

The Royal Society of Medicine
International Congress & Symposium
Series Number 47

**INTERNATIONAL WORKSHOP
ON (+) - CYANIDANOL - 3 IN
DISEASES OF THE LIVER**

HAROLD O. CONN MD. Editor

Royal Society of Medicine
International Congress and Symposium Series

Number 47

International Workshop on (+)-Cyanidanol-3 in Diseases of the Liver

Editor **HAROLD O. CONN MD**

Administrative Editor **CLIVE WOOD**

1981

Published jointly by

THE ROYAL SOCIETY OF MEDICINE
1 Wimpole Street, London

ACADEMIC PRESS
London, Toronto, Sydney

GRUNE & STRATTON
New York, San Francisco



ROYAL SOCIETY OF MEDICINE
1 Wimpole Street, London W1M 8AE

ACADEMIC PRESS INC. (LONDON) LTD.

24/28 Oval Road, London NW1 7DX

United States Edition published and distributed by
GRUNE & STRATTON INC.

111 Fifth Avenue, New York, New York 10013

Copyright © 1981 by

ROYAL SOCIETY OF MEDICINE and
ACADEMIC PRESS INC. (LONDON) LTD.

All rights reserved. No part of this book may be reproduced in any form by photostat microfilm, or any other means, without written permission from the publishers. *

This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any fair dealing under the UK Copyright Act 1956, Part 1, Section 7, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the Honorary Editors, Royal Society of Medicine.

The sponsors of this Symposium are responsible for both the scientific and literary content of this publication. The Royal Society of Medicine has only sought to assure itself of the medical and scientific reputability of the work reported. The views expressed are not necessarily those of the Society. Distribution has been in accordance with the wishes of the sponsors but a copy is available free of charge to any Fellow of the Society.

ISBN (Academic Press): 0-12-790898-6

ISBN (Grune & Stratton): 0-8089-1433-2

British Cataloguing in Publication Data

International Workshop on (+)-Cyanidanol-3 in
Diseases of the Liver (1981: Crans-Montana)
International Workshop on (+)-Cyanidanol-3
in Diseases of the Liver.—(International congress
and symposium series/Royal Society of Medicine,
ISSN 0142-2367; no. 47)

1. Liver—Diseases—Chemotherapy

I. Conn, H. O. II. Wood, C. III. Series

616.3'62061 RC846

Filmset by Northumberland Press Ltd, Gateshead, Tyne and Wear
Printed in Great Britain by Fletcher & Son Ltd, Norwich

Contents

Chairman's Introduction

| | |
|----------------------|---|
| H. O. CONN | 1 |
|----------------------|---|

PHARMACOLOGY

Moderator: C. D. KLAASSEN

State of the Art: Pharmacological and toxicological evaluation of a new therapeutic agent

| | |
|--------------------------|---|
| C. D. KLAASSEN | 3 |
|--------------------------|---|

Biochemical, pharmacological and toxicological evaluation of (+)-cyanidanol-3

| | |
|-----------------------|---|
| G. HENNINGS | 7 |
|-----------------------|---|

State of the Art: Free radical scavengers

| | |
|------------------------|----|
| T. F. SLATER | 11 |
|------------------------|----|

(+)-Cyanidanol-3 inhibition of lipid peroxidation induced by hepatotoxic chemicals in the rat

| | |
|---------------------|----|
| H. KAPPUS | 17 |
|---------------------|----|

Antihepatotoxic activity of (+)-cyanidanol-3 in acute and chronic experimental liver injury

| | |
|-------------------------|----|
| C. P. SIEGERS | 19 |
|-------------------------|----|

A morphological and biochemical study of the interaction of (+)-cyanidanol-3 with the plasma membrane and protection against phalloidin and CCl₄ toxicity in isolated rat hepatocytes

| | |
|--|----|
| D. PERRISSOUD, M. F. MAIGNAN and G. AUDERSET | 21 |
|--|----|

Interactions of (+)-cyanidanol-3 with the hepatic drug metabolizing system (abstract)

C. STEFFEN, R. BRAUN and A. SCHMOLDT 27

An ultrastructural morphometric investigation in rat and Syrian hamster liver after treatment with toxic agents and following application of (+)-cyanidanol-3

H. THEMANN, R. MEISS and K. OPITZ 29

(+)-Cyanidanol-3 and acute ethanol ingestion in the rat

L. A. VIDELA, V. FERNANDEZ, A. VALENZUELA and G. UGARTE 31

The free radical scavenging action of (+)-cyanidanol-3 in relation to the toxicity of carbon tetrachloride

T. F. SLATER and R. SCOTT. 33

CLINICAL PHARMACOLOGY

Moderator: R. PREISIG

State of the Art: Problems in phase I and phase II trials with "hepatoprotective" drugs

R. PREISIG 41

Clinical pharmacology of (+)-cyanidanol-3: a synopsis with emphasis on pharmacokinetics

L. BALANT 49

State of the Art: Drug metabolism in liver disease: an overview of basic pharmacokinetic principles and clinical investigations

R. V. PATWARDHAN and S. SCHENKER 55

State of the Art: Assessment of enzyme induction in man

D. D. BREIMER 61

State of the Art: Dilemmas in the design and execution of clinical trials

N. TYGSTRUP 67

State of the Art: The statistical anatomy of controlled clinical trials

D. L. SACKETT 69

(+)-CYANIDANOL-3 IN ACUTE HEPATITIS

Moderators: A. J. ZUCKERMAN and J. H. HOOFNAGLE

State of the Art: Therapy of acute viral hepatitis

J. H. HOOFNAGLE 77

**Treatment of acute viral hepatitis with (+)-cyanidanol-3
(published summary)**A. L. BLUM, W. DOELLE, K. KORTÜM, P. PETER, G. STROHMEYER, P.
BERTHET, H. GOEBELL, S. PELLONÍ, H. POULSEN and N. TYGSTRUP . . . 81**Influence of (+)-cyanidanol-3 on the course of acute viral
hepatitis (abstract)**W. WELLMANN, I. VIDO, F. W. SCHMIDT, R. MÜLLER, U. RANFT, E. WILD-
HIRT, E. HOLZER, H. WALLNÖFER and G. KORB 83**(+)-Cyanidanol-3 in acute viral hepatitis**H. SCHOMERUS, K. H. WIEDMANN, W. DÖLLE, H. PEERENBOOM, G. STROH-
MEYER, K. BALZER, H. GOEBELL, G. DÜRR, C. BODE, A. L. BLUM, G.
FRÖSNER, W. GERLICH, P. A. BERG and K. DIETZ 85**Effect of (+)-cyanidanol-3 in acute viral hepatitis**

G. THEODOROPOULOS, A. DINOS, P. DIMITRIOU and A. ARCHIMANDRITIS . . . 89

**The treatment of hepatitis B and other liver diseases with
(+)-cyanidanol-3**B. KAFKIAS, A. PAPACHRISTOU, M. HATZIDIMOU-MOULA, J. ZACHOS,
G. KAMBOURAKIS and S. FALTSIS 93**Effect of (+)-cyanidanol-3 in acute A, B and nA-nB
hepatitis**M. PIAZZA, V. GUADAGNINO, L. PICCIOTTO, R. DE MERCATO, A. CHIRIANNI,
R. ORLANDO and G. GOLDEN 97**(+)-Cyanidanol-3 in acute hepatitis (abstract)**

F. BEGEMANN and H. G. DAMMANN 101

State of the Art: Serological diagnosis of hepatitis Type B

A. J. ZUCKERMAN 103

(+)-Cyanidanol-3 in prolonged viral hepatitis

F. DI NOLA and B. SALASSA 107

Influence of (+)-cyanidanol-3 on the leucocyte migration inhibition test carried out in the presence of PPD and HBsAg (published abstract)

J. J. VALLOTTON and P. C. FREI 109

Influence of (+)-cyanidanol-3 on human lymphocyte proliferation *in vitro*

J. P. DESPONT and R. CORNILLE BRÖGGER 111

***In vitro* effect of (+)-cyanidanol-3 on rosette formation**

J. SIPOS, V. GÁBOR, Z. TÓTH, K. BARTÓK and P. RIBICZEY 113

(+)-CYANIDANOL-3 IN CHRONIC HEPATITIS

Moderator: H. J. ZIMMERMAN

State of the Art: The treatment of chronic hepatitis

N. TYGSTRUP 117

Effect of (+)-cyanidanol-3 on chronic persistent and chronic active hepatitisM. PIAZZA, R. DE MERCATO, V. GUADAGNINO, L. PICCIOTTO, N. ABRESIA,
A. CHIRIANNI and G. GOLDEN 123**Treatment of polyphasic hepatitis with (+)-cyanidanol-3**

C. LAVERDANT 131

Study of (+)-cyanidanol-3 in chronic active hepatitis. Results of a controlled multicentre studyF. DEMEULENAERE, V. J. DESMET, E. DUPONT, R. FIASSE, H. GISSEL-
BRECHT, F. HEULLY, R. JEANPIERRE, J. LECOMTE, G. LENNES, C. MACINOT,
P. MIGEOTTE, J. PIROTTE, G. RAUBER, L. RUYTERS and H. VAN CAUWEN-
BERGE 135**Immunological properties of (+)-cyanidanol-3 (abstract)**

S. YAMAMOTO, H. SUZUKI and T. ODA 143

Effect of (+)-cyanidanol-3 on the production and release of HBsAg in a hepatoma cell line

G. G. FRÖSNER, V. GAUSS-MÜLLER and F. DEINHARDT 145

(+)-CYANIDANOL-3 IN ALCOHOL INDUCED LIVER DISEASE

Moderator: N. TYGSTRUP

State of the Art: The management of alcoholic hepatitis

H. O. CONN 147

Treatment of alcohol related liver disease with (+)-cyanidanol-3: a three month randomized double-blind trial

M. Y. MORGAN, J. C. COLMAN, P. J. SCHEUER and S. SHERLOCK 157

Treatment of alcoholic cirrhosis with (+)-cyanidanol-3

S. PALMAS 167

(+)-Cyanidanol-3 in mild alcoholic hepatitis

J. M. SÁNCHEZ-TAPIAS, B. FIOL and J. RODÉS 173

Influence of (+)-cyanidanol-3 on chronic alcoholic liver injury

H. HENNING 177

Effect of (+)-cyanidanol-3 on alcoholic liver disease with and without overt hepatic failure

G. UGARTE, D. BUNOUT and H. ITURRIAGA 181

The effect of (+)-cyanidanol-3 on alcoholic fatty liver in the rat

P. R. RYLE, J. CHAKRABORTY, G. K. SHAW and A. D. THOMSON 185

Studies on lymphocyte subpopulations and on the receptors of hepatocytes in alcoholic liver disease

J. SIPOS, V. GÁBOR, Z. TÓTH, K. BARTÓK and P. RIBICZEY 195

(+)-CYANIDANOL-3 IN THE PREVENTION OF DRUG INDUCED HEPATOTOXICITY AND IN THE INDUCTION OF TOXICITY

Moderator: S. SCHENKER

State of the Art: Drug induced liver disease

H. J. ZIMMERMAN 201

Influence of (+)-cyanidanol-3 on liver damage during tuberculostatic therapy

G. SIEMON 217

Tuberculosis therapy, liver damage and (+)-cyanidanol-3: a prospective controlled study (abstract)

M. KNOBLAUCH, F. SUTER, B. SCHNEIDER, O. BRANDLI and J. GARTMANN 221

(+)-Cyanidanol-3 induced immune haemolytic anaemia

K. A. NEFTEL, H. JAPP, P. DIEM and M. WÄLTI 223

(+)-Cyanidanol-3 induced fever and its pathogenesis

N. BRATTIG, G. J. DIAO and P. A. BERG 227

MODERATORS' SUMMARIES

Moderator: D. L. SACKETT

Pharmacology of (+)-cyanidanol-3

C. D. KLAASSEN 235

Clinical pharmacology of (+)-cyanidanol-3

R. PREISIG 237

(+)-Cyanidanol-3 in acute viral hepatitis

A. J. ZUCKERMAN 239

Effect of (+)-cyanidanol-3 on clearance of HBsAg

J. H. HOOFNAGLE 241

(+)-Cyanidanol-3 in chronic hepatitis

H. J. ZIMMERMAN 245

(+)-Cyanidanol-3 in alcohol induced liver disease

N. TYGSTRUP 247

(+)-Cyanidanol-3 in toxic injury to the liver

S. SCHENKER 249

**Assessment of controlled clinical trials with
(+)-cyanidanol-3**

D. L. SACKETT 251

**Chairman's concluding comments: whither
(+)-cyanidanol-3?**

H. O. CONN 253

Bibliography of publications on (+)-cyanidanol-3 263



Chairman's Introduction

H. O. CONN

*Veterans Administration Medical Center, West Haven,
and Yale University School of Medicine, New Haven,
Connecticut, USA*

"The truth is more important than the facts."

Frank Lloyd Wright

Several years ago I was one of a group of hepatologists who was introduced to and asked to evaluate (+)-cyanidanol-3 (Catergen®), which has now officially been given the international non-proprietary name, *cianidanol*. At the time only a few clinical papers describing (+)-cyanidanol-3 in acute viral hepatitis and several basic pharmacological papers had been published. The panel of physicians of which I was a member was not overly impressed with these meagre data, and recommended that additional studies of the mechanism of action be obtained and that additional controlled clinical trials be performed.

About the end of 1979, Dr Gerard Golden of Zyma SA told me that a number of these controlled clinical and basic investigations had been completed or were in progress, and invited me to serve as chairman of a workshop to assess (+)-cyanidanol-3 in hepatic disorders. I agreed with certain provisions: firstly, that I be free to appoint a select committee of experienced investigators of stature in the profession and of impeccable integrity—in current sporting parlance they could be called "world class"; secondly, that my committee and I would be free to design the format and select the programme; thirdly, that all investigators who had studied this agent would be invited to submit abstracts from which the programme would be selected; fourthly, that the proceedings of the workshop would be published ("win, lose or draw", I believe were my exact words).

Without hesitation, Dr Golden replied: "Of course". It was a response that might well send a gambling man directly to his stockbroker. During the preparation for the workshop, Dr Golden and

his associate, M. Michel Yosbergue have been extremely supportive in all professional aspects of his programme and were fully in charge of all administrative aspects.

Each member of the select committee, none of whom had any special knowledge of or previous involvement with (+)-cyanidanol-3, was selected to moderate one session and to present a State of the Art lecture in an area of his expertise. They are listed in order of the sessions they moderated:

| | |
|-------------------------------|--|
| <i>Pharmacology</i> | Professor Curtis D. Klaassen of the University of Kansas School of Medicine. |
| <i>Clinical pharmacology</i> | Professor Rudolf Preisig of the University of Berne. |
| <i>Acute viral hepatitis</i> | Professor Arie J. Zuckerman of the University of London. Professor Jay H. Hoofnagle of the National Institutes of Health. |
| <i>Chronic hepatitis</i> | Professor Hyman J. Zimmerman of the George Washington School of Medicine. |
| <i>Alcoholic hepatitis</i> | Professor Niels Tygstrup of the University of Copenhagen. |
| <i>Drug induced hepatitis</i> | Professor Steven Schenker of the Veterans Administration and Vanderbilt University School of Medicine. |
| <i>Summary session</i> | Professor David Sackett of McMaster University, Ontario. |

The format of the symposium was designed as follows. Each session was initiated by a State of the Art presentation by one of the committee members. In the pharmacology sessions, it outlined the evaluation of a new therapeutic agent, focusing on those used in the treatment of liver diseases. In each

Conn, H. O., Ed., (+)-Cyanidanol-3 in Diseases of the Liver, International Workshop (Royal Society of Medicine International Congress and Symposium Series No. 47); Crans-Montana, Switzerland 1981, 284 pages; published jointly by Academic Press Inc. (London) Ltd and the Royal Society of Medicine.

clinical session the State of the Art presentation served as a yardstick against which the effects of (+)-cyanidanol-3 could be compared in the disorder under discussion. The remainder of each session consisted of shorter presentations selected from submitted abstracts. Selections were made exclusively on the basis of the excellence and objectivity of the studies. In some of the papers selected only the design of the study was known; the results of these investigations were not available at the time of selection. No controlled, randomized and double-blind study was excluded. Most presenters were allotted 10 minutes. Previously published papers were assigned 5 minutes, except when additional new information was to be presented. Discussion periods were scheduled after every group of papers.

Our goals in publishing the proceedings are to indicate the status of pharmacological and clinical knowledge about (+)-cyanidanol-3 at the time of the workshop, to permit comparison of (+)-cyanidanol-3 as a therapeutic agent with other modes of treatment in current use and to collect in one place all of the available information about

(+)-cyanidanol-3. To this end we have included a complete bibliography of all papers on the agent published including those submitted to or presented at the workshop. We hope therefore that this volume will serve as the definitive reference source about (+)-cyanidanol-3 for the foreseeable future.

Because the programme was so busy—12 State of the Art lectures, 33 scientific presentations and 8 summations—a tight schedule was necessary. All speakers regardless of colour, creed, national origin or their own assessment of the importance of their data were requested to adhere to the time assignments. To assist the speakers, M. Bernard Chevallier was assigned the thankless task of time-keeper, which he performed with aplomb and precision based on his long musical experience as a conductor, employing the clear, unignorable tones of a Swiss cowbell. Never have so many said so much so punctually.

We hope that this volume provides everything the reader wanted to know about liver disease and (+)-cyanidanol-3 but didn't know whom to ask.



State of the Art

Pharmacological and Toxicological Evaluation of a New Therapeutic Agent

C. D. KLAASSEN

*Department of Pharmacology, University of Kansas
Medical Center, Kansas City, USA*

The history of drug regulation reflects the growing involvement of governments of most countries to assure the highest degree of efficacy and safety in marketed medicinal agents. The first act in the USA, the Federal Pure Food and Drug Act of 1906, was concerned only with the purity of drugs. There were no restrictions on drug sales nor any obligations to establish efficacy and safety. However, few new drugs were marketed between 1908 and the advent of the sulphonamides in the mid-1930s. The Federal Act was amended in 1938, following a series of deaths that resulted from the marketing of a solution of sulphanilamide in diethylene glycol, an excellent but highly toxic solvent. The amended act, which is enforced by the Food and Drug Administration (FDA), was primarily concerned with the labelling and safety of drugs. Toxicity studies were required, as well as approval of a new drug application (NDA), before a drug could be promoted and distributed. However, no proof of efficacy was required. Drugs could go from the laboratory to clinical testing without approval from the FDA.

Research in basic and clinical pharmacology flourished during this era, resulting in the introduction of many new drugs. The risk-to-benefit ratio was seldom mentioned, but it emerged in a dramatic fashion early in the 1960s. At that time thalidomide, an hypnotic agent, was introduced in Europe. The incidence of a relatively rare birth defect, phocomelia, increased and soon reached epidemic proportions. Retrospective epidemiological research firmly established the causative agent to be thalidomide, taken early in the course of pregnancy. The reaction to the dramatic demonstration of the teratogenicity of thalidomide was worldwide. In the USA it resulted, in 1962, in the

Harris-Kefauver Amendment to the Federal Pure Food and Drug Act.

The Harris-Kefauver Amendment is sound legislation. It requires extensive pharmacological and toxicological research before a drug can be tested in man. The data from such studies must be submitted as an Investigational New Drug (IND) application and approved by the FDA before clinical studies can begin. Proof of efficacy and documentation of relative safety in terms of the risk-to-benefit ratio for the disease to be treated are both required. Three extensive phases of clinical testing must be completed before a new drug application (NDA) can be submitted.

Any new drug, therefore, must be submitted to a set of animal tests before administration to man. While there are no absolute requirements in the USA as to which tests are necessary before a drug can be marketed, general guidelines have developed. Information generally deemed necessary before a chemical substance can be administered to man includes data on chemical and physical properties, deposition and pharmacokinetic behaviour and pharmacological and toxicological activity.

The various chemical and physical data relating to a drug should be known before it is given to man, or even to animals. The chemical structure is extremely important because the experienced toxicologist might predict the toxic effects of the drug by comparing it to compounds with similar structures and functional groups for which the toxicology and pharmacology are known. Drug purity is also important. Whilst it is ideal to test and sell the pure chemical, this is not always feasible. If the chemical is not pure, the identity and composition of the impurities should be deter-

mined. The toxicity tests should be performed with the same purity of substance as that sold to the consumer. The stability of the drug should also be known, including its stability in the diet to be administered, before subchronic and chronic experiments are performed. Stability at various pH values, especially at low pH like those found in the stomach, to which the chemical is exposed, after oral administration and in feeding studies should be determined. The dissociation constant and the organic solvent-water partition coefficient of the drug should be known. These parameters will give the toxicologist some idea of the absorption and distribution of the substance, and of the effect of urinary pH on its excretion.

Information on the disposition and pharmacokinetics of a drug are invaluable in evaluating its safety. Data on absorption of the drug from all clinically anticipated routes of administration are desirable. Distribution to various tissues is often determined by using the radioactively labelled drug. The extent of binding to plasma proteins is important because significant drug interactions may occur with other agents that are also bound to these proteins. The identities of major biotransformation products are also highly relevant as are the major pathways of excretion. The half-life of the drug in the blood is important in determining dosage schedules and whether it is likely to be cumulative. Knowledge of the chemical agent's effects on drug metabolizing enzymes would again help to predict potential drug interactions.

The pharmacological properties of the new substance, in addition to those effects for which it is to be marketed, should be delineated. At the least, its action on the cardiovascular system, the influence of the drug on the electrocardiogram, heart rate, blood pressure and regulatory mechanisms should be delineated. Its effects on the sympathetic and cholinergic branches of the autonomic nervous system and its interaction with histamine and the histaminergic receptors should also be examined. Central nervous system effects are extremely important. It should be determined whether the drug produces sedation and/or ataxia, whether it has convulsant, anticonvulsant, and/or analgesic properties and the dose required to produce these effects. In studying any new drug's influence on renal function, its effect on urine production, urine osmolality and sodium excretion are commonly measured.

The first toxicity test performed on a new chemical substance is determination of the LD_{50} by two routes of administration (usually oral and i.v., one being the intended route of exposure) in several species. Those most often used are the mouse, rat, rabbit and dog. In the mouse and rat the LD_{50} is usually determined using considerable

numbers of animals, but in larger species only an approximation of the LD_{50} is obtained by increasing the dose until serious toxic effects are demonstrated. The number of animals that die in a 7 or 14 day period after a single dose is tabulated. In addition to mortality, periodic examination of test animals should be conducted for signs of intoxication, lethargy, behavioural modifications or morbidity.

The ability of a chemical substance to irritate the skin and eyes after acute exposure is usually determined in the rabbit. For the dermal irritation test, rabbits are prepared by removal of the fur on a section of their backs. The substance is applied to the skin under a covered patch and usually kept in contact for a period of either 4 or 24 h. The degree of skin irritation is scored for erythema and eschar formation, presence of oedema and corrosive action. These dermal irritation observations are repeated at various intervals after the covered patch is removed. To determine ocular irritation, the agent is instilled into one eye of each rabbit. Both eyes are then examined at various times after application.

Toxicity after subchronic exposure is then determined. Subchronic exposure tests may last for different periods of time, but 90 days is the most common test duration. The subchronic study is usually performed in two species (rat and dog) by the route of intended use or exposure. At least three doses are employed: a high dose which produces marked toxicity, a low dose which produces no toxic effects and an intermediate dose. Usually, 15 rats of each sex and four dogs of each sex are used per dose.

Observations on these test animals include mortality, body weight changes, dietary consumption, haematology and clinical chemistry. Haematology measurements usually include haemoglobin concentration, haematocrit, erythrocyte counts, leucocyte counts (including differential counts), clotting and prothrombin times. Clinical chemistry determinations commonly made include glucose, urea nitrogen, glutamic-pyruvic transaminase, alkaline phosphatase, creatinine, bilirubin, triglyceride and cholesterol. At the end of the experiment, the gross and microscopic condition of the organs and tissues (from 15–20) and the weight of various organs (about 12) are recorded and evaluated. Subchronic toxicity studies characterize the dose-response relationship of a test substance following repeated administration and also provide data for a more reasonable prediction of appropriate doses for the chronic exposure studies.

After completion of acute and subchronic studies and any special studies that might be required because of the known toxicity of the class of agents, one may file an Investigational New Drug or IND

to the FDA and, if approved, clinical trials can commence. At the same time that Phase I, Phase II and Phase III clinical trials are being performed, chronic exposure to the test compound can be performed in laboratory animals as well as any additional, specialized tests.

Long-term or chronic exposure studies are performed in a way similar to subchronic studies, except that the period of exposure is longer, and is somewhat dependent on the intended period of exposure in man. If the drug is planned to be used for short periods of time, as is the case with an antimicrobial agent, a chronic exposure of 6 months might be sufficient. However, if the drug will be used in man for a much longer duration, then a chronic study of 1.5–2 years is likely to be required.

Chronic exposure studies are often used to determine the carcinogenic potential of chemical substances. Such studies are usually performed in rats and mice and extend over the average lifetime of the species. Thus, the exposure time for a rat is 2 years. To assure that 30 rats per dose survive the 2 year period, 60 rats per group per sex are often started in the study. Gross and microscopic pathological examinations are made on those animals that survive the chronic exposure and also on those that die early.

The teratogenic potential of chemicals is also determined in laboratory animals. Teratology is defined as the study of malformations induced during development from conception to birth. Teratogenic studies are usually performed in rats and rabbits with two doses of the test chemical (both of which produce no maternal toxicity). Teratogens are most effective when administered during the first trimester, the period of organogenesis. Thus the animals are usually exposed on days 6–15 of gestation and the foetuses removed by Caesarean section prior to the estimated time of delivery.

The uterus is examined for the number of implantations and dead and living foetuses are counted, weighed and examined grossly. Some foetuses are examined histologically and others are stained for skeletal abnormalities. Since teratogens can produce functional as well as morphological changes, functional changes in the offspring, such as changes in behaviour, are sometimes monitored at various times after delivery.

Fertility and reproductive toxicity studies are usually performed in rats at dosage levels similar to those used for the teratology investigations. In a typical reproductive study the male parent is given the agent for 60–80 days and the female for 14 days prior to mating. The percentage of animals that become pregnant is determined, together with the number of still-born and live offspring, their weight, growth, survival and general condition

during the first 3 weeks of life. The perinatal and postnatal toxicities of chemical agents are also often examined by administering the test agent to the rat from day 15 of gestation throughout delivery and lactation and determining its effect on birth rate, survival and growth of the offspring.

Investigation of the mutagenic potential of the chemical substance is becoming increasingly important. Mutagenesis is the ability of chemicals to cause changes in the genetic material in the nucleus of the cell in ways that can be transmitted during cell division. If mutations are present in genetic material at the time of fertilization in either egg or sperm, the resulting genetic combination may not be viable and death may occur in the early stages of embryonic cell division. Alternatively, the mutation in the genetic material may not affect early embryogenesis but may result in death of the foetus at a later developmental period and abortion. Congenital abnormalities may also result from mutations.

Since initiation of chemical carcinogenesis is thought to be a mutagenic event, mutagenic tests are often used to screen for potential carcinogens. Several *in vivo* and *in vitro* procedures have been devised to test chemicals for their ability to cause mutations. Since some mutations are visible with the light microscope, cytogenetic analysis of bone marrow smears after exposure to the test agent is often employed. Since most mutagens are incompatible with normal development, the mutagenic potential of a substance can also be measured by the dominant lethal test, usually performed in rodents. The male is exposed to the test compound for 3 months and then mated with two untreated females. The females are sacrificed before term and the number of live embryos and *corpora lutea* determined.

The mutagenic test receiving the widest attention at the present time is the reverse mutation test developed by Ames and colleagues. This test uses a mutant strain of *Salmonella typhimurium* that lacks coding for the enzyme phosphoribosyl ATP synthetase, which is required for histidine synthesis. Thus, the strain is unable to grow in a histidine deficient medium unless a reverse mutation has occurred. Since many chemicals are not mutagenic or carcinogenic unless they are transformed to a toxic product by the microsomes of the endoplasmic reticulum, rat liver microsomes are usually added to the medium containing the mutant strain and reverse mutation is then quantitated by the growth of the strain in a histidine deficient medium.

The overall purpose of these tests is to determine the biological effects which a chemical substance can produce in the mammalian system. Special attention must be given to determine whether these effects also occur in man. The difference in dose

between that required to produce the therapeutic effect and that which produces undesirable effects must also be assessed, in an attempt to determine

the drug's relative safety, in terms of risk-to-benefit, for the condition to be treated.

Bibliography

- Doull, J., Klaassen, C. D. and Amdur, M. (Ed.) (1980). "Casarett and Doull's Toxicology. The Basic Science of Poisons", Second edition. Macmillan, New York.
- Gilman, A. G. (Ed.) (1980). "Goodman and Gilman's Pharmacological Basis of Therapeutics", Sixth edition. Macmillan, New York.
- Zbinden, G. (Ed.) (1973). "Progress in Toxicology", Vol. 1. Springer-Verlag, New York.



Biochemical, Pharmacological and Toxicological Evaluation of (+)-Cyanidanol-3

G. HENNINGS

*Institute of Pharmacology and Toxicology,
University of Munich,
Munich, Federal Republic of Germany*

(+)-Cyanidanol-3 (Fig. 1) is a naturally occurring flavonoid with the composition (+)-3',4',5,7-tetrahydroxyflavan-3-ol. It is also known as (+)-catechin. It has the empirical formula $C_{15}H_{14}O_6$, molecular weight 290.28 and a melting point of 210°C . It has recently been found to be active as a hepatotropic drug in a systematic biochemical, pharmacological and toxicological evaluation (Bertelli, 1975).

Pharmacology and Biochemistry

The pharmacokinetic behaviour of (+)-cyanidanol-3 was studied with regard to absorption, distribution, metabolism and excretion with oral and parenteral administration in the rat, rabbit, guinea-pig, monkey, and in man. The drug is readily absorbed from the gastrointestinal tract and metabolized mainly in the liver. The unchanged substance and metabolites are excreted in the urine and bile (Das and Sothy, 1971). One major metabolite is 3'-O-methyl-(+)-cyanidanol-3 (Shaw and Griffiths, 1980).

Numerous classical pharmacological models have been used to establish the hepatotropic activity of (+)-cyanidanol-3 on experimental steatosis, necrosis, inflammation, cholestasis and fibrosis of the liver. (+)-Cyanidanol-3 showed protective activity against specific hepatotoxic agents like CCl_4 , CCl_3Br , phalloidin, galactosamine, paracetamol, ANIT, depletors of hepatic glutathione (diethylmaleate, vinylidene chloride, phorone and ethanol), ethionine and orotic acid,

as well as against steatosis induced by specific dietary regimens (Bertelli, 1975; Köster-Albrecht *et al.*, 1979; Wünsch and Heine, 1979; Younes and Siegers, 1980). From the experimental models employed certain conclusions about possible mechanisms of action may be suggested.

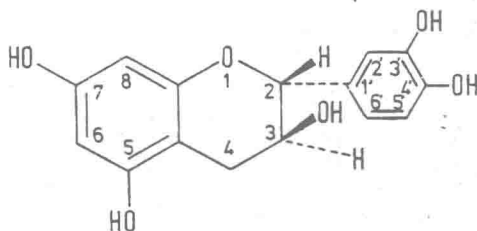


Figure 1.

(1) (+)-Cyanidanol-3 acts as a radical scavenger and anti-oxidant (Slater and Eakins, 1975) *in vitro* and *in vivo*, thus preventing lipid peroxidation (Köster-Albrecht *et al.*, 1979; Kappus *et al.*, 1979) induced either by halogenated hydrocarbons or by depletion of hepatic glutathione content (Younes and Siegers, 1980).

(2) (+)-Cyanidanol-3 changes the basic properties of cytoplasmic and other cell membranes. In particular, this substance reduces the membrane permeability of low molecular weight solutes transported by free and exchange diffusion. The agent appears to be adsorbed at the membrane site, thus exerting stabilizing properties as indicated by a shift of the transition temperature of the liquid crystal state to the solid gel state of membrane lipids (Ring *et al.*, 1976, 1977).

Some additional features of the mode of action

Conn, H. O., Ed., (+)-Cyanidanol-3 in Diseases of the Liver, International Workshop (Royal Society of Medicine International Congress and Symposium Series No. 47); Crans-Montana, Switzerland 1981, 284 pages; published jointly by Academic Press Inc. (London) Ltd and the Royal Society of Medicine.