skin keratinocytes treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and beta-propiolactone (BPL) either in vitro or in vivo. The positive results observed under both conditions of test encourage the further development of the mouse skin comet assay as a screen for direct-acting in vivo genotoxins. From the outset of the present experiments we were struck by the compacted nature of the DNA in mouse skin keratinocytes. Under similar conditions of assay, rodent hepatocytes presented a uniform 'unwound' distribution of DNA over the whole nuclear region. In order to study this effect we varied what seemed to be the most obviously related assay parameter: the DNA-unwinding time. A series of experiments was conducted in which control and MNNG-treated cells were exposed to a range of alkaline DNA-unwinding times (0.3-18 h) followed by measurement of the three comet tail parameters (length, DNA content, and their product, tail moment). Each of these parameters increased with increasing time of unwinding such that the tails observed for MNNG-treated cells with 0.3 h of DNA unwinding were similar in length to the tails of control cells exposed to an 8 h DNA-unwinding time. It is concluded that DNA-unwinding time is a critical parameter of the comet assay and that it may require optimisation for each tissue/cell type studied. Further, the data alert to the prospect that agents that uniquely affect chromosomal protein superstructure may increase comet tail length/DNA content in the absence of chemically induced DNA damage. Thus, there may be two discrete classes of chemical interaction with chromosomal DNA that yield identical comet assay results, but which have different implications for the genetic toxicity of the test agent. Similar effects were observed for rat hepatocytes or mouse lymphoma cells exposed to an 18 h DNA-unwinding time, but no comet tails were produced by exposure of cells to the lysis conditions (pH 10.0) for 18 h.

Yu et al. 1990. ADPRT-mediated decrease of cellular NAD content and the detection of chemically induced DNA damage--development of a new short-term screening test for mutagens. Proc. Chin. Acad. Med. Sci. Peking Union Med. Coll. Vol. 5(1): 19-24. It was found that the DNA-damaging agents N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methyl-methanesulphonate (MMS) and 4-nitroquinoline-N-oxide (4NQO) could stimulate ADP-ribosyl transferase (ADPRT) activity and reduce the cellular NAD content in a dose-dependent way. The reduction of NAD after DNA damage could be partially or completely prevented by ADPRT inhibitors, 3-aminobenzamide or nicotinamide, which showed no influence on reduction of NAD induced by metabolic blocking agents. Therefore, a simple and specific method to detect DNA-damaging mutagens by measuring ADPRT-mediated decrease of cellular NAD content was
explored. Using beta-naphthoflavone, a mixed function oxygenase inducer, together with induced or uninduced human amnion FL cells, it was found that aflatoxin B1, benzo(a)pyrene, 2-acetylaminofluorene, 9,10-dimethylanthracene and ethylcarbamate could induce the ADPRT-mediated decrease of cellular NAD content, while 4-acetylaminofluorene, anthracene, isopropyl-N-(3-chlorophenyl)-carbamate, beta-propiolactone, gamma-butyrolactone, cyclophosphamide and safrol could not. The results indicate that this is a cheap and specific method to detect DNA damage caused by chemical carcinogens/mutagens with a specificity approaching that of the unscheduled DNA synthesis assay.

Zakova et al. 1985. Evaluation of skin carcinogenicity of technical 2,2-bis-(p-glycidyloxyphenyl)-propane in CF1 mice. Food Chem.Toxicol. Vol. 23(12): 1081-1089. The carcinogenic potential of a technical-grade epoxy resin, Araldite GY 250, of which the diglycidyl ether of bisphenol A (DGEBA) is the main component, was investigated in CF1 mice. Groups of 50 male and 50 female mice were treated for 2 yr by repeated epidermal application of a 1 or 10% (v/v) solution in acetone. The controls, 50 mice of each sex, were treated with acetone alone. The treatment had no effect on survival and no excess incidence of skin tumours occurred. A positive control group of 50 male and 50 female CF1 mice was treated by epidermal application of 2% (v/v) beta-propiolactone in acetone. In this group there was a high incidence of malignant skin tumours at the site of application and, consequently, increased mortality. Treatment with neither DGEBA technical nor beta-propiolactone induced systemic neoplasia.