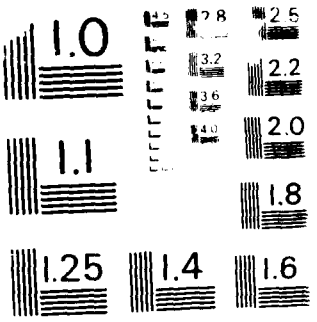
AD-A190 111 ANIMAL STUDIES IN THE MODE OF ACTION OF AGENTS THAT ARE 1/2
ANTITRANSFORMERS (..(U) OESTERREICHISCHES
FORSCHUNGSZENTRUM SEIBERSDORF G M B H VIENN.
UNCLASSIFIED H ALTMANN 28 OCT 87 AFOSR-TR-87-1989 F/G 6/15 NL



SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

DTIC FILE COPY

1a. AD-A190 111

1b. RESTRICTIVE MARKINGS

2a.

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; Distribution unlimited

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)
AFOSR-TR- 87-1909

6a. NAME OF PERFORMING ORGANIZATION
Österreichisches Forschungszentrum

6b. OFFICE SYMBOL (If applicable)
OEFZS

7a. NAME OF MONITORING ORGANIZATION
Air Force Office of Scientific Res

6c. ADDRESS (City, State, and ZIP Code)
A-2444 Seibersdorf, Austria

7b. ADDRESS (City, State, and ZIP Code)
Bolling Air Force Base, DC 20332-6448

DTIC ELECT JAN 07 19

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
AFOSR

8b. OFFICE SYMBOL (If applicable)
NL

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
AFOSR-84-390

8c. ADDRESS (City, State, and ZIP Code)
Bolling AFB, DC 20332-6448

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61102F	2312	A5	

11. TITLE (Include Security Classification)
Animal Studies in the mode of action of agents, that are antitransformers in cell cultures.

12. PERSONAL AUTHOR(S)
Hans Altmann

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM Jun.84 TO Aug.87	87 10 28	110

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Antitransformer, poly(ADP-ribose)-polymerase, DNA-repair

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The most important results of the animal studies on the mode of action of the antitransformer DADH in short and long term experiments are the following:

1. Combined treatment of DADH and γ-irradiation generated a decreased incidence of malignant lymphoma compared to γ-irradiation alone.
2. DADH itself shows some carcinogenic properties.
3. In short term experiments DADH has an immunoprotective effect with respect to γ-irradiation: a. earlier reconstitution of lymphocyte subsets, b. increase in natural killer cell activity.
4. Higher poly(ADP-ribose)-polymerase activity to a certain extent seems to control replicative DNA synthesis and specific DNA amplification determined by double minutes.
5. Spleen cells with loss of DNArepair increased remarkably with age. At the same time lymphoma incidence is increasing.

./.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Lorris G. Cockerham, Lt Col USAF	202 767-5021	AFOSR/NL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

Atch 3

Contin.

19. Abstract

6. Nucleoid sedimentation studies showed an oversedimentation phenomena rather than DNA breaks during short and long term experiments.
7. A certain correlation between basic UDS in spleen cells and the occurrance of lymphomas exists.
8. Basic UDS was highest in the combined (γ + DADH) group. But also after a single irradiation dose of 1 Gy basic UDS was elevated during the whole life time.
9. Poly(ADP-ribose)-polymerase activity parallels the poly(ADP-ribose) content in spleen and liver cells at the end of the life span of C57 bl mice.

ANIMAL STUDIES ON THE MODE OF ACTION OF AGENTS, THAT ARE ANTITRANSFORMERS IN CELL CULTURES

Institution: Austrian Research Centre Seibersdorf
A-2444 Seibersdorf, Austria

Principal investigator: Hans ALTMANN

Grant: AFOSR 84-390

page

INTRODUCTION

The aim of the work done under AFOSR grant No 84-0390 was to find out the action mechanism of N,N'-Diacetyl-1,6-diaminohexane (DADH), a substance of known antitransforming effect in vitro, in an animal experiment. Primary field of interest were molecular-biological and immunological parameters. Beside this, tumor development as well as other biological parameters were investigated.

We chose C57 bl/6 mice as experimental model because the incidence of malignant tumors is high and their mean life span is 660 days in males and 640 days in females. Immunologically the onset of decline occurs earlier and the rate of the age related decline in activity is more rapid, compared to other mouse strains. Additionally the T cell immunity may play an important role in the resistance against life shortening malignant lymphoma, which can also be induced by ionizing radiation. Radiation induced thymic lymphomas in C57 bl/K mice can produce high titers of radiation leukemia virus (Rad LV) which is able to selectively transform T lymphocytes. Studies of Rad LV induced leukemogenesis in C57 bl/K9 mice have shown that the thymus is essential for virus replication and that T cells are the cellular target of virus induced neoplastic transformation. T cell immunity may therefore play an important role in the resistance against life shortening malignant lymphoma induced by ionizing radiation.

It was reported by Hirokawa et al. that all the T cell dependent splenic immunological activities investigated, showed a decline with age. Studies from our laboratory have shown that poly(ADP-ribose)-synthesis declines as a function of age in lymphocytes. Because the resistance against radiation induced lymphoma is also dependent on the right balance of lymphocyte subsets and also of natural killer cell activity and these investigations were not done up till now, we included these determinations in our programme. The determination of PAN T cells, suppressor cells (including cytotoxic cells) and B cell population was done

using monoclonal antibodies and the killer cell activity was studied by a ^{51}Cr release method. Since for balanced lymphocyte action several differentiation steps are necessary and poly(ADP-ribose)-synthesis (PAR-synthesis) controls this important developmental mechanism, we tried to find out if any antitransforming activity in vivo is also correlated with enhanced PAR-polymerase activity and differentiation processes.

Besides poly(ADP-ribose)-polymerase activity and poly(ADP-ribose) content of cells also replicative DNA synthesis, unscheduled DNA synthesis and DNA strand breaks possibly play a role in the generation of irradiation induced lymphomas. Investigations including these parameters were also performed.

Since gene amplification can be detected by cytogenetic determination of doubleminutes we have tried to include this factor in the short term experiments with DADH.

During the initial phase of this project two "antitransformer" substances were selected to investigate some biochemical and immunological parameters in short term experiments to find out the "best" substance for the long term study.

Some valuable results were obtained with both, 3-methoxy-benzamide (3-MBA) and DADH, but DADH was choosen for the long term experiment, because the substance can be applied with drinking water and is facilitated by the characerization of its cellular uptake and metabolism.

Toxicokinetic study of 3-methoxybenzamide

14-labelled 3-methoxybenzamide was applied perorally to groups of 3 male and 3 female rats. The animals were killed 1, 4 or 18 hours after a single application of the test substance or 72 hours after the last of 5 consecutive administrations. 14C-activity in liver, lung, spleen, thymus, kidneys and blood was analysed. Highest concentrations were observed in liver and kidneys, lowest in spleen and thymus. 14C-activity decreased with an initial half time of 1 - 1.5 hours and was then slowing down. Details see enclosed report (enclosure 1).

Toxicokinetic study of N,N'-Diacetyl-1,6-diaminohexane

14-labelled N,N'-diacetyl-1,6-diaminohexane was applied peorally to groups of 3 male and 3 female rats. The animals were killed 1, 4 or 18 hours after a single application of the test substance or 72 hours after the last consecutive administration. 14C-activity in liver, lungs, spleen, thymus, kidneys and blood was analysed. Highest concentrations were observed in kidneys, thymus and spleen, lowest in blood. 14C-activity decreased with an initial half time of about 1.5 hours and was then slowing down. An accumulation of the test substance in the organs occurred after repeated administrations.
Details see enclosed report (enclosure 2).

Because antitransforming activity is also important for human skin, a percutaneous absorption study of N,N'-diacetyl-1,6-diaminohexane was performed in addition.
Details see enclosed report (enclosure 3).

ANIMAL EXPERIMENTS AND HISTOPATHOLOGY IN:
Animal studies on the mode of action of agents, that are antitransformers in cell cultures.

SUMMARY
N, N'-Diacetyl-1,6-diaminohexane (DADH), a substance of a known antitransforming effect in vitro, was used to study a possible effect when applied to mice alone or in combination with gamma irradiation. Primary field of interest were biochemical parameters. Besides this, tumor development as well as other biological parameters were investigated.
4 groups of 89 male and 98 female C57-bl mice were kept for up to 618 days.
Group 1 remained untreated as control.
Group 2 was treated with DADH (100 ppm in the water) continuously.
Group 3 was gamma irradiated with 1 Gy once initially.
Group 4 was treated with DADH (100 ppm in the water) continuously and gamma irradiated with 1 Gy once initially.

Investigations reported in this part: Clinical signs, body weight, water consumption, haematology, necropsy and histopathology of spleen, thymus and grossly changed tissues.

Summarized results:
There was no detectable effect of any kind of treatment on body weight, water consumption, clinical signs, haematologic parameters, spontaneous mortality and non neoplastic changes. Incidence of neoplasms, mainly malignant lymphoma, was higher in all treated groups compared to the untreated group 1. This gives an indication for a carcinogenic effect of the test substance alone and - as expected - of the single gamma irradiation. There was no different incidence of neoplasm bearing animals between groups 2, 3 and 4 till the termination of this study. Incidence of malignant lymphomas alone was lower in the combined treated

group than in the irradiated group and the DADH treated group. This gives an indication for a suppressive effect of DADH on irradiation induced lymphomas.

1. INTRODUCTION

The test substance, N, N'-diacetyl-1,6-diaminohexane is an antitransformer and a modulator of poly(ADP-ribose)syntheta-se. It was the aim of this study to:

- supply tissue samples for biochemical experiments and to
- reveal chronic in life effects of the test substance alone or in combination with gamma-irradiation.
- to perform a pathological examination of the treated animals for elucidating a possible inhibitory effect of the test substance to induction of spontaneous and irradiation induced neoplasms, mainly malignant lymphomas.

2. MATERIALS AND EXPERIMENTAL CONDITIONS

2.1. Test substance:

Name: DADH
Chemical name: N, N'-diacetyl-1,6-diaminohexane
Other Name: N, N'-Hexamethylenbisacetamide
Purity: 98%
Lot No.: 7715 HK
Source: Aldrich Chemical Company, Inc., Milwaukee, Wis 53233, USA
Cat. No.: 22,423-5.
Storage: In the refrigerator at about +4 C.
Appearance: White, cristalline powder, slight odour.

2.2. Preparation and application of the test substance:

DADH was applied dissolved in the drinking water in a concentration of 100 ppm (100 mg per l). Test substance solutions were prepared freshly once a week.

2.3. Irradiation:

Animals of group 3 and 4 were gamma-irradiated once with 1 Gy from a 60-Co irradiation plant. Animals were irradiated on day 15, 2 weeks after the first dosing with DADH.

2.4. Animals:

C57 bl mice.

Source: Charles River - WIGA, D8741. Sulzfeld.
20 mice were supplied by Institut für Versuchstierzucht, A-2325 Himberg to replace males that died from fighting.

Number and sex: 356 males and 356 females.

Age: approx. 9 weeks at time of arrival in the test facility.

Date of arrival at test facility: September 19, 1985 (20 additional mice arrived at Oct. 8, 1985).

2.5. Animal husbandry and environmental control:

Hygiene: behind a hygienic barrier.

Room numbers: B3-10, animals for interim sacrifice term A and B in room No. B3-11.

Room temperature: average of 21°.

Relative humidity: average of 55%.

Air exchange: 12/h.

Light: artificial light from 6 a.m. to 6 p.m.

Cages: joint caging in Makrolon cages type III (39 cm x 23 cm x 15 cm). Wire mesh lids. Initially 10 mice were caged together. From Day 9 on maximally 6 male mice were caged together to reduce fighting. Animal number per cage was reduced gradually by taking animals for scheduled sacrifices.

Food: Altromin 1314 ff, gamma irradiated with 10 kGy 60Co, ad libitum. Random samples of the food are analysed by Altromin, D-4937 Lage.

Water: tap water acidified with HCl to pH 3, ad libitum,

offered in Makrolon bottles with stainless steel canules.

Identification: Animals were not identified individually during the treatment period. Cages were labelled with group identification patches. Individual labelling with felt-tipped pen on the tail was performed only for identification at necropsy.

Acclimatisation period: 10 days.

2.6. Groups, treatment:

group	treatment
1	untreated
2	100 ppm DADH in drinking water
3	a single gamma-irradiation (1 Gy) on Day 15
4	100 ppm DADH in drinking water during the whole life span plus a single gamma-irradiation (1 Gy) on Day 15

Doses were selected by the principal investigator on the basis of preliminary experiments.

2.7. Animal numbers:

Group	Animal Numbers Females	Males
1 (control)	1 - 89*	101 - 189*
2 (DADH)	201 - 289*	301 - 389*
3 (irrad.)	401 - 489*	501 - 589*
4 (DADH, irrad.)	601 - 689*	701 - 789*

89 mice of each sex were used per group. Animals for scheduled sacrifice were assigned with a plain number (e.g. 101, 102), animals of moribund sacrifice were assigned with a number plus preceding M (e.g. M101, M102), and spontaneously died animals were assigned with a number plus preceeding T (e.g. T101, T102).

*) The total number per group was 89 animals. Scheduled and moribund sacrifices and spontaneous deaths were assigned in parallel starting with the lowest number. Therefore the highest possible number was not reached in any group.

2.8. Type and frequency of observation:

Behaviour and physical signs of the animals were checked daily except for weekends. Only abnormal findings were recorded. A more detailed examination was performed once a week.

2.9. Body weight:

Body weight sum of animals in one cage was determined weekly till Day 48, then monthly. No body weight was determined of animals of sacrifice term A and B.

2.10. Water consumption:

Water consumption was calculated by weighing the bottles in weekly intervals. No water consumption was calculated of animals of sacrifice term A and B. Determination of water consumption was performed weekly till Day 37, then monthly.

2.11. Haematology:

Blood samples were taken from all males (except for sacrifice date A, B and C) on the last 1-5 days before necropsy. Blood samples were taken from the retrobulbar plexus in ether anesthesia.

Parameters - methods: Red and white cell count, mean cell volume of red and white blood cells, haemoglobin, haematocrit by Coulter Counter, model ZF6.

Differential white cell count by microscopical analysis of blood smears stained according to Pappenheim.

2.12. Scheduled sacrifices:

To gain tissue samples for biochemical examinations, animals were sacrificed at scheduled terms. Randomly selected animals were taken out of the groups and given an individual number for necropsy and sample identification.

Tissues taken, procedures and results of the biochmical experiments are described later.

Term	Date*	Day*	Animal Nos.
A	Oct.14, 1985	15	1-4, 101-104, 201-204, 301-304, 401-404, 501-504, 601-604, 701-704
B	Oct.21, 1985	22	5-12, 105-112, 205-212, 305-312, 405-412, 505-512, 606-612, 705-712
C	Dec. 9, 1985	71	13-20, 113-120, 213-220, 312-320, 412-420, 512-520, 612-620, 712-720
D	Apr. 7, 1986	190	21-28. 121-128, 221-228, 321-328, 421-428, 521-528, 621-628, 721-728
E	Oct. 6, 1986	372	29-36, 129-136, 229-236, 329-336, 429-436, 529-536, 629-636, 729-726
F	Dec. 9, 1986	436	37-44, 137-144, 237-344, 337-344, 437-444, 537-544, 637-644, 737-744
G	Jan.19, 1987	477	45-52, 145-152, 245-252, 345-352, 445-452, 545-552, 645-652, 745-752
H	Feb.16, 1987	505	53-60, 153-160, 253-260, 353-360, 353-460, 553-560, 653-660, 753-760
I	Mar.23, 1987	540	61-68, 161-168, 261-268, 361-368, 461-468, 561-568, 661-668, 761-768
J	Apr.27, 1987	575	69-72, 169-179. 269-272, 369-370, 469-470, 569-576, 669-674, 769-772

*) Males were killed in groups of two on the days indicated and the following 3 days in each case, females in groups of four on monday and tuesday of the following week. Remaining animals were euthanized on day 618.

2.13. Moribund sacrifices:

Animals with serious and/or life threatening lesions or a marked loss of body weight were killed as moribund.

2.14. Necropsy and histological examination

All animals, spontaneous deaths and moribund or scheduled sacrifices were dissected and examined macroscopically to

identify gross changes or tissue masses.
A sample of the spleen, thymus (except for animals of sacrifice term A and B and those with complete thymic involution), macroscopically changed organs and tissue masses were fixed with 7% formaldehyde, trimmed and embedded in paraplast. Sections of 5 µm were stained with hematoxilin/eosin/alcian blue and examined histopathologically using a Zeiss-Universal microscope.

2.15. Time schedule:

Animals arrived on: September 19, 1985.
Date of first dosing: September 30, 1985.
Date of irradiation: October 14, 1985 (some animals of interim sacrifice date B were irradiated from October 15 through 22, 1985).
Date of last sacrifice: June 9, 1987.
The day of the first dosing with DADH was called the first Day of experiment.

2.16. Bias control:

Assigning of animals to their groups was performed using random numbers. Cages were positioned by partial randomisation on the cage racks. Animals for interim sacrifice were taken in a way to keep a constant number of cages and to reduce the number of animals per cage.

2.17. Statistical analysis:

Arithmetic mean and standard deviation were calculated and are presented in the Tables. The one way analysis of variance followed by Scheffé test was used to evaluate differences between means. Countable events were analysed with Chi-square test or with Fisher's Exact test. Males and females were analysed separately except for the pathological data. P=0.05 was chosen in each test. A significant difference between any groups is marked in the Tables.

2.18. Archives:

All raw data, blood smears, histological slides and a copy of the final report are retained for a period of 12 years.

The data are not destroyed without the prior consent of the sponsor.

3. RESULTS

3.1. Body weight (Table 1, Fig. 1, 2):

There were some scattered significant differences in body weights, especially in female mice, but there was no group with a significant lesser body weight compared with the untreated control group 1. None of the treatments had therefore a negative influence on body weight.

3.2. Water consumption (Table 2, Fig. 3, 4):

There was no significant difference in mean water consumption between the different groups, except for one single case, which is not regarded to be of importance. No indication for a poor palatibility of DADH solutions can be derived from this data, as there was no lower water consumption in the DADH treated group.

3.3. DADH uptake (Table 3):

DADH uptake was calculated from water consumption and actual body weight. As expected, it decreased slightly with increasing age because of the relatively lower water consumption of elder animals.

3.4. Animal observation (Table 6):

Fighting was a major problem in the first weeks of the study and cause of death for several animals. After reducing the number of males per cage no further fighting and cannibalism was seen.

Except for this the most important changes observed were lesions of the skin, like alopecia, skin ulceration or thikkened skin.

None of the changes observed is attributable to any kind of treatment.

3.5. Haematology (Table 4):

There were a few scattered differences in haematology parameters, but there is no indication for a haematologic effect of any kind of treatment.

3.6. Mortality:

Number, group and time of death of the spontaneously died animals and of the moribund sacrifices are listed in Table 5, the actual number of animals in the study at any time is given in Figs. 5 and 6.

There was no significant difference in the total number of unscheduled deaths between the groups. More males than females died spontaneously in all groups. Main causes of death of the spontaneously died animals were neoplasms. Main reason for moribund kill was obvious lethargy and serious dermal changes.

3.7. Necropsy findings (Tables 7 and 8):

Except for two congenital changes (kidney, hypoplasia and aplasia) and several tissue masses, the most prominent finding were white foci on the liver.

None of the these changes gives an indication for a treatment related effect.

3.8. Histopathology findings (Tables 9, 10 and 11):

Non neoplastic lesions were found in several animals of all groups. A statistical comparison between the groups was only possible for changes of the spleen, as in all other cases the number of examined organs was not representative and there was no random selection of tissues. None of the non neoplastic lesions gives an indication for an effect of any kind of treatment.

Skin changes and focal liver necroses possibly indicate a chronic bacterial infection.

Renal amyloid deposits were seen in nearly all samples of mice in the last months of the experiment, indicating a very high incidence of this change.

The most frequent neoplasms observed were different types of the malignant lymphoma.

Only single cases of other neoplasms were found except for lymphomas. As only grossly identified tissue masses were examined microscopically, no tumors of incretory glands or of the brain have been found and the total number of tumors described in tissues except for spleen and thymus does not represent the total number effectively present.
Tumor incidence was lowest in the untreated control group 1. It was higher in the other three groups.

3.9. Miscellaneous:

Cannibalism victims, died within the first days of the study, have been replaced by additional animals between Day 9 and 15.

4. CONCLUSION

There is no evidence for a treatment related effect on bodyweight, water consumption, haematology, clinical signs, and non neoplastic pathological lesions.
The number of animals with any kind of neoplasm is lowest in the untreated control group 1, compared to all treated groups.

Incidence of malignant lymphoma is increased in DADH treated group 2 and in the irradiated group 3 compared to the untreated control group 1. Though there is no statistical significance, incidence of malignant lymphoma in the group 4 with combined treatment (DADH plus irradiation) is lower than in both groups 2 and 3. This gives an indication for:

- a carcinogenic effect of the treatment with DADH alone.
- a carcinogenic effect, as expected, of a single gamma irradiation with 1 Gy.
- a inhibitory effect on lymphoma induction by combined treatment with DADH and gamma irradiation.

Table 1: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Body weight data (g).
Data are presented by mean/standard devation

FEMALES

Day	Group 1 untreated	Group 2 DADH	Group 3 irrad.	Group 4 DADH,irrad.	sign.*
1	18.5/0.2	18.0/0.8	18.4/0.5	18.2/0.9	-
8	19.4/0.4	19.2/0.3	19.5/0.5	15.6/0.6	-
15	21.4/1.4	19.3/1.1	20.0/0.5	20.8/1.0	1-2
22	20.4/0.2	18.9/3.6	20.6/0.5	20.8/0.2	-
29	21.1/0.4	20.7/0.2	21.3/0.4	21.0/0.3	2-3
36	21.4/0.3	21.1/0.2	21.3/0.3	20.9/0.2	1-4,3-4
43	21.7/0.3	21.1/0.9	21.6/0.4	21.4/0.7	-
50	22.1/0.3	21.8/0.4	22.5/0.4	22.3/0.2	2-3
57	22.3/0.2	21.7/1.1	22.7/0.5	22.5/0.3	2-3
64	22.5/0.6	22.0/1.3	22.7/0.6	22.4/0.3	-
95	24.4/0.4	24.0/0.3	24.2/0.6	24.2/0.3	-
127	25.0/0.3	24.8/0.4	24.9/0.5	25.0/0.5	-
155	25.6/0.3	25.3/0.4	25.8/0.6	25.8/0.3	-
204	26.4/0.5	26.0/1.1	27.0/0.8	27.3/0.6	2-4
234	26.6/0.6	26.6/0.4	27.8/0.9	28.5/1.0	1-3,1-4, 2-3,2-4
260	26.8/0.6	27.5/1.8	28.5/0.8	29.0/1.0	1-4
288	27.2/0.7	27.0/0.4	29.0/1.2	28.9/1.3	1-3,1-4, 2-3,2-4
316	27.0/1.4	26.5/2.9	29.2/1.2	28.7/2.0	-
345	27.9/0.8	27.2/2.0	29.1/1.1	29.6/1.6	2-4
386	27.5/0.7	27.8/0.7	28.8/1.0	29.3/1.1	1-4,2-4
420	27.7/0.7	28.5/1.6	29.2/1.3	29.5/1.9	-
470	27.5/0.9	27.6/0.8	29.0/1.0	28.7/1.2	1-3
498	27.4/1.0	27.0/0.8	28.9/0.7	27.9/0.6	2-3
526	27.3/1.2	28.9/0.8	30.0/1.9	29.3/1.0	1-3,2-3 1-4
554	27.8/2.7	29.5/1.6	30.8/1.4	28.4/1.2	-

sign.*: significant differences between groups indicated

Table 1, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Body weight data (g).
Data are presented by mean/standard devation.

MAES

Day	Group 1 untreated	Group 2 DADH	Group 3 irrad.	Group 4 DADH,irrad.	sign.*
1	21.4/0.4	21.6/0.4	21.3/0.9	21.4/0.5	-
8	22.4/0.5	22.6/0.3	22.4/0.7	22.6/0.5	-
15	23.1/1.0	20.8/1.6	22.1/2.1	24.9/1.7	1-2,1-4 2-4,3-4
22	23.5/1.8	23.9/0.6	23.7/0.9	23.5/0.7	-
29	24.8/0.8	24.3/0.4	24.9/0.5	24.4/0.7	-
36	25.1/1.0	24.2/0.6	24.9/0.7	24.3/0.7	1-2
43	25.4/1.3	25.1/0.4	25.3/0.5	24.9/1.0	-
50	26.0/1.6	25.5/0.8	25.6/0.6	25.0/0.9	-
57	26.2/1.4	25.9/0.8	25.9/0.5	25.4/0.9	-
64	26.8/1.8	26.4/0.7	26.2/0.7	25.7/1.4	-
95	28.4/2.1	27.6/0.8	27.8/0.4	27.5/1.1	-
127	29.1/2.2	28.4/0.8	28.5/0.5	27.7/1.5	-
155	29.9/2.2	29.2/0.9	29.7/0.6	29.1/1.4	-
204	30.9/2.2	30.9/1.3	30.4/0.8	29.9/1.7	-
234	32.4/2.0	32.2/1.6	31.7/1.3	31.2/1.7	-
260	32.5/2.3	32.7/1.7	32.4/1.1	32.3/1.9	-
288	33.6/2.3	33.3/1.9	32.9/1.4	31.8/1.3	-
316	33.6/2.5	33.6/2.3	32.7/1.9	32.1/1.9	-
345	33.7/2.2	33.9/1.9	32.9/1.2	32.6/2.2	-
386	33.3/2.7	33.3/1.8	32.3/0.9	32.4/2.4	-
420	33.3/2.6	33.0/1.6	32.0/0.9	31.6/2.1	-
470	32.6/2.4	32.4/1.2	32.2/1.0	31.7/1.9	-
498	32.2/2.5	30.9/1.2	31.6/1.4	30.2/1.6	1-4
526	31.3/2.9	33.5/1.8	32.8/1.7	32.6/2.7	-
554	32.7/2.8	32.3/2.9	32.3/2.0	31.3/1.1	-

sign.*: significant differences between groups indicated

Table 2: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Water consumption (ml per day per animal)
Data are presented by mean/standard devation.

FEMALES

Week before Day	Group 1 untreated	Group 2 DADH	Group 3 irrad.	Group 4 DADH,irrad.	sign.*
4	4.3/0.4	4.1/0.4	4.3/0.3	4.2/0.4	-
11	4.1/0.2	4.3/1.0	4.1/0.2	4.2/0.2	-
18	4.0/0.3	4.2/0.2	4.1/0.3	4.2/0.2	-
25	3.7/0.4	3.8/0.5	3.9/0.3	4.0/0.2	-
32	4.2/0.1	4.0/0.5	4.2/0.1	4.0/0.3	-
39	4.1/0.3	4.1/0.4	4.3/0.2	4.2/0.4	-
46	4.1/0.3	4.1/0.5	4.3/0.2	4.0/0.2	-
53	4.4/0.2	4.2/0.4	4.1/0.2	4.0/0.2	-
60	4.3/0.2	4.0/0.7	3.9/0.2	3.9/0.2	-
67	4.4/0.3	4.0/.06	4.1/0.2	4.0/0.2	-
102	4.8/0.3	4.1/0.5	4.0/0.4	3.9/0.2	-
137	4.8/0.2	4.4/0.5	4.3/0.4	4.0/0.3	-
165	4.4/0.2	4.3/0.5	4.2/0.2	4.1/0.1	-
207	4.7/0.3	4.7/0.4	4.3/0.3	4.1/0.1	-
235	4.2/0.3	4.3/0.4	3.9/0.3	4.0/0.2	-
263	3.9/0.2	4.2/0.4	3.9/0.2	3.8/0.3	-
291	3.7/0.3	3.9/0.5	3.5/0.4	3.6/0.3	-
319	3.6/0.3	3.6/0.4	3.5/0.3	3.4/0.3	-
347	3.7/0.2	3.6/0.3	3.6/0.2	3.4/0.2	-
389	4.2/0.4	3.7/0.6	4.0/0.5	3.8/0.3	-
424	4.0/0.3	4.0/0.4	4.1/0.9	3.7/0.3	-
473	5.0/1.0	4.6/0.5	5.3/0.9	4.1/0.4	3-4
501	5.3/1.0	5.3/0.8	6.1/1.5	4.6/0.4	3-4
529	7.3/2.5	6.7/1.8	8.5/2.5	6.0/1.4	-

sign.*: significant differences between groups indicated

Table 2, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Water consumption (ml per day per animal).
Data are presented by mean/standard devation.

MALES

Week before Day	Group 1 untreated	Group 2 DADH	Group 3 irrad.	Group 4 DADH,irrad.	sign.*
	4.2/0.4	4.1/0.34	4.5/0.23	4.2/0.34	-
11	-------	-------	-------	-------	-
18	4.1/0.3	4.0/0.8	3.8/0.4	3.9/0.5	-
25	3.9/0.5	3.7/0.5	3.7/0.7	3.9/0.9	-
32	4.0/04.	3.7/0.7	3.9/0.3	3.7/0.6	-
39	4.0/0.5	3.7/0.6	4.0/0.3	3.8/0.5	-
46	3.8/0.6	3.6/0.5	4.0/0.3	3.6/0.6	-
53	4.0/0.6	3.8/0.7	4.0/0.4	3.7/0.5	-
60	3.8/0.5	3.6/0.6	3.8/0.3	3.6/0.5	-
67	4.1/0.6	3.9/0.6	3.9/0.3	3.8/0.6	-
102	4,1/0.8	3.7/0.6	3.9/0.2	3.6/0.5	-
137	4.1/0.7	3.9/0.5	3.8/0.4	3.8/0.8	-
165	3.9/0.7	3.9/0.6	3.7/0.4	3.8/0.7	-
207	4.5/1.4	4.1/0.4	4.0/0.3	4.2/1.1	-
235	3.9/0.6	4.0/0.6	3.8/0.3	4.2/0.6	-
263	4.1/1.4	3.8/0.3	3.7/0.2	4.0/0.8	-
291	3.6/1.0	3.5/0.6	3.2/0.4	3.4/0.6	-
319	3.4/0.7	3.3/0.4	3.2/0.3	3.4/0.6	-
347	3.4/0.7	3.3/0.5	3.2/0.3	3.4/0.6	-
389	3.5/0.9	3.7/0.6	3.5/0.4	3.8/0.7	-
424	3.8/0.7	4.0/0.9	3.9/0.9	4.2/0.9	-
473	4.5/1.3	5.5/1.5	4.8/1.8	5.0/1.6	-
501	5.3/2.0	6.3/2.3	5.3/2.0	5.8/2.0	-
529	6.1/2.7	8.0/3.3	6.1/2.3	7.3/3.5	-

sign.*: significant differences between groups indicated

Table 3: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Calculated DADH uptake (mg DADH/kg bodyweight/day).

MALES

Day	Group 2 DADH	Group 4 DADH,irrad.
1	19	20
15	19	16
22	15	17
29	15	15
36	15	16
43	14	14
50	15	15
57	14	14
64	15	15
95	13	13
204	13	14
234	12	13
260	12	12
288	11	11
316	10	11
345	10	10
386	11	12
420	12	13
470	17	16

Table 3, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Calculated DADH uptake (mg DADH/kg bodyweight /day).

FEMALES

Day	Group 2 DADH	Group 4 DADH, irrad.
1	23	23
8	22	27
15	22	20
22	20	19
29	19	19
36	19	20
43	19	19
50	19	18
57	18	17
64	18	18
95	17	16
204	18	15
234	16	14
260	15	13
288	14	12
316	14	12
345	13	11
386	13	13
420	14	13
470	17	14

Table 4: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Haematology data. Data are presented by mean and standard deviation.

Term:D Day: 191-193

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	4.49 0.37	4.18 0.25	4.27 0.56	4.18 0.38	---
mean cell volume, erythrocytes(fl)	61.3 3.2	60.4 2.3	60.4 3.3	60.9 2.4	---
leukocytes (10^{6}/l)	4.7 1.9	4.7 1.0	5.6 4.5	4.5 1.0	---
mean cell volume, leukocytes(fl)	73.3 7.7	66.3 5.7	68.3 7.7	69.5 6.1	---
haemoglobin (g/l)	15.5 0.9	14.8 0.8	14.8 0.5	14.7 0.6	---
packed cell volume (%)	not measured at this term				
differential count (%):					
neutrophils	4.1 2.7	4.5 3.7	4.4 3.9	6.0 3.4	---
lymphocytes	93.6 4.2	95.0 3.7	93.1 6.2	93.1 4.0	---
eosinophils	0	0	0	0	---
monocytes	0	0	0	0	---

sign.*: significant differences between groups indicated

Table 4, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Haematology data. Data are presented by mean and standard deviation.

Term:E Day:366

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	4.37 0.39	4.39 0.96	4.87 1.96	4.78 1.56	---
mean cell volume, erythrocytes(fl)	60.4 1.1	60.3 1.9	61.8 2.0	62.5 2.1	---
leukocytes (10^{6}/l)	7.21 1.0	7.3 1.0	7.3 1.3	6.8 0.8	---
mean cell volume, leukocytes(fl)	68.8 5.4	76.9 8.9	82.5 10.5	71.3 7.5	1-3
haemoglobin (g/l)	14.2 0.4	13.3 1.1	13.8 0.8	13.4 0.5	---
packed cell volume (%)	0.24 0.02	0.25 0.06	0.28 0.09	0.28 0.10	---
differential count (%):					
neutrophils	5.3 5.0	7.1 4.2	4.5 1.3	5.4 4.3	---
lymphocytes	94.1 4.9	91.0 5.2	9[illegible].4 4.7	92.0 5.0	---
eosinophils	0.6 0.7	0.9 0.8	0.6 0.9	0.9 1.4	---
monocytes	0.1 0.4	1.1 1.4	0.5 1.1	1.3 0.9	---

sign.*: significant differences between groups indicated

Table 4, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Haematology data. Data are presented by mean and standard deviation.

Term:F Day:429

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	3.53 0.28	3.32 0.29	3.25 0.35	3.32 0.32	---
mean cell volume, erythrocytes(fl)	68.5 2.0	68.3 1.5	68.0 0.8	67.1 1.5	---
leukocytes (10^{9}/l)	8.1 2.0	7.5 0.9	6.7 1.0	7.1 3.4	---
mean cell volume, leukocytes(fl)	102.6 22.9	95.8 17.0	86.0 12.7	90.5 12.4	---
haemoglobin (g/l)	14.6 0.5	14.1 0.6	14.6 0.9	13.3 1.7	---
packed cell volume (%)	0.22 0.02	0.21 0.02	0.20 0.02	0.20 0.03	---
differential count (%):					
neutrophils	7.0 2.6	4.9 4.4	6.6 3.9	4.4 5.8	---
lymphocytes	91.9 2.9	93.3 5.3	92.8 4.9	94.5 7.3	---
eosinophils	0.6 0.9	1.3 1.0	0.9 2.1	1.6 2.4	---
monocytes	0.5 0.8	0.6 0.5	0.5 0.5	0.3 0.5	---

sign.*: significant differences between groups indicated

Table 4, cont.: Animal experiments and pathology in:
Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Haematology data. Data are presented by mean and standard deviation.

Term:G Day:473

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	3.64 0.19	3.48 0.44	3.34 0.20	3.14 0.33	1-4
mean cell volume, erythrocytes(fl)	67.6 2.7	67.3 2.7	67.4 3.6	66.3 1.4	---
leukocytes (10^{9}/l)	5.6 1.4	5.6 1.5	4.4 1.1	6.3 1.0	3-4
mean cell volume, leukocytes(fl)	82.9 11.5	79.9 6.6	81.1 9.8	84.8 10.2	---
haemoglobin (g/l)	14.1 0.8	14.0 0.7	13.5 0.8	13.3 1.3	---
packed cell volume (%)	0.23 0.02	0.22 0.04	0.21 0.02	0.19 0.20	1-4
differential count (%):					
neutrophils	7.6 5.7	5.4 3.0	6.5 9.4	2.6 2.8	---
lymphocytes	90.8 7.0	93.3 3.4	91.9 10.1	97.4 2.8	---
eosinophils	0.8 1.0	0.5 0.5	0.25 0.5	--- ---	---
monocytes	0.9 0.8	0.5 0.5	1.4 0.9	0.4 1.0	---

sign.*: significant differences between groups indicated

Table 4, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Haematology data. Data are presented by mean and standard deviation.

Term:H Day:501

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	3.53 0.40	3.43 0.20	3.30 0.20	3.24 0.23	---
mean cell volume, erythrocytes(fl)	67.3 3.5	65.6 1.2	67.9 4.4	65.8 1.2	---
leukocytes (10^{9}/l)	7.4 1.6	7.4 1.1	6.1 1.3	6.1 1.3	---
mean cell volume, leukocytes(fl)	86.9 12.6	85.0 8.0	88.9 11.8	80.8 8.5	---
haemoglobin (g/l)	14.0 0.9	14.0 0.5	13.4 1.0	13.3 0.3	---
packed cell volume (%)	20.5 2.9	19.1 1.1	19.1 1.6	18.0 1.6	---
differential count (%):					
neutrophils	4.8 4.7	5.5 3.3	5.5 5.6	5.5 3.6	---
lymphocytes	94.0 5.4	93.5 4.2	94.3 6.0	93.8 3.4	---
eosinophils	0.4 1.0	0.5 0.8	0.1 0.4	0.1 0.4	---
monocytes	0.9 0.6	0.4 0.7	0.1 0.4	0.6 1.0	---

sign.*: significant differences between groups indicated

Table 4, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Haematology data. Data are presented by mean and standard deviation.

Term:I Day:536

Parameter	Group 1	Group 2	Group 3	Group 4	sign.*
erythrocytes (10^{12}/l)	3.68 0.28	3.56 0.47	3.62 0.27	3.39 0.33	---
mean cell volume, erythrocytes(fl)	65.5 1.4	64.8 0.7	65.8 1.7	64.5 0.53	---
leukocytes (10^{6}/l)	7.9 1.7	6.8 2.3	7.5 0.5	5.1 2.0	1-4
mean cell volume, leukocytes(fl)	77.5 5.2	84.1 10.7	80.9 5.9	76.9 6.6	---
haemoglobin (g/l)	13.7 0.84	13.1 2.1	14.0 0.5	12.6 1.7	---
packed cell volume (%)	20.7 2.0	19.9 2.5	20.1 1.2	18.9 1.9	---
differential count (%):					
neutrophils	8.8 3.5	6.6 4.6	8.4 4.3	6.4 5.6	---
lymphocytes	89.5 3.6	92.0 4.3	90.9 4.9	92.9 5.8	---
eosinophils	0.5 0.5	0.5 0.5	0.6 0.7	0.4 0.5	---
monocytes	1.2 1.6	0.6 0.7	0.1 0.4	0.5 0.5	---

sign.*: significant differences between groups indicated

Table 5: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Number and time of death of spontaneous deaths and moribund sacrifices. Females.

Group 1 (control)		Group 2 (DADH)	
Animal No.	Day of death	Animal No.	Day of death
M 1	197	M 201	81
M 2	282	M 202	113
M 3	338	M 203	351
M 4	414	M 204	432
M 5	414	M 205	484
M 6	436	M 206	541
M 7	484	M 207	554
M 8	512	M 208	554
M 9	512	M 209	555
M 10	569	M 210	562
		M 211	583
		M 212	583
		M 213	589
		M 214	598
		M 215	618
T 1	382	T 201	358
T 2	533	T 202	618
T 3	554		
T 4	615		

Table 5, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Number and time of death of spontaneous deaths and moribund sacrifices. Females.

Group 3 (irrad.)		Group 4 (DADH, irrad.)	
Animal No.	Day of death	Animal No.	Day of death
M 401	283	M 601	149
M 402	345	M 602	409
M 403	414	M 603	414
M 404	414	M 604	470
M 405	414	M 605	491
M 406	414	M 606	526
M 407	414	M 607	562
M 408	449	M 608	589
M 409	512	M 609	618
M 410	515	M 610	618
M 411	524	M 611	618
M 412	569		
M 413	598		
M 414	598		
T 401	513	T 601	519
T 402	582	T 602	540

Table 5, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Number and time of death of spontaneous deaths and moribund sacrifices. Males.

Group 1 (control)		Group 2 (DADH)	
Animal No.	Day of death	Animal No.	Day of death
M 101	484	M 301	190
M 102	491	M 302	358
M 103	519	M 303	319
		M 304	506
		M 305	540
		M 306	543
		M 307	551
		M 308	555
		M 309	557
T 101	225	T 301	326
T 102	456	T 302	396
T 103	540	T 303	456
T 104	551	T 304	456
T 105	551	T 305	509
		T 306	523

Table 5, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Number and time of death of spontaneous deaths and moribund sacrifices. Males.

Group 3 (irrad.)		Group 4 (DADH, irrad.)	
Animal No.	Day of death	Animal No.	Day of death
M 501	204	M 701	143
M 502	444	M 702	414
M 503	485	M 703	536
M 504	512	M 704	536
M 505	533	M 705	537
		M 706	537
		M 707	540
		M 708	541
		M 709	554
		M 710	557
		M 711	662
		M 712	662
T 501	484	T 701	400
T 502	484	T 702	425
T 503	505	T 703	540
T 504	505		
T 505	531		
T 506	540		

Table 6: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Animal observation.
Data are presented by number and sex of affected animals (Day of observation).

Finding	Group 1	Group 2	Group 3	Group 4
Cannibalism victims	1 m (57)	some m (8) 1 m each (15, 22, 29, 37) some m (45) 1 f (15)	---	1 f (8) 1 m (15)
Alopecia	some f(57, 95, 101, 127, 204, 234, 260, 288, 317, 331, 345, 386), some m(127,288, 317, 331, 345)	some f(57, 64, 71, 101, 127, 203, 234, 260, 288, 317, 345, 394), some m(43, 57, 78, 101, 127, 204, 234, 260, 268, 317, 331, 345)	some f(43, 78, 101, 127, 204, 234, 260, 288, 317, 331, 345) some m(57, 101, 127, 204, 260, 288, 317, 331, 345)	some f(50, 57, 89, 101, 127, 204, 234, 260, 288, 317, 331, 345), some m(57, 81, 101, 127, 204, 234, 260, 288, 317, 331, 345)
Skin ulcer	1 f(372), 2 f(379), 1 f(386)	---	1 f(346), 1 f(356), 2 f(372), 2 f(379), 2 f(386), 1 f(400), 2 f(408), 2 f(410), 1 m(372), 1 m(415)	1 f(400) 1 f(408)
Tail, thickened	---	some m (22)	---	1 m(50)
Head, thickened	1 m(500)	---	---	1 m(394) 1 m(397)
Limbs lame	1 f(408)	---	1 f(408) 1 f(436)	---
Dyspnoea	---	---	---	1 m(71)

Table 7: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Necropsy findings of scheduled sacrifices.
Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
Alopecia	26, 32, 34, 36, 40, 48, 53, 57, 60, 62, 63, 68	225, 245, 246, 247, 248, 251, 253, 255, 256, 257, 260, 262, 265	432, 437, 440, 443, 447, 454, 457, 460, 462, 466	636, 641, 643, 653, 658
Liver, white foci	29, 33, 36 37, 39, 40, 41, 43, 45, 48, 51, 55, 57, 58, 66	232, 234, 239, 241, 242, 243, 244, 245, 246, 247, 249, 250, 252, 253, 254, 255, 256, 257, 258, 263, 266, 267, 268, 269	430, 433, 434, 436, 438, 440, 441, 442, 443, 444, 445, 446, 451, 453, 455, 456, 458, 461, 465	630, 633, 634, 636, 639, 641, 642, 643, 644, 645, 646, 647, 649, 651, 654, 655, 659, 661, 663, 664, 666, 667, 668, 669
Liver, mass	---	---	457	650, 705
Liver, large	---	269	---	658, 706
Spleen, dark foci	13	268	447, 463	---
Spleen, large	46, 58, 61 65	259, 261, 269	527, 445, 457, 459, 453, 463, 466	642, 655, 658, 663, 668
Kidney, cysts	---	---	501	---
Kidney, unilat. aplasia	---	---	---	703
Kidney, unilat. hypoplasia	---	---	507	---
Thymus, large	62	---	---	614, 666
Mesentery, mass	53	---	464	---
Peritonitis chron.	---	---	---	705
Ovary, mass	---	---	468	---

Table 7, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Necropsy findings of scheduled sacrifices. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
General, anemia	---	---	409	---
General, obesiatas	---	---	---	708

Table 8: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Necropsy findings of moribund sacrifices and spontaneous deaths.
Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
GENERAL				
Cachexia	M2, M9	M201	M409	---
Anasarca	M8, T104	M206, M304 M307, T202	T506	---
Lymph nodes large	M4, M6	M201, M210	M404, M405, M406, M410, M414	M603
SKIN				
Alopecia	M1, M2, M3, M5, M7	M202, M210, M215, M301, M307, T201	M402, M405, M407, M408, M409, M501	M614, M702, M709
Ulcer	M4, M5, M7, M101, M102	M207, M208, M213	M404, M405, M406, M407, M412	M603, M604, M702
Subcut. tissue mass	M2, M7, M10	M203, M207, M303, M309	M402, M410	M601, M702
Subcut. abscess	M5	M204	---	---
Preputial gland large	M102	---	---	---
Clitoral gland large	M12	---	---	---
THORACAL CAVITY				
serous content	---	M201, T201, T202	M408	---
Hemorrhage	T102	---	---	---
Mass	---	---	---	M602
ABDOMINAL CAVITY				
Ascites	---	T202	---	---
MESENTERY				
Cyst	---	---	M408	---
Mass	---	---	M503	---

Table 8, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Necropsy findings of moribund sacrifices and spontaneous deaths. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
SMALL INTESTINE				
Meteorism	---	M205	---	---
Intussusception	---	M302, T304	M502	---
Mass	M7, M10	M214	M408	M607, M614
ADRENAL				
Mass	---	M213	---	---
KIDNEY				
light yellow	M6	M205, M207, M212, M307	M406, M503	M608, M709, M710, M711
Mass	---	---	M408	---
large	---	M307	---	M604
LIVER				
large	M7, M13, M102	M209, M211, M304, M308, M309	---	M607, M614, M703, M704, M711
Mass	---	M213	M503	M607, M712
white foci	M12, M13, T103	M209, M213, M305, M306, M308, M309	M408, M410, M413, T401	M703, M704, M707, M708, M709, M710, M711, T703
OVARY				
Cyst	M3	---	M413	---
Mass	M7, M11	M215	M408	M607, M609
UTERUS				
Mass	M7	---	---	---
SPLEEN				
large	M1, M2, M4, M5, M7, M10, M102, M103	M201, M20, M203, M204, M209, M214, M215, M301, M302, M303, M304, M305, T201	M402, M404, M405, M406, M407, M408, M501	M603, M607, M708

Table 8, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Necropsy findings of moribund sacrifices and spontaneous deaths. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
HEART				
large	---	T201	M408	---
LUNG				
Mass	M2	---	---	M602, M712
THYMUS				
large	M4, M7, M10, M103	M201, M402	---	---

Table 9: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Neoplastic changes. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
SPLEEN				
Malignant lymphoma lymphocytic	M102	M205,M209, M211,M213, M215,M304, T305	M503	M703,M707, M710,M712
Malignant lymphoma mixed cell type	136,152, M1,M4,M5, M7,M10,M101, M103	M201,M202, M204,M301, M302,M303, T301,T302	424,436, 442,527, 531,532, M401,M402, M403,M404, M405,M406, M407,M410, M411,M501	658,722,724 733,M601, M606,M702, M704,T701
Malignant lymphoma histiocytic	152	---	463	---
Hemangioma	---	T306	---	767
THYMUS				
Malignant lymphoma mixed cell type	M7,M101, M103	232,M201, M208,M301	425,447, 539,M407, M501	---
KIDNEY				
Malignant lymphoma NOS	---	M201,M301, T305	M406,M501	M606
LYMPH NODES				
Malignant lymphoma lymphocytic	---	---	M503	---
Malignant lymphoma mixed cell type	152,M1,M7, M10	M207,M210	572,573, M403,M405, M406,M414	M607
GL.MANDIB.				
Malignant lymphoma mixed cell type	---	---	M411	---

Table 9, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Neoplastic lesions. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
LIVER				
Hemangioma	---	M305	---	M708,M712
Hemangiosarcoma	---	M213	---	748
Malignant lymphoma lymphocytic	---	206,M211, M304,M309	M409,M503	M605,M610
Malignant lymphoma mixed cell type	---	M301	M401,M402, M403,M411, M501	722,M606, M607
Adenoma hepatocell.	---	---	---	650
Carcinoma hepatocell.	---	---	---	M602
LUNG				
Carcinoma NOS, metastatic	---	---	---	712,771, M602
TISSUE NOS				
Hemangioma	---	---	---	M705
Malignant lymphoma NOS	---	---	---	M601
Sarcoma NOS	---	---	M401,M402	---
PANCREAS				
Malignant lymphoma NOS	---	---	527	---
OVARY				
Granulosa cell tumor benign	---	---	---	607
SMALL INTESTINE				
Malignant lymphoma NOS	---	---	463,464	---
Adenocarcinoma	M2	---	---	---
Sarcoma NOS	---	---	M408	---

Table 9, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Neoplastic lesions. Data are presented by number of affected animals.

Finding	Group 1	Group 2	Group 3	Group 4
UTERUS				
Leiomyosarcoma	M7	---	---	---
EYE, LID				
Chondroma	---	---	M403	---
SKIN				
Squamous cell carcinoma	---	---	---	M702

The abbreviation NOS means not otherwise specified.

Table 10: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Non neoplastic lesions. Data are presented by number of affected animals (Score). Scoring scheme s. Table 12.

Finding	Group 1	Group 2	Group 3	Group 4
SPLEEN				
Lymphoid hyperplasia	117(3),	221(2), 245(2), 305(1), 308(1), 314(2), 325(3)	420(2), 501(2), 526(3)	620(2), 709(3)
Lymphoid depletion	16(3),43(3), 52(1),64(3), 123(3), 125(3), 137(2), 148(1)	217(3), 223(3), 226(3), 230(3), 241(1), 246(1), 248(1), 249(1), 345(2), 349(2), 350(2), M212(3), M305(3)	403(2), 451(1), 466(2), 513(3), 523(2), 546(2), 547(2), 550(2)	711(2), 737(1), 745(2), 747(2), 749(2), 756(2),
Hematopoiesis	9(3),14(3), 16(3),20(2), 22(4),23(3), 24(2),28(2), 60(1),64(4), 67(3), 102(3), 103(3), 104(1), 113(3), 122(4), 123(2), 137(2), 140(3), 145(2), 156(3), 158(1), 162(2), M8(2),M9(2), M101(2), M102(2)	217(3), 219(1), 223(3), 226(3), 227(2), 228(2), 255(2), 259(2), 261(3), 262(2), 266(1), 301(2), 314(2), 316(3), 320, 225(2), 304(2), 349(2), 351(1), 352(2), 354(2), 356(2), 357(2), 366(4), 361(2),	403(2), 405(3), 418(3), 453(3), 459(4), 462(1), 502(3), 510(3), 511(2), 513(3), 520(2), 523(3), 537(2), 539,4), 545(3), 553(2), 555(3), 556(4), 558(2), 560(2), 561(3), 568(2), M412(2), M502(2), M503(4),	603(3), 604(1), 609(2), 658()3), 663(3), 674(2), 701(3), 704(3), 707(4), 711(3), 719(2), 721(3), 723(3), 737(2), 739(3), 742(4), 748(3), 750(3), 753(3), 754(2), 755(1), 756(4), 758(2), 760(2), 761(2),

Table 10, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Non neoplastic lesions. Data are presented by number of affected animals (Score). Scoring scheme s. Table 12.

Finding	Group 1	Group 2	Group 3	Group 4
Hematopoiesis cont.		362(3), 364(2), 365(3), 366(1), M207(3), M210(2), M306(2), M309(3)	M505(3),	762(1), 763(1), 764(2), 765(2), M608(1), M701(2), M706(1), M708(3), 714(3), 744(5)
Hematocyst	---	---	569	---
THYMUS				
Lymphoid depletion	52(1)	238(2)	425(4), 437(2), 521(2),	673(3)
Lymphoid hyperplasia	---	261(4)	---	639(2)
Cyst	13,27,32, 34,36,37, 39,41,49, 50,51,57, 62,68,121	223,233, 239,244, 246,264, 268,316, 318,319, 271	439,452, 454,462, 466,467, 517,522	639,640, 645,647, 649,650, 652,654, 655,660, 662,666, 672
SKIN				
Abscess, subcut.	M7,M12	M309	---	M601
Dermatitis, chronic, ulcerative	M7(4), M101(4), M102(4)	M203(4), M204(4), M213(4), M303(4)	M303(4)	M604(4)
Dermatitis chronic active	M5(3),M102	---	539(4), M404(4)	---
Dermatitis chronic	---	---	M403(3), M405(3), M406(3)	M603(4)
Hair follicle atrophy	---	---	M402(3)	---

Table 10, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Non neoplastic lesions. Data are presented by number of affected animals (Score). Scoring scheme see Table 12.

Finding	Group 1	Group 2	Group 3	Group 4
HEART				
Myocardium degeneration	---	T202(3)	---	---
KIDNEY				
Amyloid deposit	M6(4), M9, M13, T4(4), T104(3)	206(4), M205(3), M207(3), M208(3), M210(3), M211(4), M212(3), M213(3), M215(3), M304(3), M305(4), M306(3), M307(3), M308(3), T202(3), T305(3)	M408(4), M413(3), M503(3), M505(3), T402(3), T504(3), T505(3), T506(3)	M606(3), M608(3), M707(3), M708(3), M709(3), M710(3), M711(3), M712(3), T703(4)
Inflammation interstit. chronic	---	M305(2), M307(1)	M408()	M608(2), M712(1)
Hydronephrosis	M101(4)	---	---	---
Papillary necrosis	---	M212(3) M307(2)	M408(2)	---
Tubuli cytoplasmatic alteration	---	---	---	M711(3)
Tubuli dilatation	---	M307(2)	---	---
Tubuli mineralzation	---	---	T402(1)	---
Tubuli protein casts	T104(3)	M305(2), M307(2), T305(2)	M604(1), T504(2)	M708(1), M711(2), T703(2)
ADRENAL GLAND				
Amyloid deposit	---	---	573(3)	---

Table 10, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Non neoplastic lesions. Data are presented by number of affected animals (Score). Scoring scheme see Table 12.

Finding	Group 1	Group 2	Group 3	Group 4
LACRIMAL GLAND Inflammation acute	M10(4)	---	---	---
LIVER Cytoplasmatic alteration	---	---	571(3)	---
Focal cellular change (basoph.)	---	M305(3)	M412(3)	---
Focal cellular change (eosinoph.)	---	M215(3)	---	---
Necrosis focal	M6(3), M13(3), T103(3)	234(3), M209(3), M213(3), M304(3), M306(3), M308(3), M309(3), T304(3)	433(3), 434(3), 571(3), M408(3), M409(2), M410(2), M502(3), T402(3), T502(2), T503(4)	M605(2), M607(3), M608(2), M703(4), M704(3), M706(3), M707(2), M709(3), M710(3), M711(3)
Cytoplsmatic vacuolization	M6(3) T103	---	---	---
OVARY Amyloid deposit	---	T305	---	---
Cyst	M3	M305	M412	---
Hematocyst	53	M215,T305	---	---
SMALL INTESTINE Amyloid deposit	---	---	M408(4)	---
Intussusception	---	M302	M502	---

Table 10, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Non neoplastic lesions. Data are presented by number of affected animals (Score). Scoring scheme see Table 12.

Finding	Group 1	Group 2	Group 3	Group 4
UTERUS				
Amyloid deposit	---	---	468,M408	---
Endometrium cystic hyperplasia	---	M208	---	---

Table 11: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Survey of the incidence of neoplastic lesions.

Finding	Number of affected animals in : Group 1	Group 2	Group 3	Group 4	sign.*
Animals with a neoplasm	11	23	26	24	1-2,1-3,1-4
Malignant lymphoma, total	10	21	25	16	1-2,1-3
Other neoplasms total	2	3	4	9	---
SPLEEN					
Hemangioma	0	1	0	1	---
Malignant lymphoma lymphocytic	1	7	1	4	1-2, 2-3
Malignant lymphoma mixed cell type	9	8	16	9	---
Malignant lymphoma histiocytic	1	0	1	0	---
THYMUS					
Malignant lymphoma mixed cell type	3	4	5	5	---
KIDNEY					
Malignant lymphoma NOS	0	3	2	1	
LYMPH NODES					
Malignant lymphoma mixed cell type	4	2	6	1	
Malignant lymphoma lymphocytic	0	0	1	0	
GL. MANDIBULARIS					
Malignant lymphoma mixed cell type	0	0	1	0	

sign.*: significant differences between groups indicated

Table 11, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Survey of the incidence of neoplastic lesions.

Finding	Number of affected animals in : Group 1	Group 2	Group 3	Group 4
LIVER				
Hemangioma	0	1	0	2
Hemangiosarcoma	0	1	0	1
Malignant lymphoma lymphocytic	0	4	2	2
Malignant lymphoma mixed cell type	0	1	5	3
Hepatocellular adenoma	0	0	0	1
Hepatocellular carcinoma	0	0	0	1
LUNG				
Carcinoma NOS, metastatic	0	0	0	3
TISSUE NOS				
Hemangioma	0	0	0	1
Malignant lymphoma NOS	0	0	0	1
Sarcoma NOS	0	0	2	0
PANCREAS				
Malignant lymphoma NOS	0	0	1	0
OVARY				
Granulosa cell tumor, benign	0	0	0	1

Table 11, cont.: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures. Histopathology findings. Survey of the incidence of neoplastic lesions.

Finding	Number of affected animals in : Group 1	Group 2	Group 3	Group 4
SMALL INTESTINE				
Malignant lymphoma NOS	0	0	2	0
Adenocarcinoma	1	0	0	0
Sarcoma NOS	0	0	1	0
UTERUS				
Leiomyosarcoma	1	0	0	0
EYE, LID				
Chondroma	0	0	1	0
SKIN				
Squamous cell carcinoma	0	0	0	1

Table 12: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Scoring scheme of histopathology findings.

Score	Verbal description
1	minimal
2	mild
3	moderate
4	marked
5	severe

Figure 1: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Body weight, males.

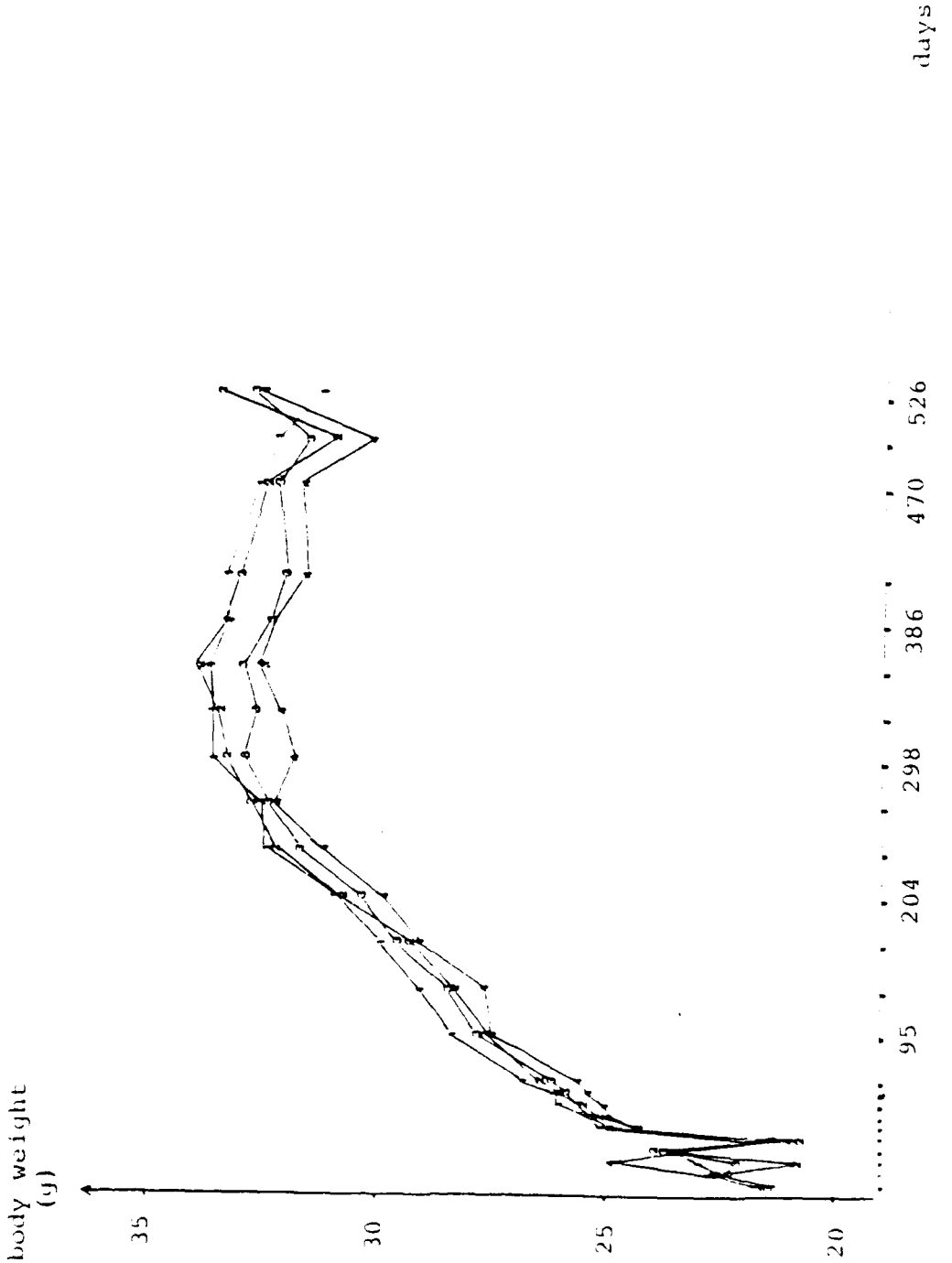


Figure 2: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Body weight, females.

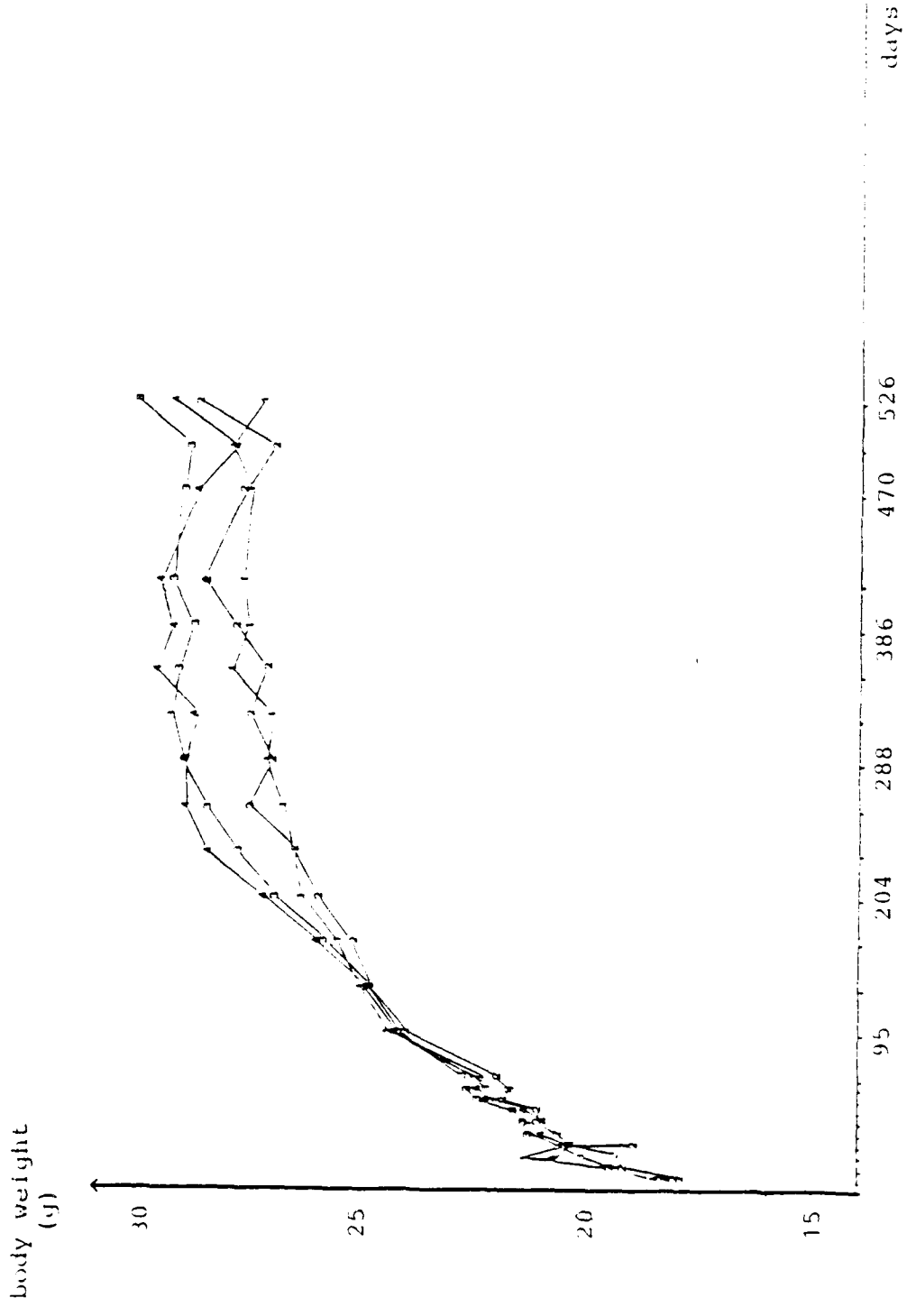


Figure 3: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Water consumtion, males.

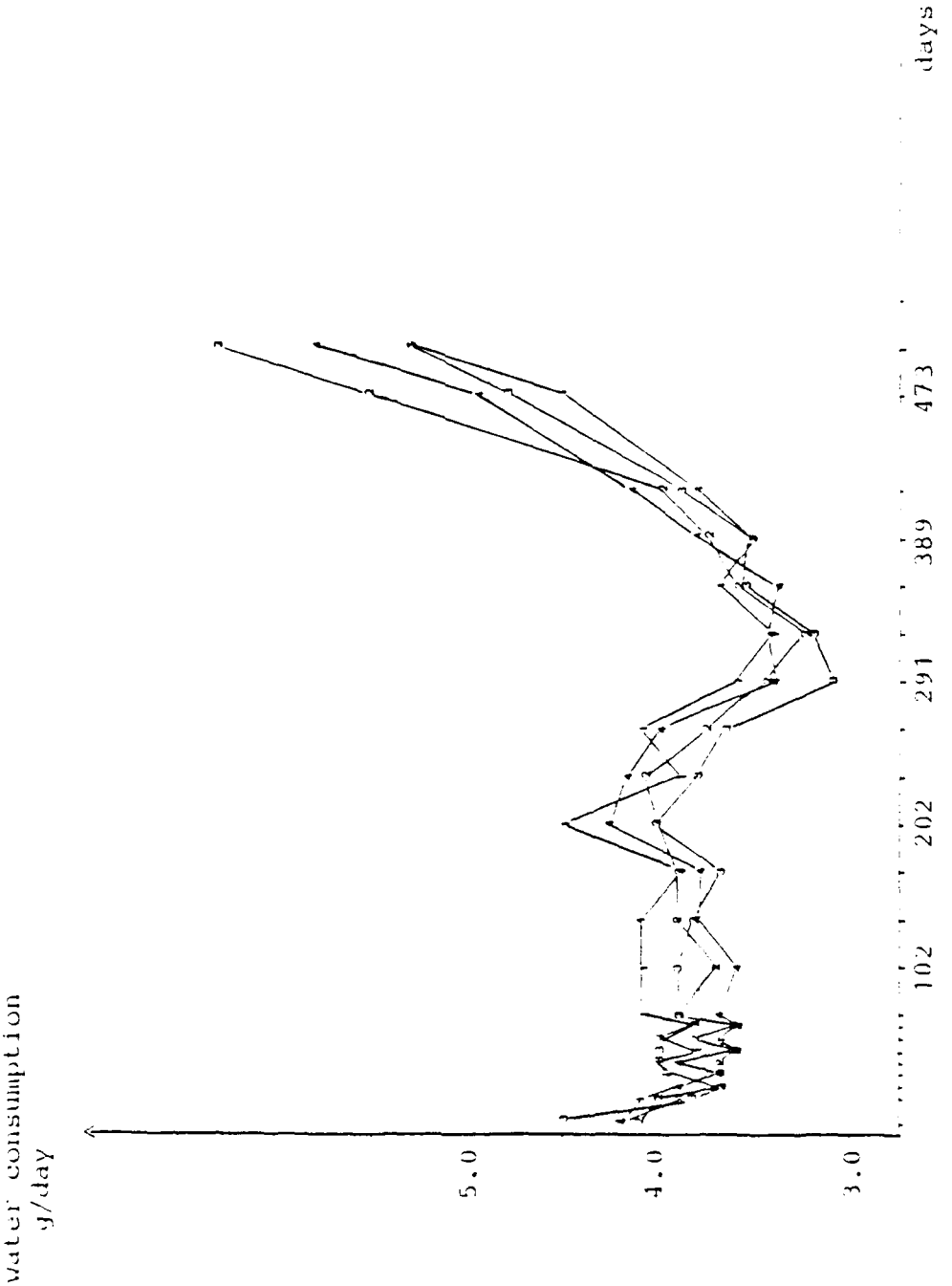


Figure 4: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Water consumption, females.

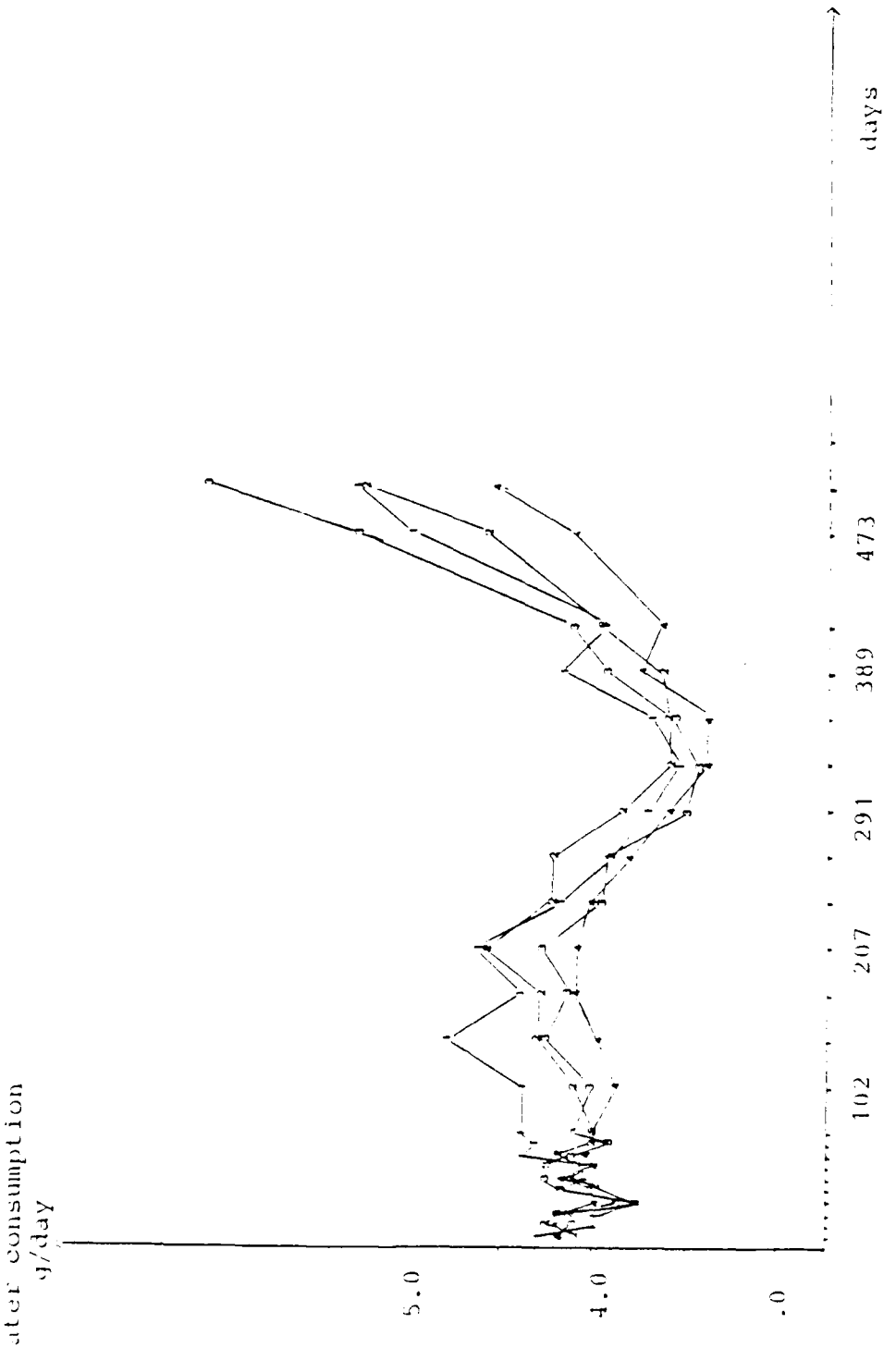


Figure 5: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Total number of animals in study, males.

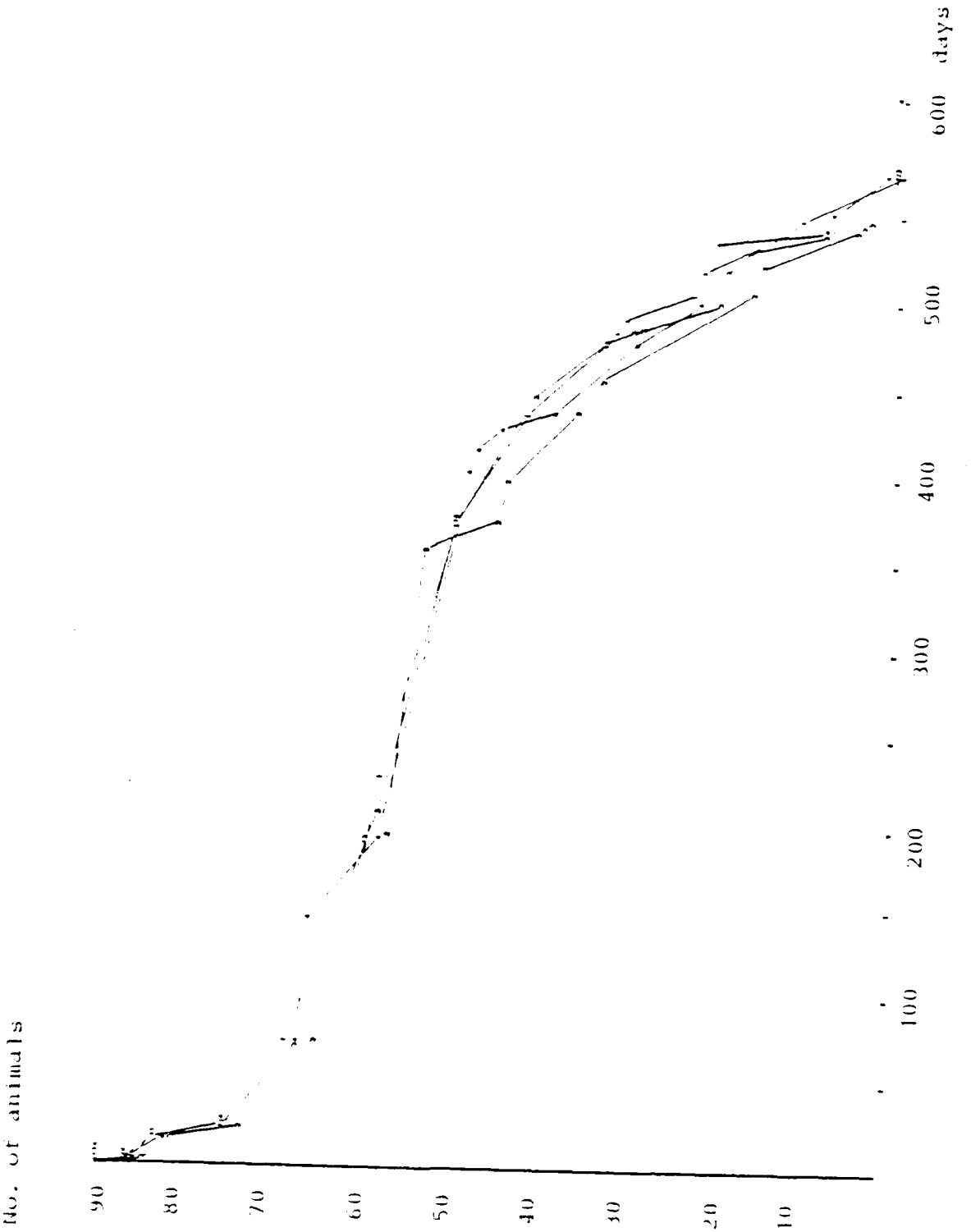


Figure 6: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Total number of animals in study, females.

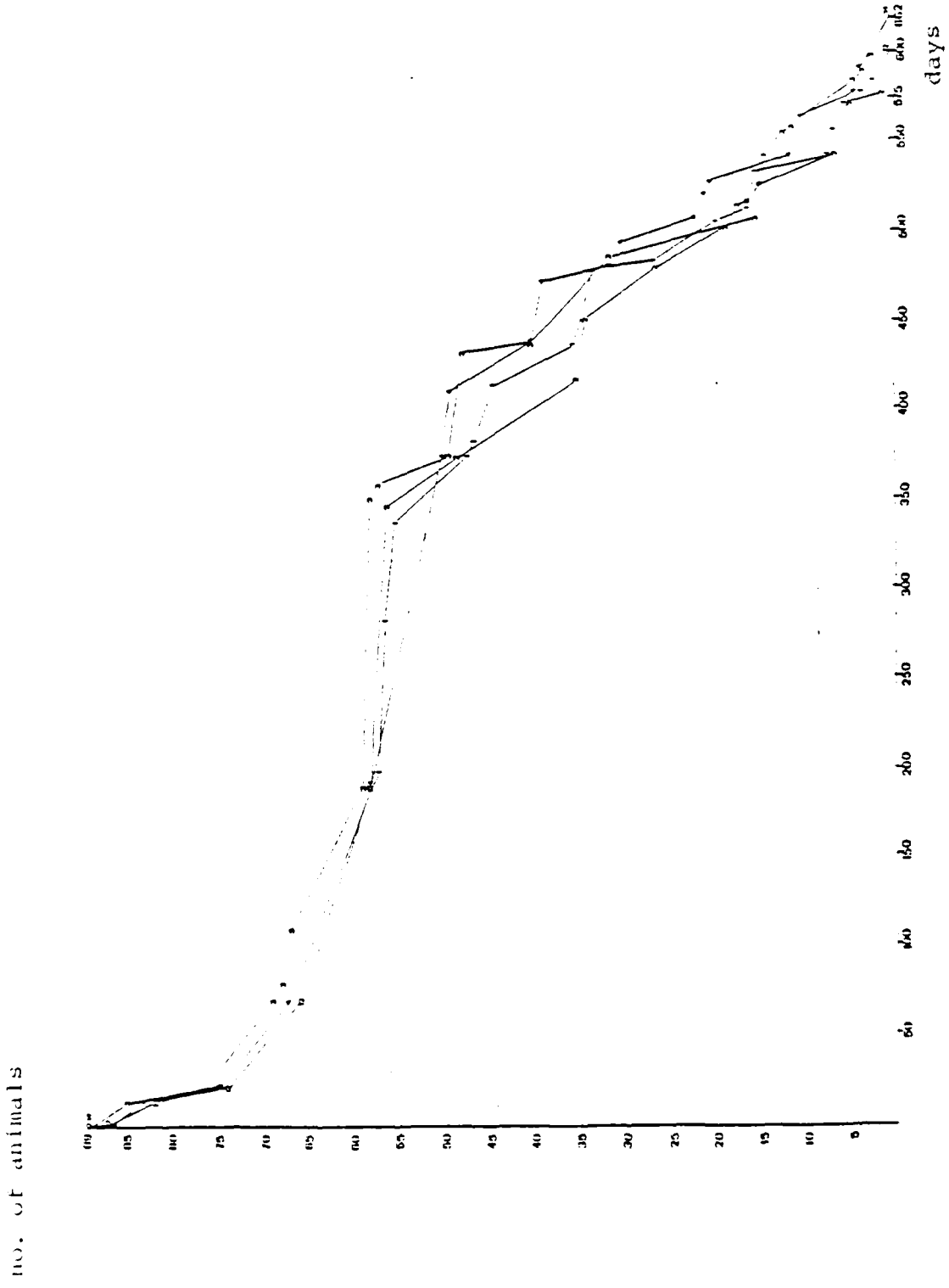
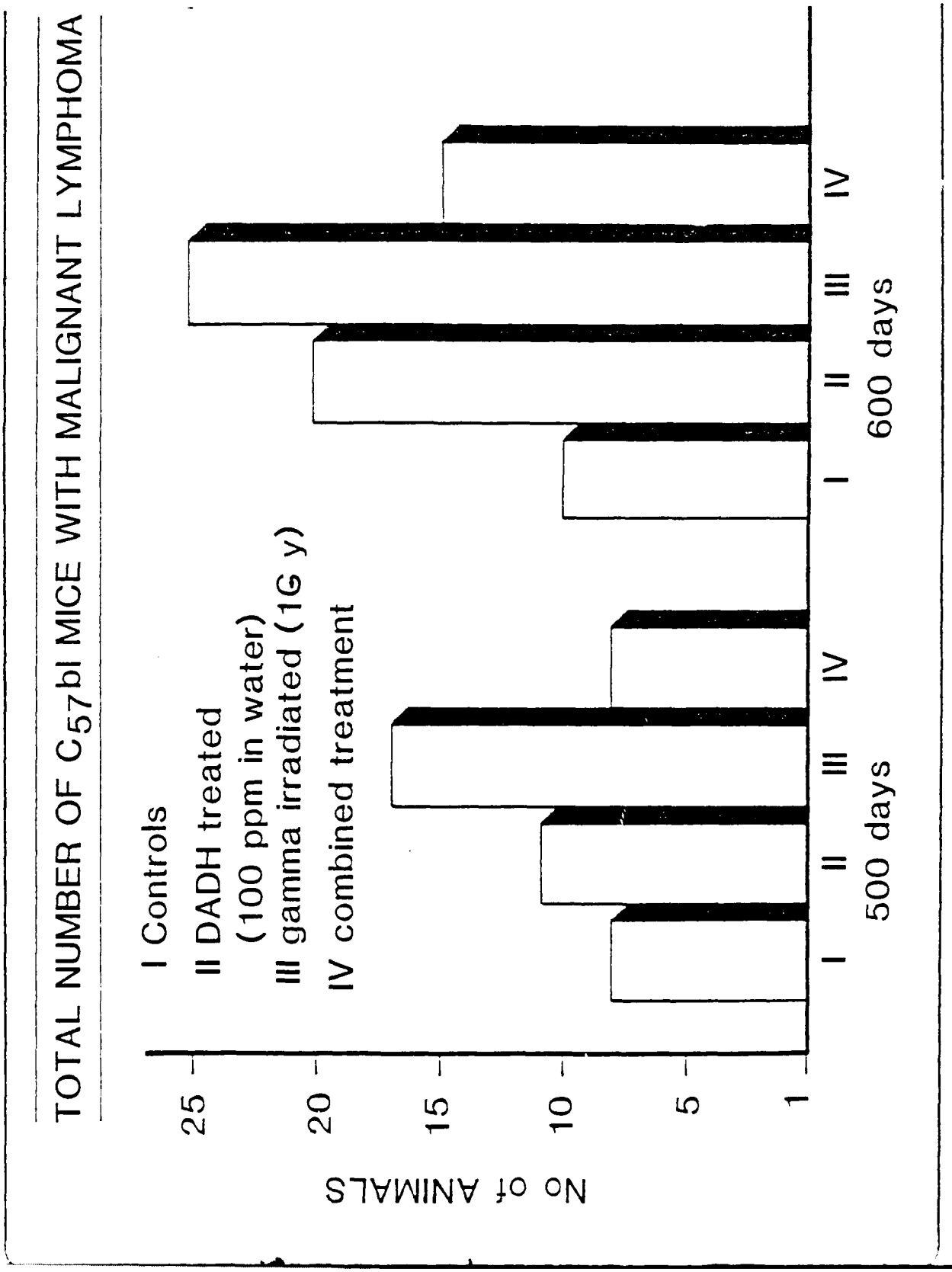


Figure 7: Animal experiments and pathology in: Animal studies on the mode of action of agents, that are antitransformers in cell cultures.
Number of animals with malignant lymphoma at typical Days.



LYMPHOCYTE SUBPOPULATIONS

The regulation of an induced immune response occurs by the interaction of different lymphocyte subsets. In the absence of one of these necessary cell subpopulations the circuit is interrupted and a deficient immune response will take place. Recently Jerne pointed out that the immune system in its ability to respond to antigen is complete, even when 90% of specific antibodies are lost. In mice, 100 rad ionizing radiation can decrease the peripheral lymphocyte count up to 25%. However, if the lymphocyte subsets have the correct cellular composition error free immune reactions can occur.

Views on the immunological consequences of radiation damage are still controversial because multiplicity of lymphocyte subsets and factors are required for immunoregulation. The radio-sensitivity of helper T cell subpopulations has been determined but that of suppressor T cells is unknown. It is also unknown whether or not precursors of helpers and suppressor T cells have the same radio-sensitivity. The radiation induced impairment of specific functions of lymphocyte subsets appears to be an important factor in the immune suppression and in the pathogenesis of viral infections, especially in immune deficient and autoimmune diseases as well as in carcinogenesis. Cytotoxic lymphocytes are believed to play a crucial role in the development of immunity to virus infections und tumors.

Differentiation of lymphocytic subpopulations

Lymphocytic subpopulations were investigated after application of 1 mM 3-MBA/d and 1 Gy 60-Co-gamma radiation and application of 1 mM DADH/d and 1 Gy 60-Co-gamma radiation.

The total T cell (Pan T) population in spleen cell suspensions was identified in a direct immunofluorescence test using the monoclonal antibody anti-Thy 1.2 FITC (Becton-Dickinson). Spleen

cell suspensions prepared to 10^6 cells/ml in cytotoxicity medium (Cedarlaue) were incubated at 37°C for 60 min to reduce background fluorescence due to unspecific binding. After centrifugation, the cell sediment was incubated with 10/ul anti-Thy 1.2 FITC at 4°C for 30 min. After incubation, cells were washed twice with PBS and then suspended in a solution of PBS and polyvinylalcohol in glycerol. Five to 10/ul of cell suspension were dropped onto slides and examined for percentage of fluorescent cells. Suppressor/cytotoxic T cells were differentiated by anti-Lyt 2 antibody (Becton Dickinson). For experiments with 3 MBA, a direct test was performed using the FITC-conjugate of anti-Lyt 2.

Since the fluorescence of cell membranes was very dim an indirect test was performed in the experiments done with DADH; instead of the FITC conjugate, biotinylated anti-Lyt 2 was used. After 30 min incubation and the washings of cells, Avidin FITC (1/ug/10^6 cells) was added and cells incubated for further 30 min. Further preparation was the same as for direct tests.

B cells were stained by anti-mouse Igh + M (Becton Dickenson) FITC in a direct test using 100/ul of 1:20 diluted antibody.

1 mM 3-MBA/d administered on 5 consecutive days did not show any effect on the percentage of Pan T cells, suppressor T cells and B cells (table 1), three days after termination of treatment. 60-Co radiation of 1 Gy caused a significant drop in the Pan T cell population, a small diminuation of suppressor-cytotoxic T cells, which was statistically not significant (table 1), and left B cell rates unchanged. The combination of 1 mM 3-MBA/d and 60-Co-gamma radiation signals an influence of 3-MBA on the balance of lymphocytes: Pretreatment with 3-MBA of irradiated animals prevented the reduction of T cells by radiation to a significant extent, and showed the tendency to render also suppressor-cytotoxic T cell values to normal rates (table 1).

Table 1: Lymphocytic subpopulations; C57 bl mice treated by 3-MBA 1 mM/kg/d and 1 Gy 60-Co-gamma radiation;
variance analysis, significance = 5%,
factor A: control (1), 3-MBA (2), irradiation (3),
3-MBA + irradiation (4)

	ANOVA	RANKV		1	2	3	4
Pan T	0.000	0.0001	m	32.5	32.4	20.8	36.2
	+	+	s	7.0	6.0	4.5	4.6
			n	10	10	10	10
	3 < 1, 4 > 3, differences significant						
Suppr/Cytotox	0.0017	0.0031	m	13.3	14.7	10.4	13.3
	+	+	s	2.4	2.1	2.1	2.6
			n	9	10	10	9
	3 < 1, difference significant						
B	0.0377	0.0922	m	54.3	50.3	58.7	49.5
	-	-	s	8.6	4.6	9.6	6.5
			n	10	10	10	10

The same tendency could be observed with DADH (table 2): no effect of DADH when applied on five consecutive days, a significant drop of Pan T cells three days after irradiation, a small, but statistically not significant increase after combined treatment in comparison with animals only irradiated without DADH pretreatment. The small B cell values observed (table 2) with the last series of experiments could be attributed to the fact that male animals were used, while only females had been used for all other experiments.

Table 2: Lymphocytic subpopulations; C57 bl mice treated with 1 mM/kg/d DADH and 1 Gy 60-Co-gamma radiation;
variance analysis, significance = 5%,
factor A: control (1), DAH (2), irradiation (3),
DAH + irradiation (4)

	ANOVA	RANKV		1	2	3	4
Pan T	0.0223	0,0668	m	29.1	27.0	23.3	25.7
	+		s	3.7	4.3	4.6	3.7
			n	10	10	10	10
	3 < 1, differences significant						
Suppr/Cytotox	0.5099	0.3809	m	11.9	11.0	9.7	12.1
	-	-	s	3.6	4.1	3.2	3.6
			n	9	9	9	9
	no significant difference						
B	0.5455	0.3278	m	40.3	40.8	40.1	36.1
	-	-	s	9.3	7.6	7.2	7.7
			n	10	10	10	10
	no significant difference						

An aspect of radioprotection 3-MBA on the immunesystem during radiation therapy see enclosure 4.

In the long term experiment lymphocyte subsets were investigated over the whole life span of C67 bl mice.

The results obtained on the number of Pan T cells, suppressor-cytotocxic T cells and B cells are illustrated in figures 1-3.

They are submitted to the following statistical analysis: Firstly, results of the four testgroups were compared with one another at any of the ten terms of ivestigation. No significant difference between testgroups could be established at a significance level of 3%. In a second analysis the terms of investigations were compared within each testgroup and subpopulation. Since significant differences were found between various terms of investigations, the linear regression between percentage of cells and age was studied:

a significant negative correlation between the number of Pan T cells and suppressor/cytotoxic T cells and the age of the animals was established.
Pan T cells - coefficient of regression = - 0.00655
suppressor/cytotoxic T cells - -"- = - 0.01213
significance level 5%.

No significance was found for the percentage of B cells.

These findings are in good accordance with the data of Hirokawa et al. (Gerontology 30 (1984) 223-233). The latter found a pronounced age related decrease in T cell mediated immune functions, while B cell activities showed no age-related change. At the very end of their life-span (670 days), animals showed an increase in the percentage of Pan T cells - an effect that was also observed by the Japanese authors for the mixed lymphocyte reaction.

In any case no effect of the test substance on lymphocytic subpopulations could be observed, although DADH showed a slight effect on the re-establishing of lymphocyte subsets in the short-term experiments. This results may by attributed to the fact that within the short-term experiments subsets were investigated three days after irradiation, whereas in the long-term investigations the first sampling took place seven days after irradiation. At that time lymphocytic subsets probably have already redressed their balance.

Figure 1

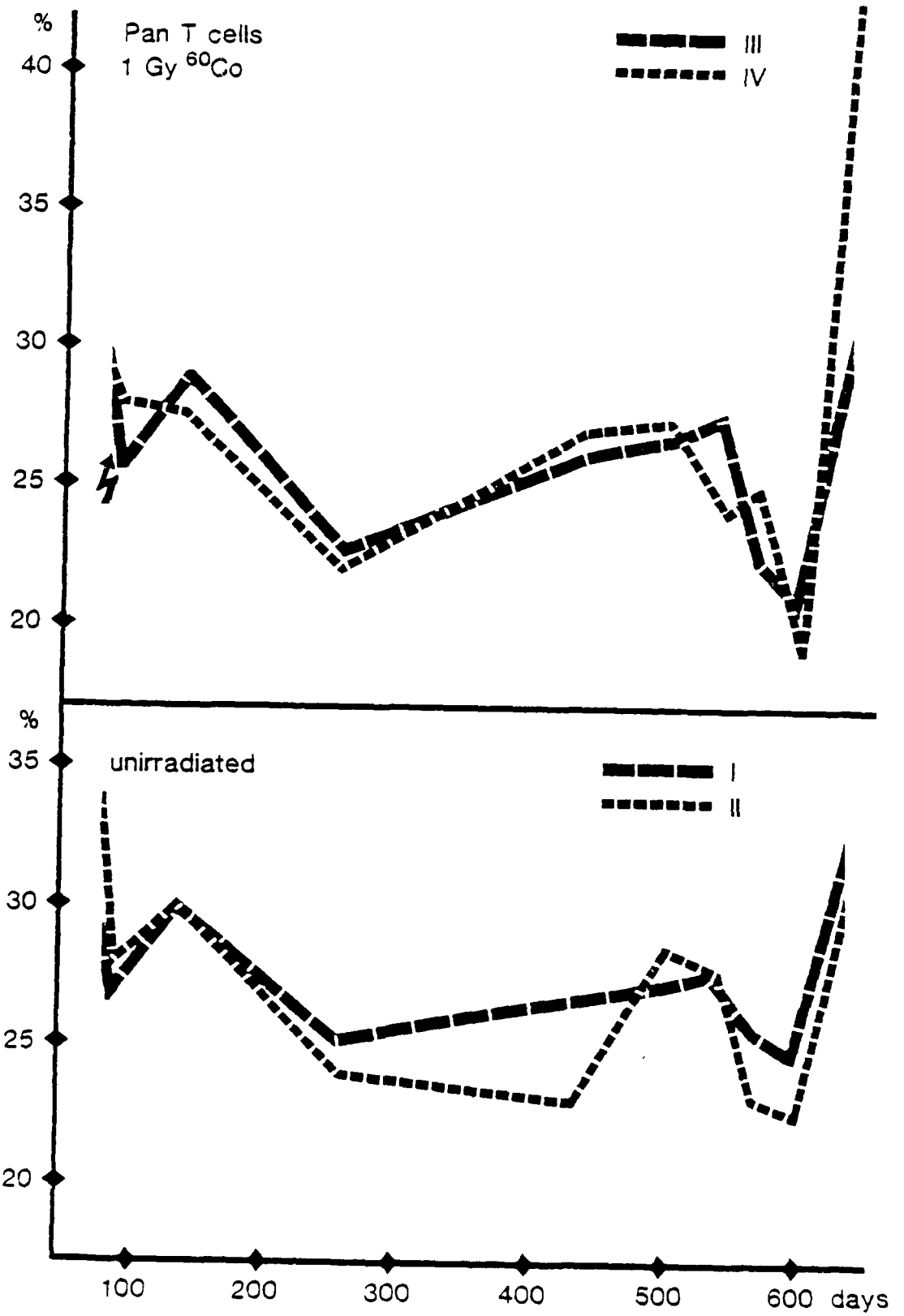

%
Pan T cells
1 Gy 60Co
III
IV
40
35
30
25
20
%
unirradiated
I
II
35
30
25
20
100
200
300
400
500
600 days

Figure 2

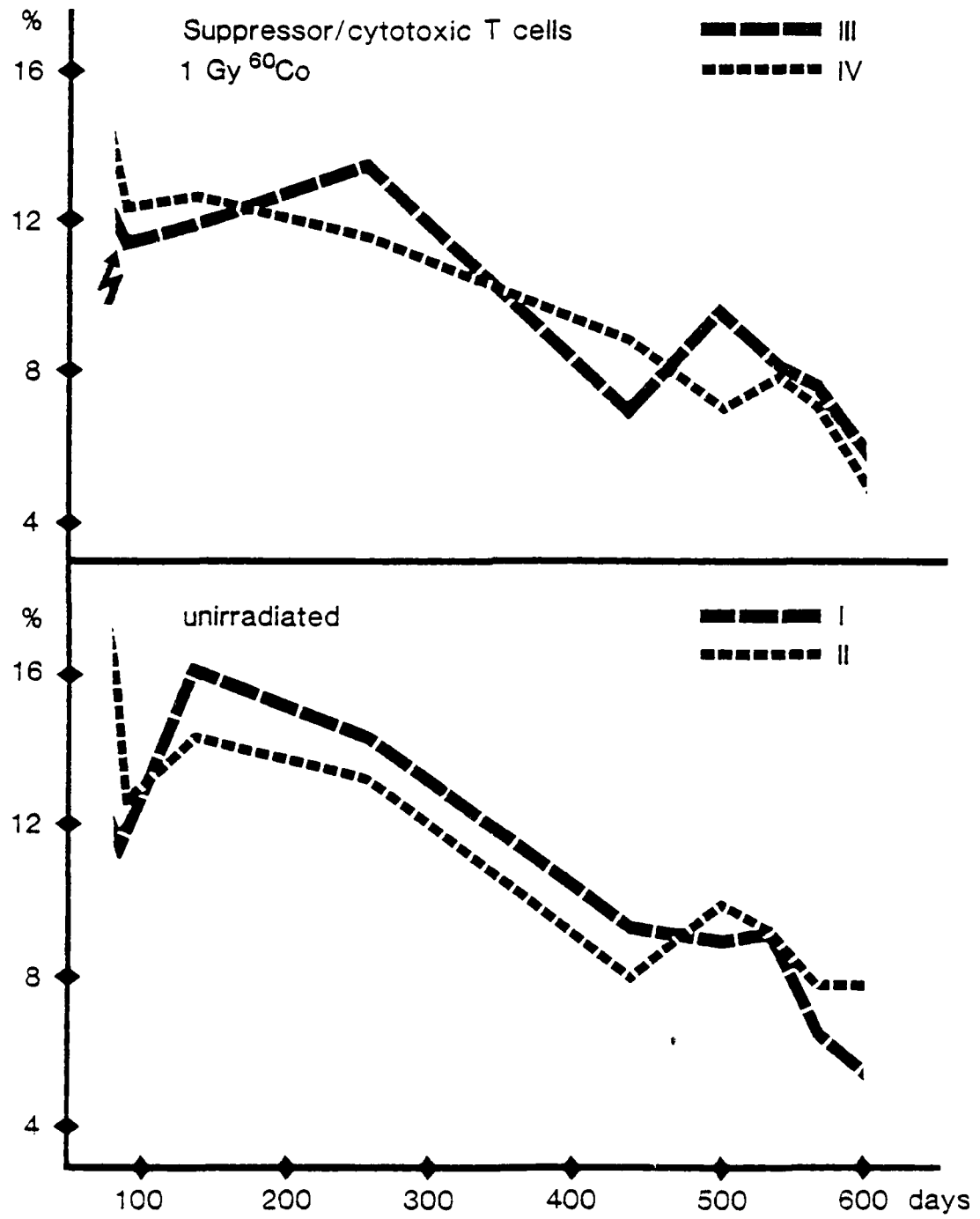
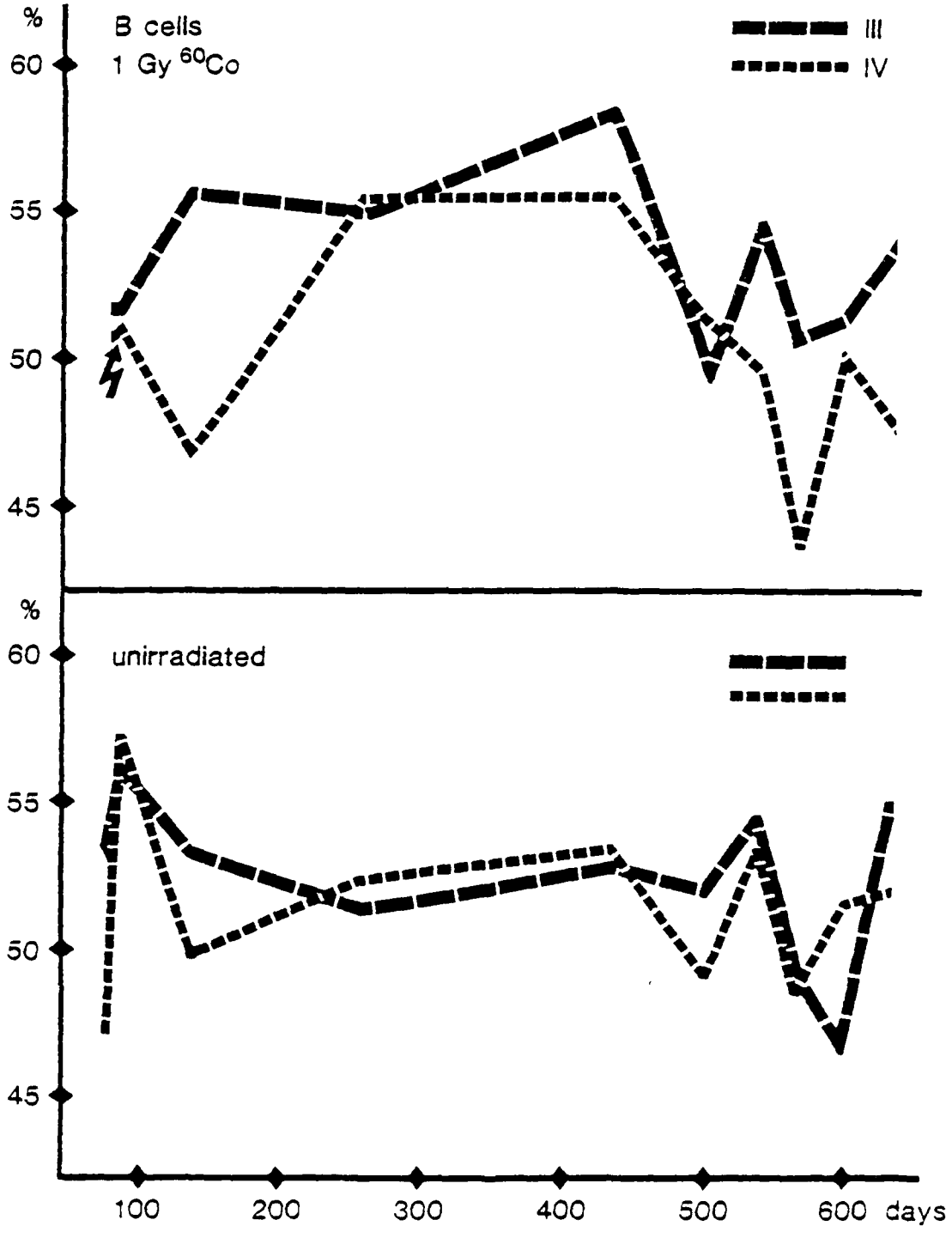


Figure 3


%
B cells
1 Gy ^{60}Co
III
IV
60
55
50
45
%
unirradiated
60
55
50
45
100
200
300
400
500
600 days

MODEL: VARIANCE ANALYSIS, ONE-FACTORIAL SIGNIFICANCE: 5.0 %

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: A
FACTOR: TEST GROUP

NAME	FACTOR A		1	2	3	4
NO	ANOVA	RANKV	M/S/N	M/S/N	M/S/N	M/S/N
FANT	.2648	.3138	29.30	33.50	29.70	29.35
8	-	-	1.65	3.74	1.55	5.00
A 1	4	4	4	4	4	4
SUPP	.2329	.1638	12.00	16.90	11.90	14.10
6	-	-	2.49	0.99	3.54	1.98
A 2	4	4	3	2	2	2
B	.1073	.1355	58.55	47.25	51.37	50.42
7	-	-	3.21	2.60	3.87	3.38
A 3	4	4	4	4	4	4

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: B
FACTOR: TEST GROUP

NAME	FACTOR A		1	2	3	4
NR	ANOVA	RANKV	M/S/N	M/S/N	M/S/N	M/S/N
FANT	.6998	.7167	26.47	28.09	25.86	27.16
5	-	-	3.71	3.46	4.94	3.13
A 4	4	4	8	8	8	7
SUPP	.7484	.8339	11.20	12.42	11.24	12.19
6	-	-	2.83	3.45	2.68	2.13
A 5	4	4	8	8	8	8
B	.1351	.1048	56.06	57.27	51.40	50.86
7	-	-	7.37	5.27	4.44	8.08
A 6	4	4	8	8	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: C
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	FACTOR A RANKV	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.8080 -	.8020 -	29.92 6.15	29.99 6.15	28.50 7.28	27.44 4.49
A 7	4	4	8	8	8	8
SUPP 6	.1601 -	.1491 -	15.92 4.65	14.30 4.12	11.80 2.74	12.41 3.81
A 8	4	4	8	8	8	8
B 7	.1292 -	.1521 -	53.47 6.08	48.90 8.55	55.56 9.98	46.99 5.77
A 9	4	4	8	7	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: D
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	FACTOR A RANKV	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.3541 -	.5585 -	24.89 3.82	23.91 4.37	22.34 4.17	21.53 2.98
A 10	4	4	8	8	7	7
SUPP 6	.4257 -	.5392 -	14.05 2.46	13.11 3.11	13.32 3.78	11.51 2.86
A 11	4	4	8	8	8	8
B 7	.7236 -	.9818 -	51.66 7.54	52.46 5.44	54.94 8.64	55.06 7.1
A 12	4	4	8	8	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: E
FACTOR: TEST GROUP

NAME	FACTOR A		1	2	3	4
NR	ANOVA	RANKV	M/S/N	M/S/N	M/S/N	M/S/N
PANT	.7349	.7533	26.30	22.59	25.72	26.45
5	-	-	8.74	7.08	6.52	8.77
A 13	4	4	8	8	8	8
SUPP	.3882	.2952	8.94	7.92	6.64	8.50
6	-	-	3.17	1.92	2.62	3.15
A 14	4	4	8	8	8	8
B	.4754	.5667	53.17	53.41	58.35	55.45
7	-	-	5.31	7.59	9.28	6.56
A 15	4	4	8	8	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: F
FACTOR: TEST GROUP

NAME	FACTOR A		1	2	3	4
NR	ANOVA	RANKV	M/S/N	M/S/N	M/S/N	M/S/N
PANT	.8627	.5485	26.75	28.41	26.16	26.96
5	-	-	5.11	3.07	6.16	6.69
A 16	4	4	8	8	8	8
SUPP	.3240	.3669	8.51	9.29	9.26	6.70
6	-	-	2.99	3.03	3.33	3.13
A 17	4	4	8	8	8	8
B	.7369	.6672	52.22	48.94	49.40	51.11
7	-	-	6.22	6.83	8.16	4.84
A 18	4	4	8	8	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: G
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	RANKVPAM	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.2667 -	.1852 -	27.60 6.71	28.04 5.32	27.37 4.93	23.46 2.79
A 19	4	4	7	8	8	8
SUPP 6	.9630 -	.9081 -	8.47 2.82	8.51 3.26	8.10 2.21	7.92 2.19
A 20	4	4	8	8	8	8
B 7	.2778 -	.2202 -	54.29 4.35	53.67 4.49	54.40 8.56	49.42 4.89
A 21	4	4	8	8	7	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: H
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	RANKV	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.3109 -	.2784 -	25.27 4.11	22.45 3.91	21.81 4.04	24.93 4.91
A 22	4	4	8	8	7	7
SUPP 6	.8061 -	.7647 -	6.36 2.33	7.35 2.50	7.40 2.19	7.00 2.29
A 23	4	4	8	8	7	7
B 7	.7079 -	.5940 -	48.70 8.30	48.22 8.05	50.36 6.70	44.98 5.75
A 24	4	4	6	6	5	5

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: I
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	RANKV	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.2411 -	.3684 -	24.15 6.75	20.95 3.83	20.09 5.50	18.84 4.49
A 25	4	4	8	8	7	8
SUPP 8	.3943 -	.6592 -	5.34 2.68	7.39 4.52	5.51 3.11	4.50 2.29
A 26	4	4	7	8	8	7
B 7	.4986 -	.5169 -	46.60 8.42	51.63 6.72	51.04 7.36	50.04 5.68
A 27	4	4	8	8	8	8

SELECTION: VARIANCE ANALYSIS, ONE-FACTORIAL
TERM: J
FACTOR: TEST GROUP

NAME NR	FACTOR A ANOVA	RANKV	1 M/S/N	2 M/S/N	3 M/S/N	4 M/S/N
PANT 5	.1360 -	.2615 -	30.90 5.09	29.45 0.49	30.10 8.20	42.75 1.34
A 28	4	4	2	2	2	2
SUPP 6	.6933 -	.2995 -	8.80 5.52	13.50 0.99	9.50 4.24	9.50 4.67
A 29	4	4	2	2	2	2

MODEL: LINEAR REGRESSION SIGNIFICANCE: 5.0 %

SELECTION: ANALYSIS OF REGRESSION
INDEPENDENT VARIABLE: AGE
DEPENDENT VARIABLE: PAN T CELLS

NAME NO	R ANZ	YM KONST.	ALT 4
PANT 5	.2238 * + *	35.84	391.2 * + *
A 39	280	28.4046	-.006551

SELECTION: ANALYSIS OF REGRESSION
INDEPENDENT VARIABLE: AGE
DEPENDENT VARIABLE: PAN T CELLS

NAME NR	R ANZ	YM KONST.	ALT 4
SUPP 6	.5972 * + *	9.63	396.8 * + *
A 40	276	14.4404	-.012129

SELECTION: ANALYSIS OF REGRESSION
INDEPENDENT VARIABLE: AGE
DEPENDENT VARIABLE: PAN T CELLS

NAME NR	R ANZ	YM KONST.	ALT 4
B 7	.0752 -	52.09	384.4 -
A 41	274	53.0918	-.002603

NATURAL KILLER CELL ACTIVITY

Natural killer (NK) cells capable of spontaneous lysis of tumor cells, and killer (K) cells that can lyse antibody-coated target cells have been demonstrated in many species. Much attention has been focused on these cells because of their possible role in the elimination of tumors and virus-infected cells. Although NK and K cells have been characterized as "non-T, non-B cells" because they are not generated in the thymus and do not express the usual array of T and B cell surface markers, their cell lineage remains an enigma. In mice natural killer cell activity declines several weeks after birth.

Target cells were YAC-1 cells obtained from FLOW (Moloney virus induced lymphoma). 5.10^6/ml target cells were labeled with $Na_2{}^{51}CrO_4$ (spec.act. 459 mCi/mg) using 100/uCi/ml at 37°C, for 1 h. After two washings, 4.10^4 target cells were mixed with effector cells at the ratio 1:25, 1:50 and 1:100 in a total of 2 ml complete medium (= RPMI 1640, 20% fetal calf serum, 100 U/ml penicillin, 100/ug/ml streptomycin, 292/ug/ml L-glutamine 5.10^{-5} M mercaptoethanol). Effector cells were spleen cells of C57 bl mice that had been irradiated by 1 Gy 60-Co with/without application of DADH (groups III and IV). The test was performed 17 days after irradiation. Lymphocytes were obtained after Ficoll-Urografin centrifugation of spleen suspensions.

Target and responder cells were incubated at 37°C in 5% CO_2 humidified incubator for 3 h. After 5 min centrifugation at 3000 rpm, 100/ul were removed from each supernatant and ^{51}Cr activity determined in a gamma counter. Spontaneous ^{51}Cr-release was measured in supernatants of YAC cell suspensions that had been incubated without effector cells for 3 h. Maximum release was counted after treatment of 4.10^4 target cells with 5% solution of sodium laurylsulfate for 5 min, followed by aqua dest and sedimentation of cells by centrifugation at 3000 rpm. Percent

specific ^{51}Cr-release was determined by the formula:

$$\% = \frac{\text{experimental release - spontaneous release}}{\text{maximum release - spontaneous release}} \cdot 100$$

Results: Treatment of animals by DADH per se did not cause any significant difference in natural killer cell activity (Tab. 1). When DAH was applied together with irradiation, it restored natural killer cell activity to almost normal values (Tab. 1) two weeks after irradiation. A further follow up of NK activity did not seem useful, since it is well established that NK cells subside to undetectable levels in adult mice beyond 12 weeks of age (Djeu, J.Y. et al., J.Immunol. 122 (1979) 182). The above mentioned results could be reaffirmed with a second group of animals (Tab. 2); in this second experiment all three ratios (1:100), 1:50, 1:25) produced different means for groups III and IV. But only the difference for the highest ratio (1:100) was statistically highly significant. The generally higher values of experiment 2 are due to the fact that a new charge of YAC-1 cells was used which showed much better ^{51}Cr-labelling.

Table 1: Natural killer cell activity determined by spontaneous ^{51}Cr release from YAC-1 target cells; target: effector cell ratio: 1:100, tests were performed 17 days after irradiation.

	% ^{51}Cr-release			
group	I	II	III	IV
animal 1	19.5	13.6	4.1	14.1
animal 2	10.8	11.3	9.0	12.7
animal 3	20.8	23.7	5.0	7.7
animal 4	14.7	13.9	1.4	9.5
animal 5	13.3	9.5	2.7	8.1
	m_I =	m_{II} =	m_{III} =	m_{IV} =
	15.8 ± 4.2	14.4 ± 5.5	4.4 ± 2.9	10.4 ± 2.8

(t-test: $m_{III} < m_{IV}$ - P < 0.1%, difference significant.

Table 2: Natural killer cell activity determined by spontaneous ^{51}Cr release from YAC-1 target cells 17 days after irradiation.

group	%Cr-release III		
ratio	1:100	1:50	1:25
animal 1	15.7	14.4	10.9
animal 2	19.4	14.1	11.8
animal 3	16.7	15.3	n.d. (<1)
animal 4	20.1	14.8	14.3
animal 5	19.5	19.2	17.1
	m_{III_1} = 18.3	m_{III_2} = 15.6	m_{III_3} = 13.5
s.d.	±1.9	±2.1	±2.8

group	%Cr-release IV		
ratio	1:100	1:50	1:25
animal 1	31.4	18.3	14.4
animal 2	54.5	41.7	31.6
animal 3	29.5	16.3	16.2
animal 4	43.1	10.7	n.d.(<1)
animal 5	26.9	14.1	8.8
	m_1 = 37.1	m_2 = 20.2	m_3 = 17.8
s.d.	±11.5	±12.3	±9.8

(t-test: $m_{III_1} < m_{IV_1}$, $P < 0.05\%$, difference highly significant)

It is of special interest, that in the combined group (IV) the NK activity is much higher compared to the irradiated group (III).

POLY(ADP-RIBOSE)-POLYMERASE ACTIVITY AND REPLICATIVE DNA SYNTHESIS: C57 bl MICE TREATED WITH DADH AND/OR γ-IRRADIATION.

INTRODUCTION

C57 bl mice permanently carry the information of a retrovirus in their genome. After γ-irradiation oncogene activation occurs and there is a high incidence for malignant lymphomas in these animals (1). The tranforming genes of retroviruses are c-onc genes derived from normal cellular genes and there is evidence that virus induced tranformation is correlated with increased level of expression of these genes (2). The cell derived onc sequences of thymic lymphomas in C57 bl mice are designated K and N ras and probably myc (I. Guerro, personal communication). With induction of differentiation a certain diminuation in virus related onc message was observed (2). The expression of related onc sequences was also detected in an neoplastic T-cell line which produces the human T cell lymphoma retrovirus HTLV. The expression of protooncogenes is induced following induction of terminal differentiation in vitro. Inappropriate expression of protooncogenes prevents the cell from reaching terminal differentiation. There is a close relationship between the molecular mechanisms underlying both proliferation and differentiation.

There is also a close association of certain protooncogene products with both, differentiation and proliferation. Point mutations in protooncogenes, which can result from defective or misrepair, are the reason for aberrant growth factors which can increase the tumorigenic potential.

A variety of oncogenes are amplified during tumor progression, and the frequency of gene amplification can be increased by agents which generate a transient inhibition of DNA synthesis, including radiation and carcinogens. When DNA synthesis is stopped during the first 2-4 hours of the S-phase, specific DNA amplification occurs, coding for enzymes involved in DNA metabolism.

Amplification of these oncogenes can enhance their expression, but mutation does not seem to be a necessary compagion of oncogene amplification. For the amplification process of oncogenes chromosomal rearrangements are necessary to bring the oncogene in close connection to a replication origin of DNA synthesis. In ealier experiments we could show that short repetitive interspersed DNA sequences called ALU sequences are present in DNA replication origins (3). In an in vitro system, enriched replication origins enhanced the activity of the poly(ADP-ribose)-polymerase present in chromatin (4).

Antitranforming drug action of modulators of poly(ADP-ribose)-polymerase (E.C.2.4.99) appeared to be connected to augmented enzyme activity in vitro (5) and in vivo (6) and seems to be restricted to the early S phase, possible by the biosynthesis of new poly(ADP-ribose)-polymerase protein (7). Inhibitors of poly(ADP-ribose)-polymerase may have promoting or inhibitory effects on the transformation process dependent on treatment schedule, concentration and cell cycle (5, 9 - 13). We have choosen the antitransforming and differentiation inducing drug hexamethylene bis-acetamide (diacetyldiaminohexane = DADH), because in vitro it prevented almost completely carcinogen induced transformation at 1 mM external concentration and even at 20 mM had no appreciable inhibitory effect on nuclear poly(ADP-ribose)-polymerase (5). Simultaneously with the differentiation process an increase in poly(ADP-ribose)-polymerase-activity was detected in erythroleukemia cells (14), in myoblasts (15), and in the present experiment in spleen cells of C57 bl mice. DADH is a potent inducer of differentiation in some cell types. The main part of this compound is deacetylated upon cell entry (16). DADH is well water soluble and a highly stable compound.

Marks et al. made daily injections of up to 80 mg/kg/d to tumored mice and saw an altered survival pattern, but not an increase in overall survival (17).

The fact that in our experiments DADH seems to be also a cancerogenic drug is of interest in connection with the deacylated product, because polyamines stimulate DNA directed DNA synthesis catalysed by mammalian type C retroviral DNA polymerase (18).

The control of DNA synthesis seems to be one of the key points in carcinogenesis. In all phases of this process, after initiation, during promotion and progression, inhibition of DNA synthesis can interrupt carcinogenesis.

In the present paper poly(ADP-ribose)-synthesis was compared with DNA synthesis (including DNA amplification) in relation to the occurrance of malignant lymphomas in C57 bl mice.

MATERIAL AND METHODS

C57 bl/6 mice, 8-10 weeks old (supplied by the Forschungsinstitut für Versuchstierzucht Himberg, Austria), were used for the experiments. Feed (altromin diet 131ff) and bedding material were γ-irradiated with 10 kGy for sterilization. The animals were divided into 4 test groups:

1. Controls,
2. Administration of DADH (100 ppm in drinking water, during whole life span).
3. Whole body irradiation with 1 Gy 60-Co with a dose rate of 0.2 Gy/min, once initially.
4. Administration of the test substance, during the whole life span and whole body irradiation, once initially after the first week of drug treatment.

In the short term experiments animals were sacrified 18 h after irradiation, the spleens removed and cell suspensions prepared at 4°C. The cell suspensions were washed twice with medium 199. Living cells were counted by Trypanblue staining.

PAR-synthesis: Immediately after preparing the cell suspensions raw chromatin was isolated by lysis in a solution of 0.1M EDTA, 0.002M Tris and 0.5% Triton X-100 for 5 min at 20°C. After washing the raw chromatin once with distilled water, 1 µCi ^{3}H-NAD (NEN, NET-443, 1.3 X 10^{11} Bq/nmol specific activity) was added in a mixture of 0.15M sucrose, 0.001M EDTA, 0.003M DTE, 0.04M

Tris, 0.04M $MgCl_2$ and 0.000125M NAD^+ and then incubated at 37°C for 30 min. The reaction was stopped by the addition of 40% perchloric acid (final concentration 20%) in the cold. The pellet was hydrolysed at 90°C in 6% PCA for 30 min. The DNA content (optical density at 260 nm and 290 nm) and the radioactivity (liquid scintillation counting) of each sample was measured in aliquots of the supernatant, obtained after centrifugation. The radioactivity (counts/min) of the samples was calculated as specific radioactivity (counts.min^{-1}./ug^{-1} DNA).

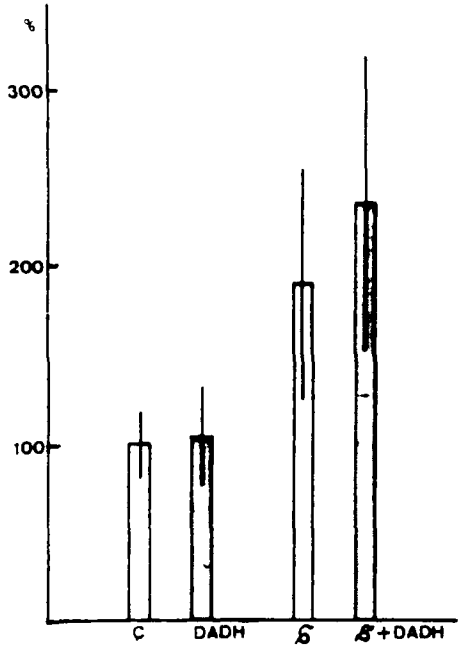
Replicative DNA-synthesis: 2.5/uCi ^3H-thymidine (NEN, specific activity 72 Ci/mmole) were added to the cell suspensions. This incorporation of thymidine in the DNA was stopped after incubation at 37°C for 90 min with icecold perchloric acid (PCA final concentration 6%). The precipitate was washed three times with 6% PCA. The pellet was hydrolysed at 90°C in 6% PCA for 30 min. The DNA content and the radioactivity of each sample was measured in aliquots of the supernatant after centrifugation. The specific radioactivity (cpm/ug DNA) was calculated.

Gene amplification measured from the occurrance of double minutes: Animals were sacrified and femoral bone marrow cells were cultured for 3 hours in complete medium in presence of colcemid and lymphocyte chromosome analysis were done after staining the slides with Giemsa (pH 6.8).

RESULTS

18 hours after whole body irradiation the DADH treated animals showed significant increase in poly(ADP-ribose)-polymerase activity (fig 1) in spleen cells.

Figure 1



At the same time replicative DNA synthesis is low, probable due to a block early in S-phase of spleen cells (fig. 2).

Gene amplification seems to be also restricted to the early S-phase and double minutes were counted in bone marrow cells (fig. 3). For details see enclosure 5.

Figure 2

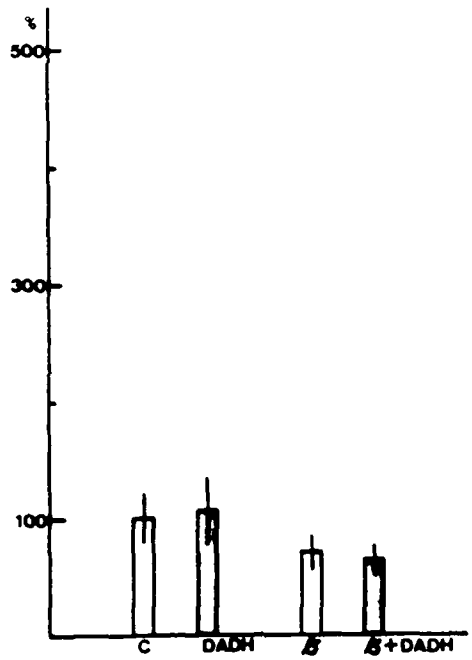
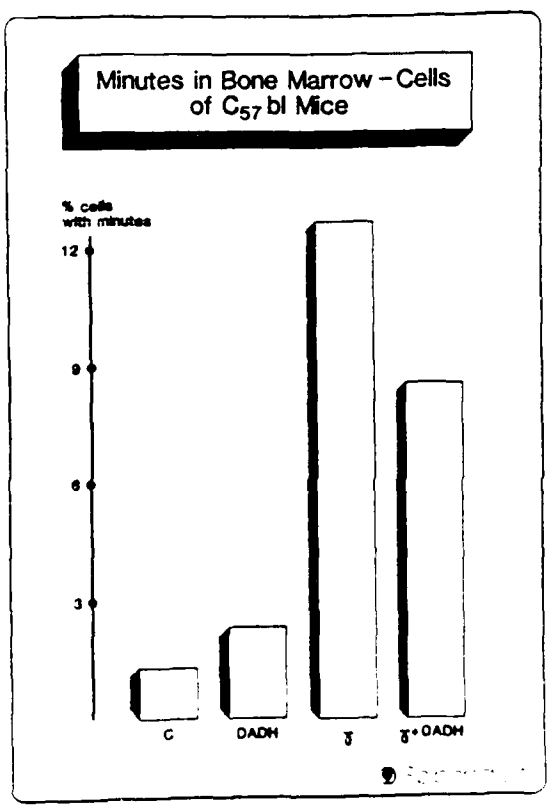
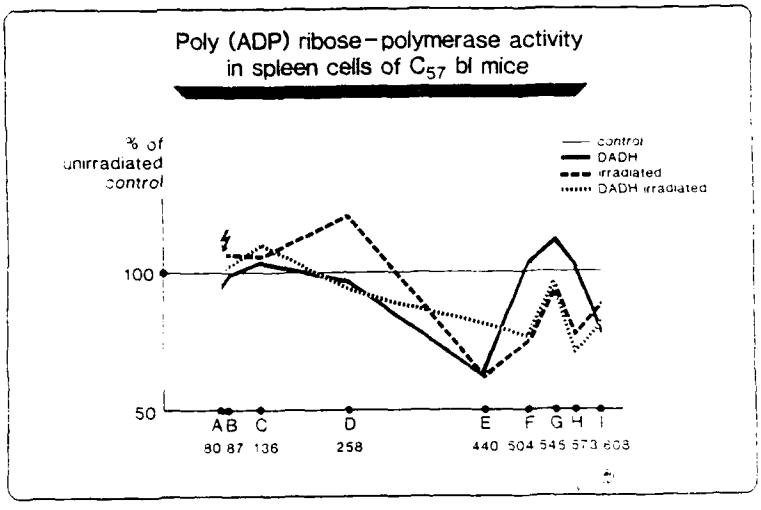


Figure 3



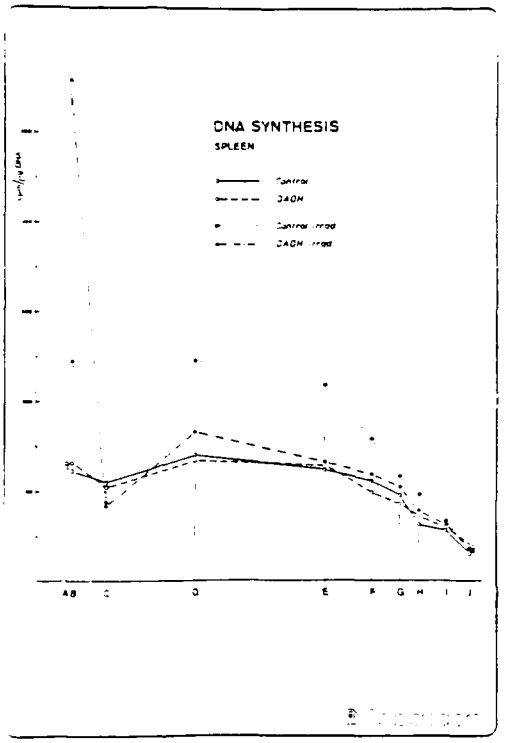
In our long term experiments poly(ADP-ribose)-polymerase activity was highest in 2/3 of the lifespan in irradiated animals, only in the last phase DADH treated mice showed higher values (fig. 4).

Figure 4



Replicative DNA synthesis was highest at the beginning of the experiments in the group with combined treatment (DADH + γ). Starting with age C, it increased in the irradiated group to the highest level of all groups (fig. 5).

Figure 5



DISCUSSION

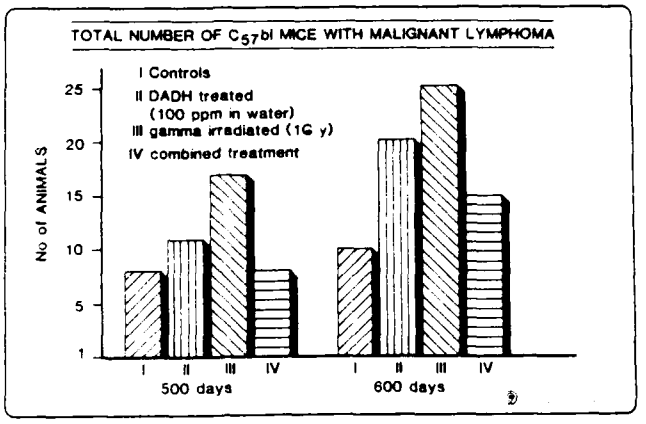
Cancer formation by γ-irradiation of 1 Gy is almost always a slow process requiring most of the life span of C57 bl mice. The long latency appears due to a stepwise, sequential transformation of normal into malignant cells via several intermediate cell populations. Differentiation steps are certainly of importance in these mechanisms. A relationship exists between inhibition of the cell cycle and differentiation of certain cells. The role of ADP-ribosylation in differentiation was first suggested by Caplan and Rosenberg (19). After an initial S-phase [illegible] poly(ADP-ribose)-synthesis increased (15), which was seen [illegible] our short term experiments (figs. 1 and 2). The increase [illegible] enzymological activity of the poly(ADP-ribose)-polymerase [illegible] an increase in the number of enzyme molecules, [illegible] activitation (20). But from our results

we will not exclude also de novo synthesis of the enzyme.

After DADH treatment of mice there was no significant increase in DNA strandbreaks. The activity of poly(ADP-ribose)-polymerase is not only dependent on the NAD^+ level or DNA strandbreaks, but also on an enzyme-associated DNA defined as s-DNA or coenzymatic DNA. The factor NAD^+ level or NAD^+-glycohydrolase activity could be excluded in our experiments, since we measured the enzyme activity in crude chromatin. From our amplification or double minutes data we know that replication origins are enriched in cells which show higher specific DNA amplification, because only DNA sequences located close to replication origins can be amplified. (For details see enclosure 6). Poly(ADP-ribose)-polymerase activity is in higher irradiated mice compared with the group of combined treatment. It is known that premalignant cells show higher poly(ADP-ribose)-polymerase activity. Only at the end of the life span DADH, which causes also a higher risk for cancer, produced higher values.

Replicative DNA synthesis was initially higher in the group of combined treatment, but during the rest of lifespan significantly lower than in the irradiated group (figure 5). There is a good correlation of the DNA synthesis in spleen cells with the occurrance of lymphomas, because 500 days after starting the experiments the number of malignant lymphomas was the same in the group of combined treatment compared with the control group. At the end of the life span of C57 bl mice, when the number of spontaneously occurring lymphomas was already high, the combined group was still lower compared with the DADH group and the irradiated group (figure 6). Poly(ADP-ribosylation) of proteins certainly plays a regulatory role in DNA metabolism and ultimatly new insights into the cause of cancer and its expression can be connected with this regulation process.

Figure 6



REFERENCES

1. GUERRERO, I., L.E. DIAMOND, E.W. NEWCOMB, J. LEON, J.W. BERMAN, R. LAKE and A. PELLICER: In vivo activation of mouse oncogenes by carcinogenic agents. Int.Cancer Congr.Abstr., C13 Mechanisms of Radiation Carcinogenesis. 1986, p. 486.

2. WESTIN, E.H., F. WANG-STALL, E.P. GELMANN, R.D. FAVERA, T.S. PAPAS, J.A. LAUTENBERGER, A. EVA, E.P. REDDY, S.R. TRONICK, S.A. AARANSON and R.C. GALLO: Expression of cellular homologues of retroviral onc genes in human hematopaietic cells. PNAS 79 (1982) 2490-2494.

3. ANACHKOVA, B., G. RUSSSEV and H. ALTMANN: Identification of the short dispersed repetitive DNA sequences isolated from the zones of initiation of DNA synthesis in human cells as ALU-elements. Biochem.Biophys.Res.Comm. 128 (1985) 101-106.

4. ALTMANN, H., A. TOPALOLGOU, G. BRKIC: Poly(ADP-ribose) present at the replication origin of DNA. In: Faktoren, die den DNA Metabolismus beeinflussen. Eds: L. Varga und H. Altmann, Sopron Dr. 1986, pp 3-13.

5. MILO, G., P. KURIAN, E. KIRSTEN und E. KUN: Inhibition of carcinogen induced cellular tranformation of human fibroblasts by drugs that interact with the poly(ADP-ribose)-polymerase system. Initial evidence for the development of transformation resistance. FEBS Lett. 179 (1985) 333-336.

6. GRIFFIN, M.J., W. KIRSTEN, R. CARUBELLI, R.B. PALAKODETY, J. McLICK and E. KUN: The in vivo effect of benzamide and phenobarbital on liver enzymes: poly(ADP-ribose) polymerase, cytochrome P-450, syrene oxide hydrolase, cholesterol oxide hydrolase, glutathione S-transferase and UDP-glucuronyl transferase. BBRC 122 (1984) 770-775.

7. SOOKI-TOTH, A., F. ASGHARI, E. KIRSTEN and E. KUN: Cellular regulation of poly ADP-ribosylation of proteins. II. Augmentation of poly(ADP-ribose)polymerase in SV40 3T3 cells following methotrexate-induced G1/S inhibition of cell cycle progession. Exp.Cell Res. 170 (1987) 93-102.

8. KUN, E., E. KIRSTEN, G.E. MILO, P. KURIAN and H.L. KUMARI: Cell cycle-dependent intervention by benzamide of carcinogen-induced neoplastic transformation and in vitro poly(ADP-ribosyl)ation of nuclear proteins in human fibroblasts. PNAS 80 (1983) 7219-7223.

9. BOREK, C., A. ONG, W.F. MORGAN and J.E. CLEAVER: Inhibition of x-ray and ultraviolet light induced transformation in vitro by modifiers of poly(ADP-ribose) synthesis. Rad.Res. 99 (1984) 219-227.

10. BOREK, C., W.F. MORGAN, A. ONG and J.E. CLEAVER: Inhibitrion of malignant tranformation in vitro by inhibitors of poly(ADP-ribose) synthesis. PNAS 81 (1984) 243-247.

11. LUBERT, R.A., J.T. McCARVILL and J.L. SCHWARTZ: Effects of 3-aminobenzamide on the induction of toxicity and transformation by various chemicals in BALB/3T3 ClA31-1 cells. In: ADP-ribosylation of proteins. Eds: F.A. Althaus, H. Hilz and S.Shall. Springer Verlag Berlin-Heidelberg, 1985, pp. 471-474.

12. MIWA, M., T. ISHIKAWA, T. KONDO, S. TAKAYAMA and T. SUGIMURA: Enhancement by m-aminobenzamide of methylazoxymethanol-acetat-induced hepatoma of the small fish "Medaka" (Oryzias latipes). In: ADP-ribosylation of proteins. Eds: F.A. Althaus, H. Hilz and S.Shall. Springer Verlag Berlin-Heidelberg, 1985, pp. 480-483.

13. MILLER, E.: Influence od dietary nicotinamide on N-Nitrosodimethylamine carcinogenesis in rats. In: ADP-ribosylation of proteins. Eds: F.A. Althaus, H. Hilz and S.Shall. Springer Verlag Berlin-Heidelberg, 1985, pp. 484-488.

14. RASTL, E. and P. SWETLY: Expression of Poly(adenosine diphosphate-ribose)polymerase activity in erythroleukemic mouse cells during cell cycle and erythropoietic differentiation. J.Biol.Chem. 253 (1978) 4333-4340.

15. TÖRÖK, O. and H. ALTMANN: Chromatinchanges in skeletal muscle cells during the fusion to myotubes. In: Growth, Cancer and the Cell Cycle. Eds: P. Shekan, S.J. Friedman. Humana Press Inc. Clifton/New Jersey, 1984, pp 27-39.

16. REUBEN, R.C.: Studies on the mechanism of action of hexamethylenbisacetamide, a potent inducer of erythroleucemia differentiation. BBA 588 (1979) 310-321.

17. MARKS, P.A., R. REUBEN, E. EPNER, R. BRESLOW, W. COBB, A.E. BOGDEN and R,A, RIFKIND: Induction of murine erythroleukemia cells to differentiate: A model for the detection of new antitumor drugs. Antibiotics, Chemotherapy 23 (1978) 33-41.

18. MARCUS , St.L., St.W. SMITH and C.J. BACEKI: Polyanmines stimulate DNA-directed DNA synthesis catalysed by mammalian type C retroviral DNA polymerases. J.Biol.Chem. 256 (1981) 3460-3464.

19. CAPLAN, A.I. and M.J. ROSENBERG: Interrelationship between poly(ADP-ribose)-synthesis intracellular NAD levels and muscle or cartilage differentiation from mesodermal cells of embryonic chick limb. Proc.Nat.Acad.Sci. 72 (1975) 1852-1857.

20. SHALL, S.: ADP-ribosylation, DNA repair, cell differentiation and cancer. In: ADP-ribosylation, DNA repair and cancer. Eds.: M. Miwa et al. Japan Sci.Soc.Press, Tokyo/VNU Science Press, Utrecht, 1983, pp. 3-25.

DNA REPAIR AFTER UV-IRRADIATION

Unscheduled DNA synthesis (UDS) was nonsemiconservative repair of damage to DNA, has been shown to occur over the entire genome. The process was first revealed by autoradiography when UV irradiation was shown to induce the uptake of labeled thymidine into non-S-phase cells. At least three steps are required: DNA damage, excision of the damage and DNA-strand polimerization and ligation.

The misrepair or incomplete repair of DNA damage may be an initial step leading to many types of genetic alterations and, indeed, many known chemical mutagens and/or carcinogens and radiation have shown to induce UDS. More recently, attention has been focussed on the use of primary cell cultures from various tissues for UDS assays. These systems may provide more relevant information on the genotocixicity of chemicals or irradaition to selected target cells.

Comparison of the response observed in cells following in vivo and in vitro treatment underscores some basic differences in these two methodologies. Treatment of the whole animal generally provides a more accurate measure of the response in a particular target tissue than in vitro assays; however, these tissue-specific assays may frequently miss genotoxic compounds that produce tumors in other target tissues.

Cell suspensions prepared from spleens of 5 ml PBS, containing about 5.10^6 cells/ml, were irradiated by a 15W germicidal lamp at 254 nm and an incident dose rate of $1J/m^2$ sec at 4^oC for 20 sec. Immediately after irradiation 10 uCi/ml ^3H-thymidine (NEN, spec.act. 80 Ci/mM) was added and cell suspensions incubated at 37^oC for 90 min. Unirradiated samples treated in the same way served as controls for the evaluation of background and replicative DNA synthesis (= SDS). After 90 min incubation,

^{3}H-thymidine uptake was stopped by addition of excess cold thymidine, the cells washed several times and fixed in icecold methanol-acetic acid (3:1). Autoradiograms were prepared by Kodak NTB 3 liquid emulsion, exposed for 13 days; after development, slides were stained with Giemsa.

One hundred cells were scored on each slide, the relative reflexion of silver grains per labeled cell was determined photometrically by a Zeiss microscope photometer OIK.

It was pointed out in the report of the grant period 1984/1985 that in the short term experiments 1 mM 3-MBA did not affect the amount of UDS induced by UV radiation; no differences in the relative incorporation of ^{3}H-thymidine per cell and the percentage of labeled cells were found between the control group and the group receiving 3-MBA. 1 Gy of gamma radiation caused a slight decrease in UV-induced UDS; combined treatment of 1 Gy gamma irradiation and 3-MBA affected UDS to the same extent; but in both cases no significance of this effect could be demonstrated by variance analysis.

In the long term experiment we could find that spleen cells without grains increased remarkable with age. The cells with 50 - 150 grains/cell and cells with <50 grains dont show great changes during the life time. In the control group cells with more than 150 grains are decreasing with age, but in the DADH, γ- and γ+ DADH groups there is again an increase at the end of the life span. It is of interest that in this period the lymphoma incidence is increasing (fig. 1).

If we compare the ratio between cells with silvergrains to cells without silvergrains, we can recognize that in all groups at the end of the life span a great number of cells without repair incorporation of ^{3}H-thymidine exists. In both irradiated groups there is also a drop of grains/cell soon after irradiation . In the γ+DADH group the reconstitution of cells, which show repair goes quicker and reaches at points C and E the critical values (fig. 2, 3).

Figure 1

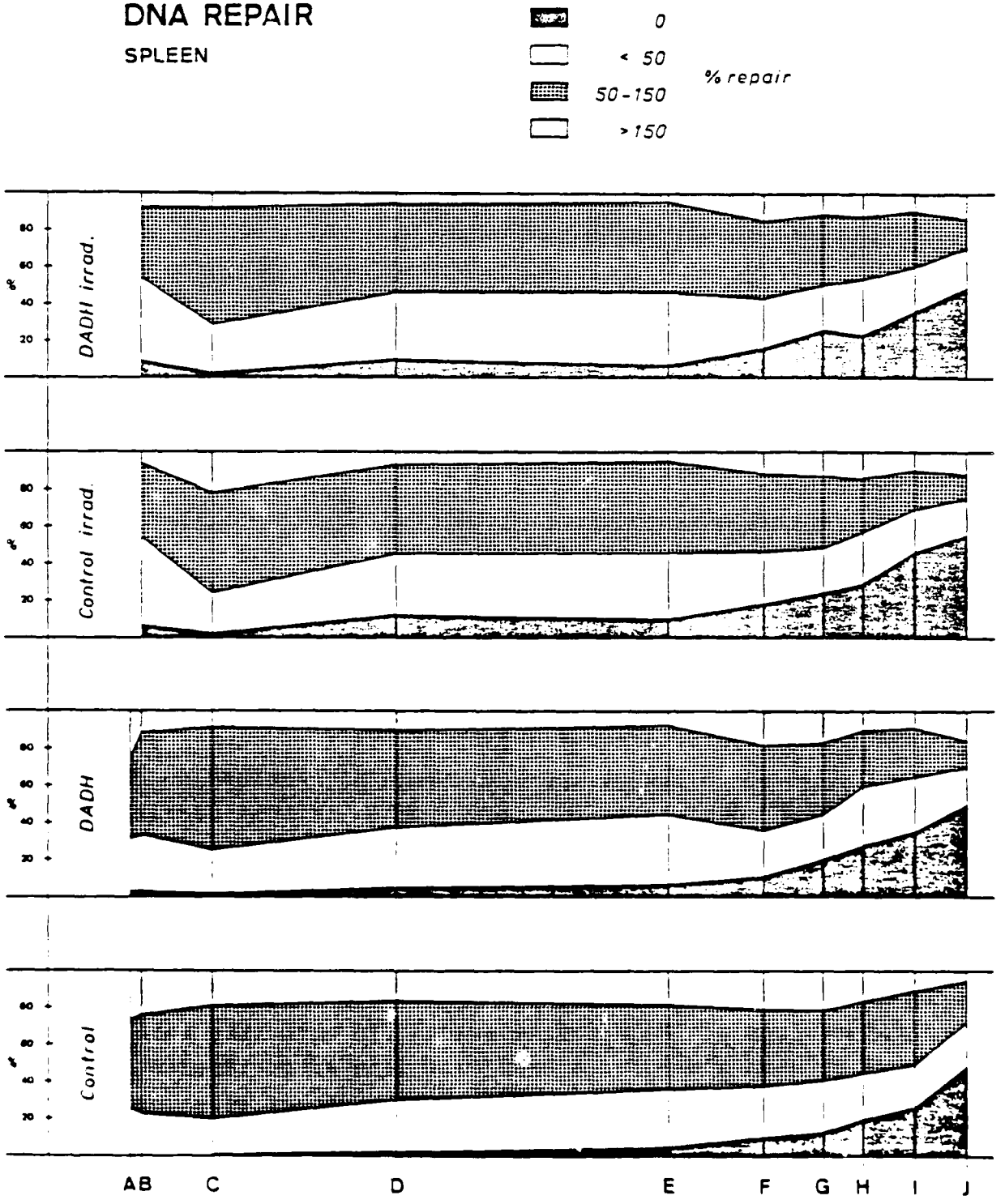

DNA REPAIR
SPLEEN
0
< 50
50-150
>150
% repair
DADH irrad.
Control irrad.
DADH
Control
AB
C
D
E
F
G
H
I
J

Figure 2

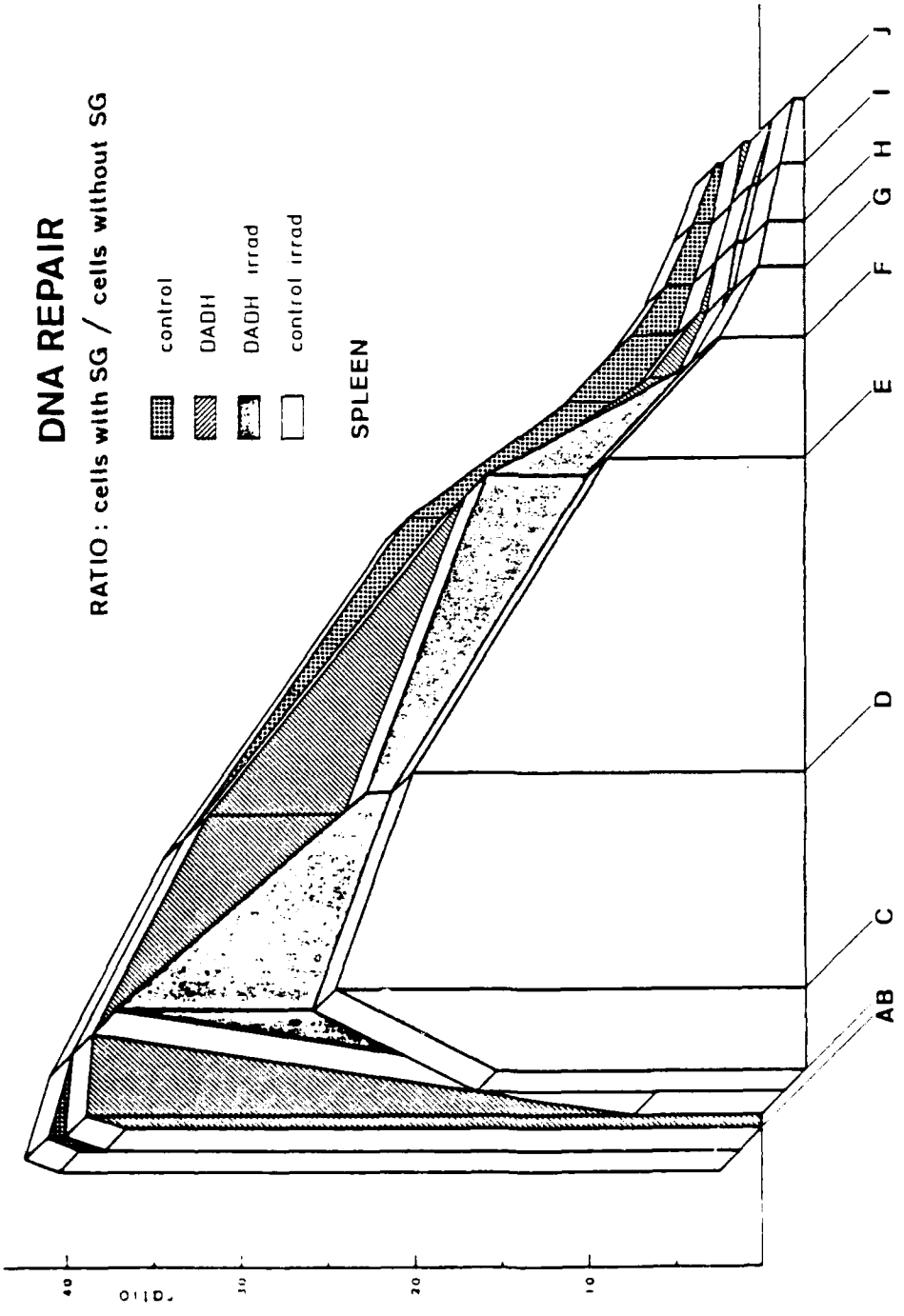
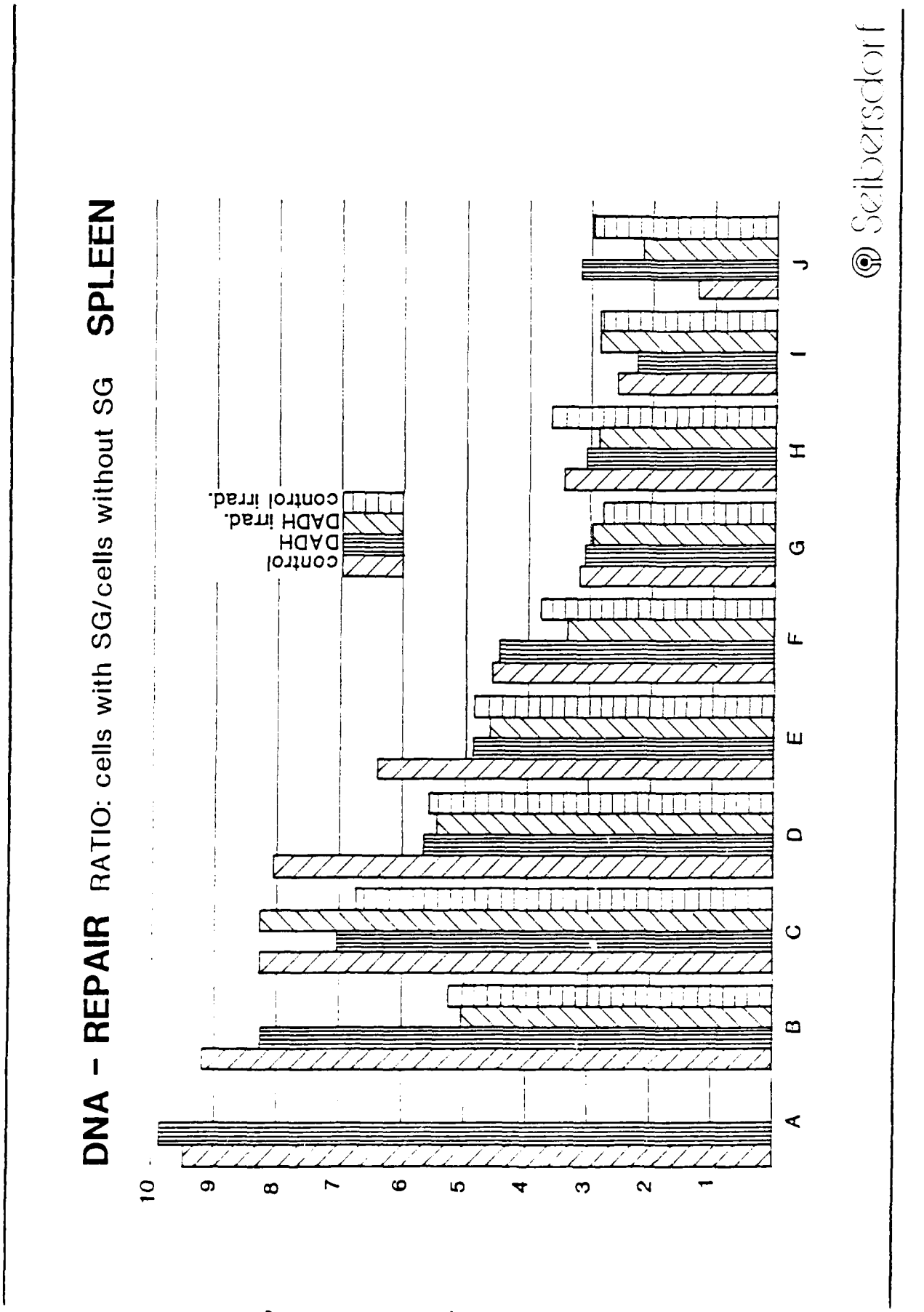

DNA REPAIR
RATIO : cells with SG / cells without SG
control
DADH
DADH irrad
control irrad
SPLEEN
J
I
H
G
F
E
D
C
AB
40
30
20
10

Figure 3


DNA - REPAIR RATIO: cells with SG/cells without SG SPLEEN
control
DADH
DADH irrad.
control irrad.
A
B
C
D
E
F
G
H
I
J
1
2
3
4
5
6
7
8
9
10
© Seibersdorf

It seems that there is a certain correlation between this obtained data and the occurrance of lymphomas.

INFLUENCE OF ANTITRANSFORMER ON NUCLEOIDSEDIMENTATION

In C57 bl mice a C-type retrovirus is latent present and thymus is essential for virus replication. In the short term experiments we used thymus cells for our nucleoidsedimentation studies. Since the thymus degenerates during life span spleen cells were investigated in the long term experiments.

Nucleoid sedimentation (supercoiled DNA)

Nucleoids can be obtained by lysing cells with non ionic detergents in the presence of high salt concentration. The secondary and tertiary structure (supercoils) of DNA remains intact under these conditions. Besides of DNA, nucleoids contain nuclear RNA and small amounts of nuclear proteins. A loss of supercoils caused by DNA incisions, DNA strand breaks or intercalating agents leads to changes of the sedimentation velocity of nucleoids.

A linear gradient was prepared (15 to 30% sucrose, 1.95 M NaCl, 0.001 M EDTA Na_2, 0.01 M Tris, pH 8.0) in centrifuge tubes and overlayered by a lysing mixture (1.95 M CaCl, 0.1 M EDTA Na_2, 2 mM Tris, 0.5% Triton X-100). Cell suspensions, non irradiated, immediately and 90 minutes, respectively, after an in vitro irradiation (1 Gy 60-Co) were lysed on the top of the gradients for 20 min and afterwards centrifuged at 132.000 x g and 20°C for 55 min. The sedimentation profile (position of the DNA maximum) was determined by measuring the extinction at 254 nm in a flow photometer. The sedimentation distance of the nucleoids as measured from the top of the gradients was related to the whole length of the gradient (R_s-values) (fig. 1).

Both substances behave similarly with respect to the nucleoid sedimentation profiles. An increased sedimentation velocity can be seen especially in the combined group. Proteaseinhibition or increased DNA content per nucleoid generated by gene amplifica-

tion could be an explanation for this effect.

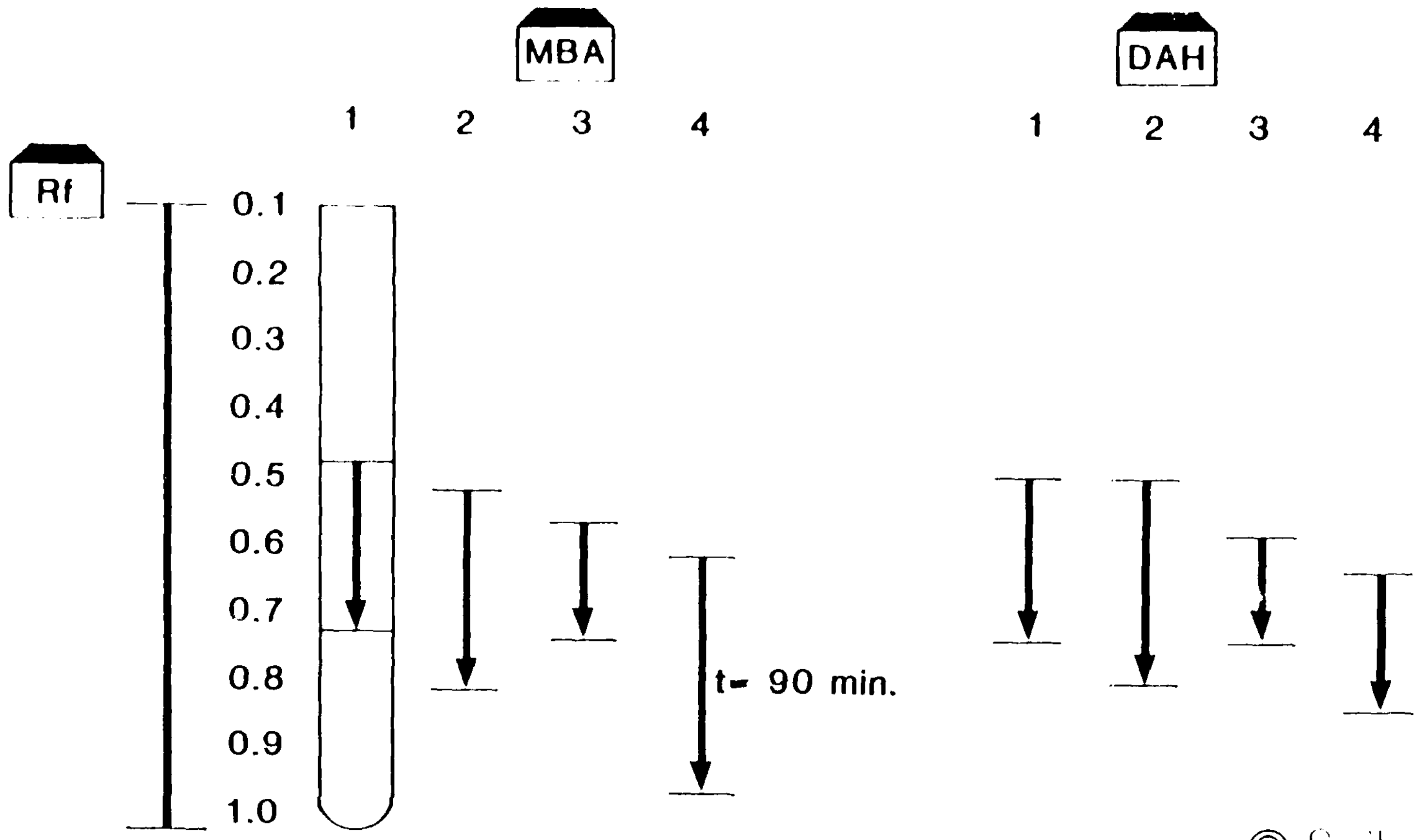
In the long term study a similar effect could be observed in the second part of the life span of irradiated mice (fig. 2).

If we calculate the ratio between nucleoidsedimentation and poly(ADP-ribose)-polymerase activity the irradiated group shows the highest values, a moderate effect can be observed in the γ-DADH group (fig. 3). Further experiments are necessary to explain these effects.

Figure 1

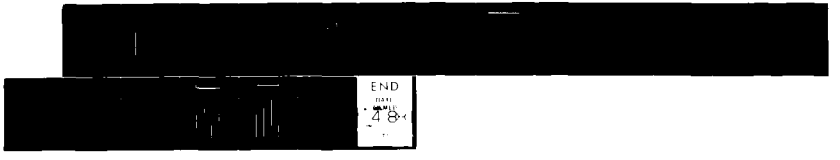
NUCLEOIDSEDIMENTATION OF MOUSE THYMUS CELLS

1) CONTROL
2) MBA/DAH TREATED
3) 1Gy Co^{60} IRRADIATED
4) MBA/DAH TREATED AND IRRADIATED

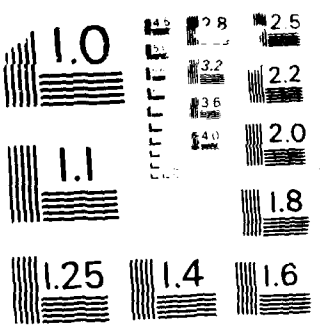


Seibersdorf

AD-A190 111 ANIMAL STUDIES IN THE MODE OF ACTION OF AGENTS THAT ARE 2/2
ANTITRANSFORMERS I. (U) OESTERREICHISCHES
FORSCHUNGSZENTRUM SEIBERSDORF G M B H VIENN.
UNCLASSIFIED H ALTMANN 28 OCT 87 AFOSR-IR-87-1989 F/G 6/15 NL



END
DATE
FILMED
4-88
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Figure 2

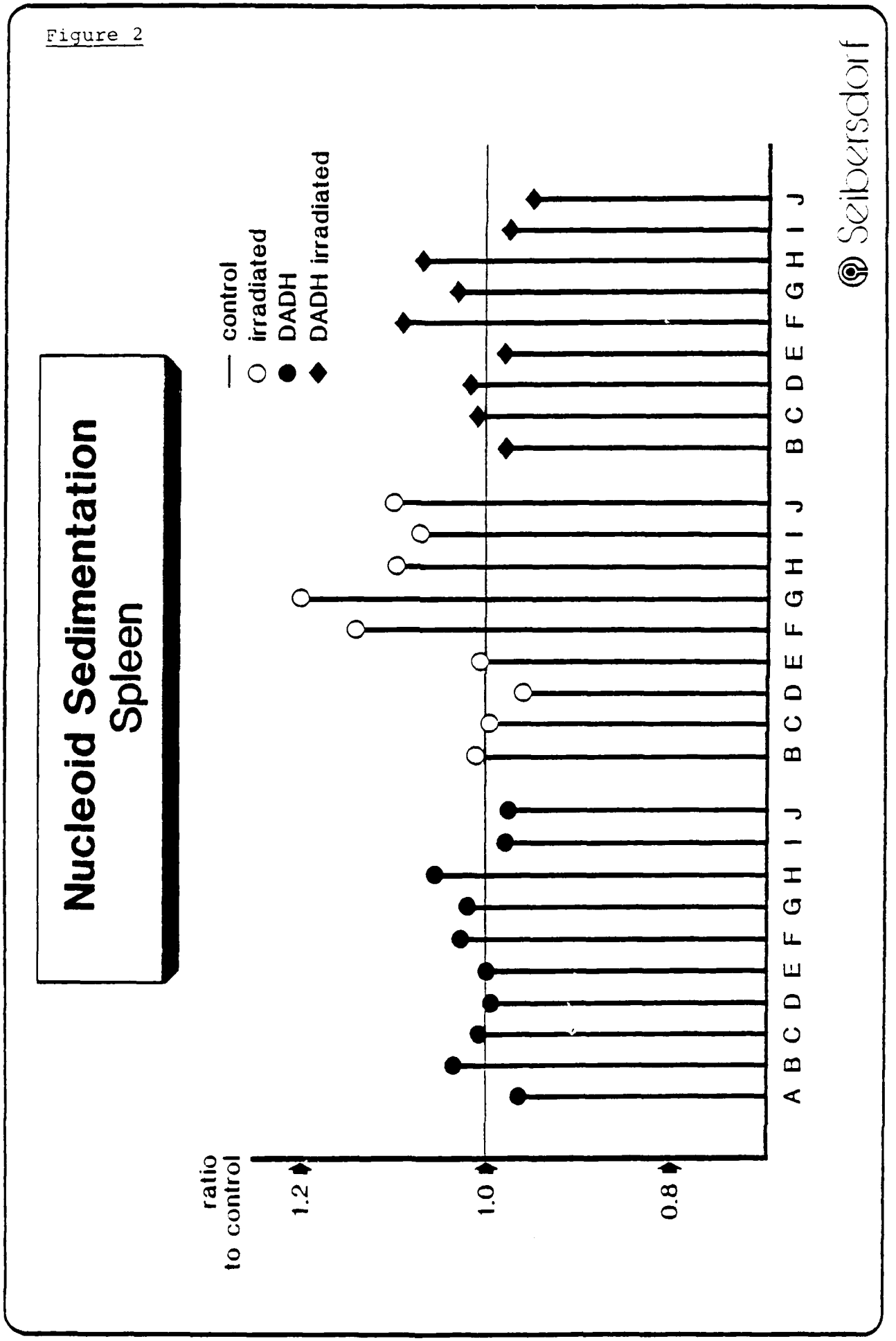
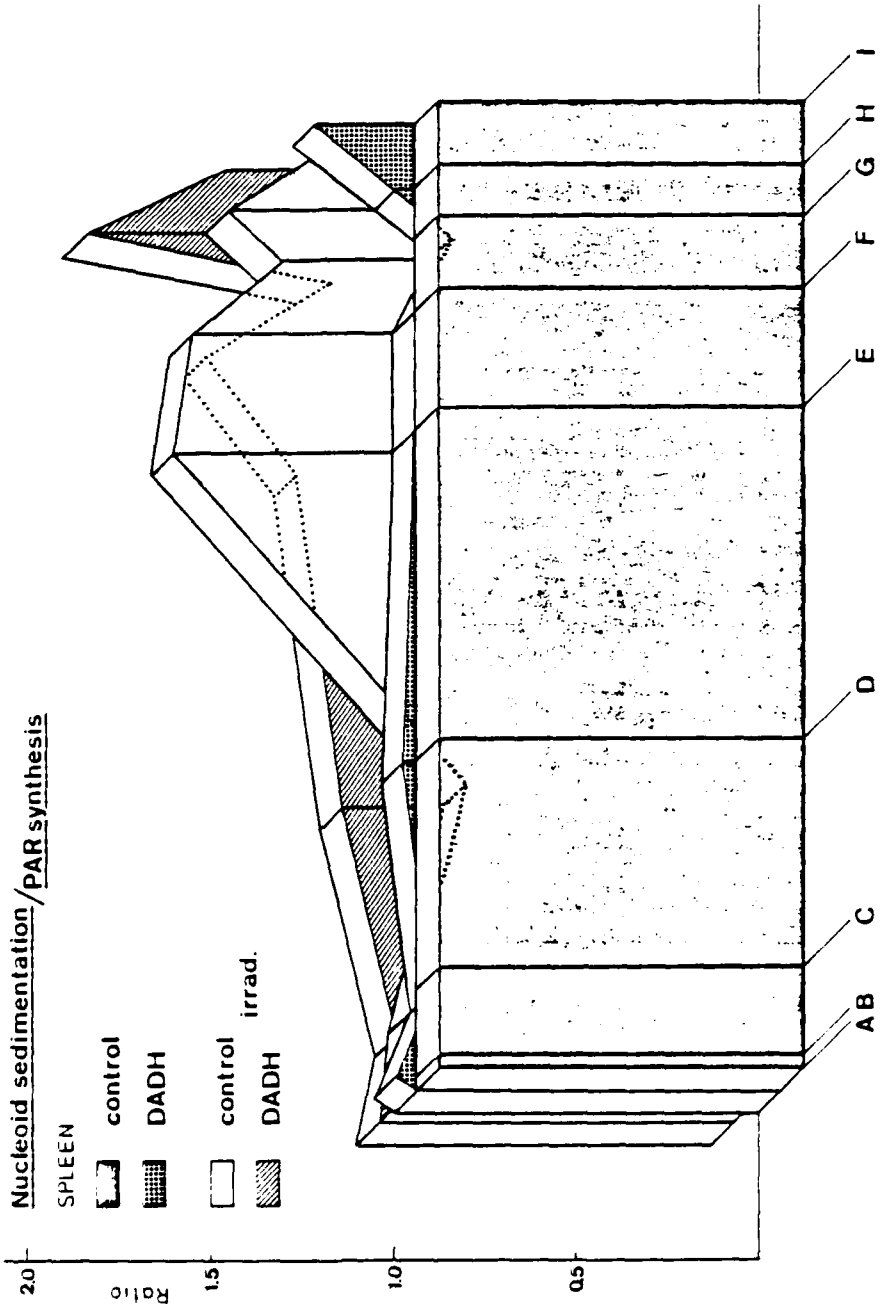

Nucleoid Sedimentation
Spleen
control
irradiated
DADH
DADH irradiated
ratio
to control
1.2
1.0
0.8
A B C D E F G H I J B C D E F G H I J B C D E F G H I J
© Seibersdorf

Figure 3

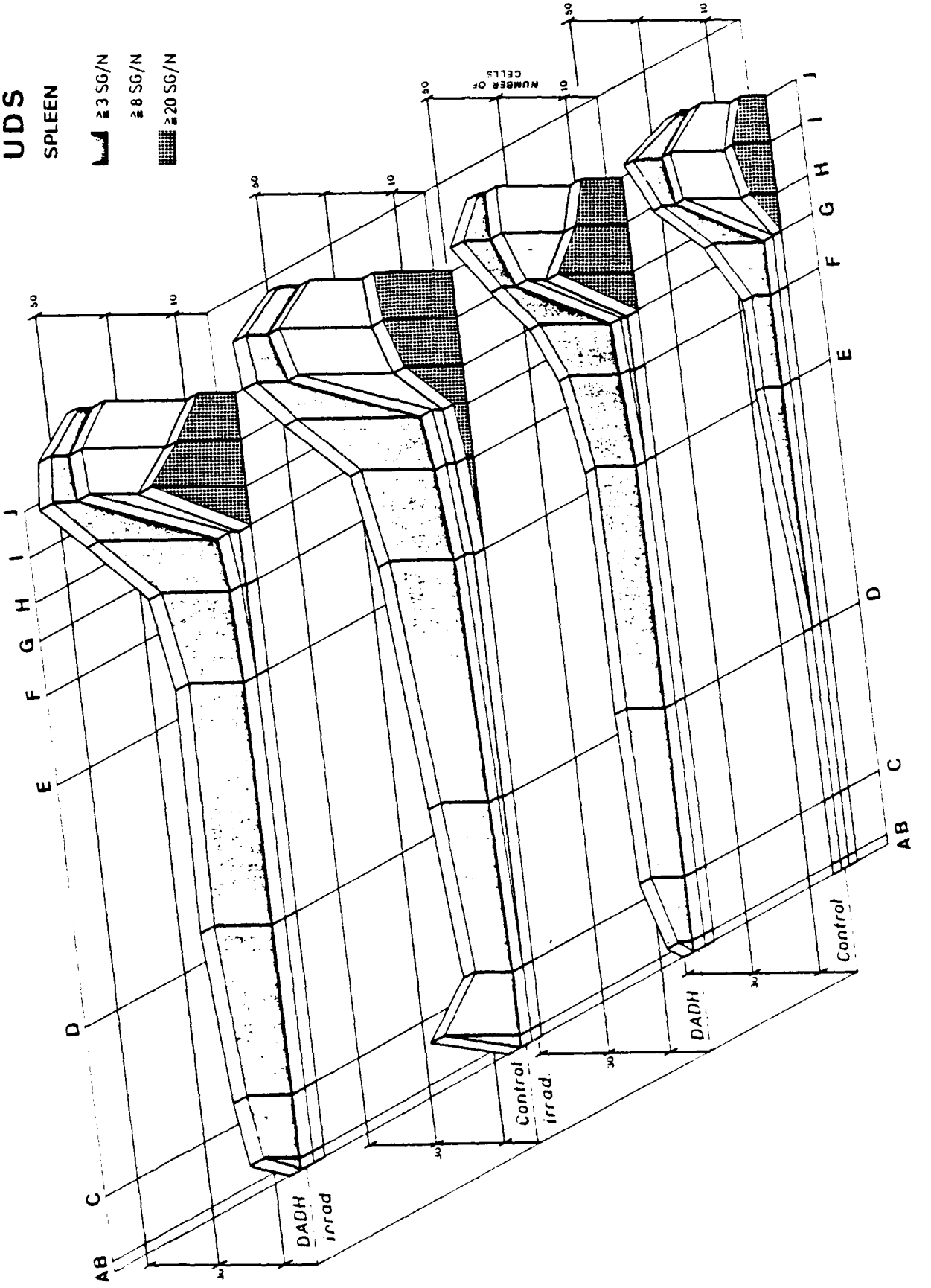

Nucleoid sedimentation/PAR synthesis
SPLEEN
control
DADH
control
DADH
irrad.
Ratio
2.0
1.5
1.0
0.5
A B
C
D
E
F
G
H
I

BASIC UDS AND POLY(ADP-RIBOSE)-POLYMERASE ACTIVITY

Basic UDS in spleen cells

Basic UDS in healthy cells is generally very low, and silver grains between 10 and 20 per nucleus can be seen only in premalignant and malignant cells. Under certain selective conditions also specific DNA amplification can contribute to higher silver grain numbers per nucleus. Figure 1 shows that in the control group cells with more than 3 grains/nucleus only appear at point D. In all other groups low level UDS starts already at the beginning of the experiments. In the irradiated group cells with = 8 SG/N occur early after irradiation, in the γ + DADH group there is a delay in the onset of higher level UDS. Cells with = 20 SG/N show the same tendency, only in the last step of the life span UDS in the irradiated group increased till the end of the study. In animals, receiving only DADH, also higher UDS values were obtained compared with controls. A certain correlation between basic UDS and the occurrence of lymphomas seems to exist. (Compare also poly(ADP-ribose)-polymerase activity in spleen cells, page 78).

Figure 1


UDS
SPLEEN
≥3 SG/N
≥8 SG/N
≥20 SG/N
NUMBER OF CELLS
50
10
AB
C
D
E
F
G
H
I
J
3
DADH irrad
Control irrad
DADH
Control

Basic UDS in liver cells

Cancer formation by low dose radiation is almost always a slow process requiring a long part of the total life span of mice. The liver offers a major advantage for studies on cancerogenesis. The earliest sign for transformed cells are discrete foci of phenotypically altered cells in the liver. It takes very often more than 1 year of promotion, before 50% of normal liver tissue is replaced by focal γ-GT positive cells. If there is no promotion after the early occurrance of foci, this premalignant cells have no chance to survive (R. Schulte-Hermann, personal communication). Premalignant cells have already a higher poly(ADP-ribose)-polymerase capacity. In our experiments poly(ADP-ribose)-polymerase activity was followed after isolation of crude chromatin. In figure 2 we can see that in contrast to spleen cells, the combined group (γ + DADH) showed the highest ADP-ribosylation of proteins. We cannot exclude that premalignant cells occurred in the liver of mice in all treated groups in the first 1/3 of the life span, but they died after a certain time of persistance. Tumor promotors are often hormones or compounds with hormone like effects. Some preliminary experiments with glutaurine have shown that this hormone like compound can increase the poly(ADP-ribose)-polymerase activity, without introducing DNA breaks (see also enclosure 7).

For UDS determination in hepatocytes a cell-suspension was prepared. The liver of the animals - while in strong ether anaestesia - was perfused first with EDTA containing PBS and then with a solution of 0.05 U/ml disphase and 10 U/ml collagenase in BME medium with 1& serum. The livers were incubated for about one hour in this solution. The organs were cut into small pieces and transferred to a solution of 0.5 U/ml dispase and 1 U/ml collagenase in BME medium with 2% serum. A single cell suspension was made by slowly stirring this solution. The cells were cleaned from debries by repeated centrifugation in PBS.

15 μCi ^{3}H-thymidine were added to 1 million of cells in BME medium with 3% serum and incubated at 37°C for 90 minutes. The cells were washed in BPS and incubated in medium containing 0.1 mMol/l inactivate thymidine for 30 min. After more washings in PBS the cells were allowed to swell 5 min in 1% trisodiumcitrate

solution and then fixed with methanol - acetic acid.

The fixed cells were dropped on to slides and covered with photographic emulsion. After 3 weeks of exposure in the dark the slides were developed and stained with Giemsa. The number of silver grains in 50 cells per animal were counted in a micrscope.

Figure 3 shows that UDS was highest in the combined group (γ + DADH), followed by the γ-irradiated group and DADH treated group.

If DADH has some carcinogenic potential it seems clear that UDS may be higher in the DADH treated animals because this substance was given during the whole life span. But it is of special interest, that mice which received only once, at the beginning of the study, 1 Gy γ-irradiation, 500 days later still showed augmented basic UDS. DNA damage produced by 1 Gy 60-Co-irradiation in mouse cells is normally repaired within 1/2 h. Therefore a special mechanism (induction of error prone repair?) should be responsible for this increased UDS, which is present during the rest of the life. The following tables show the single values.

Figure 2

Poly (ADP ribose) – polymerase activity in liver cells of C_{57} bl mice

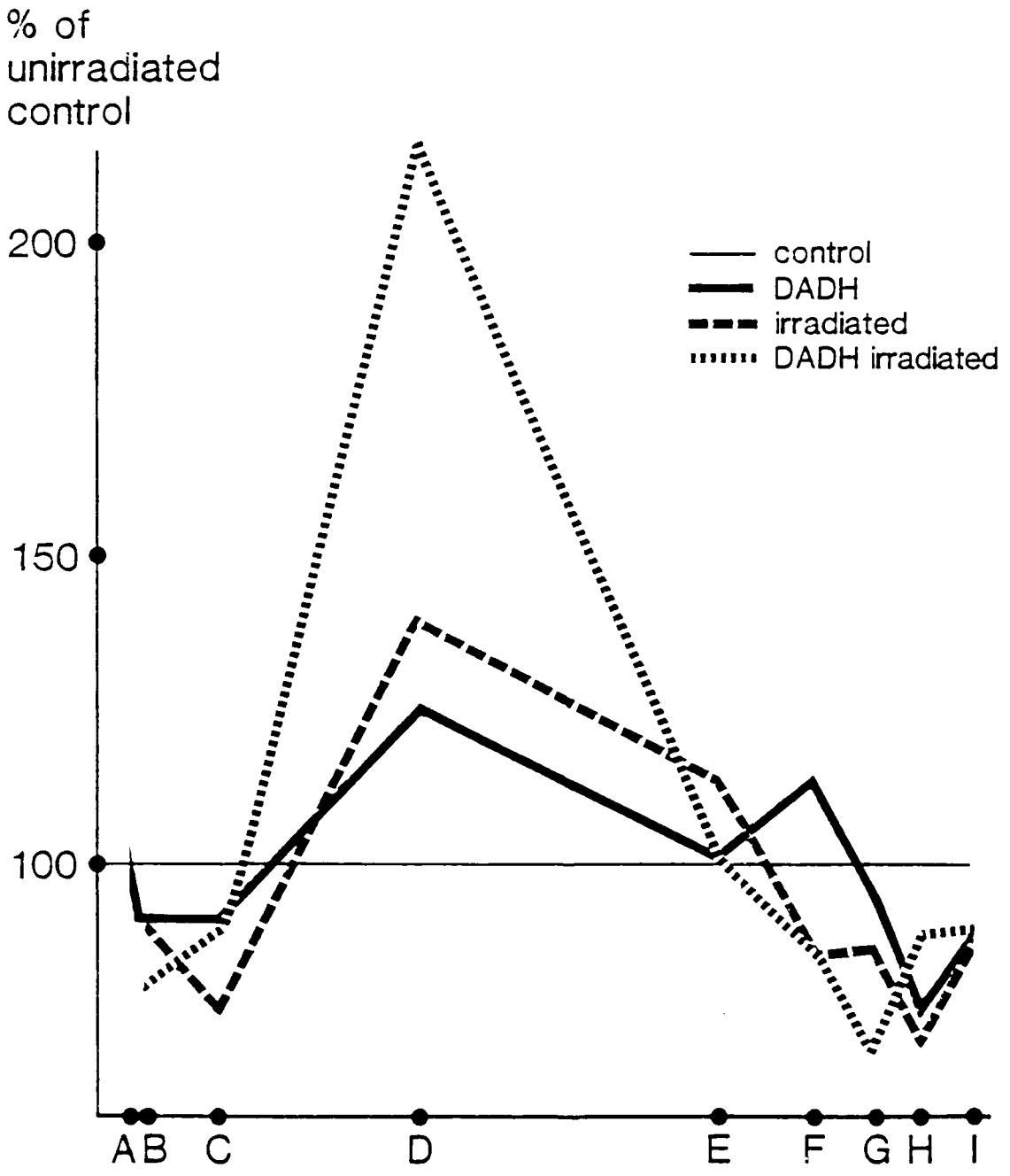
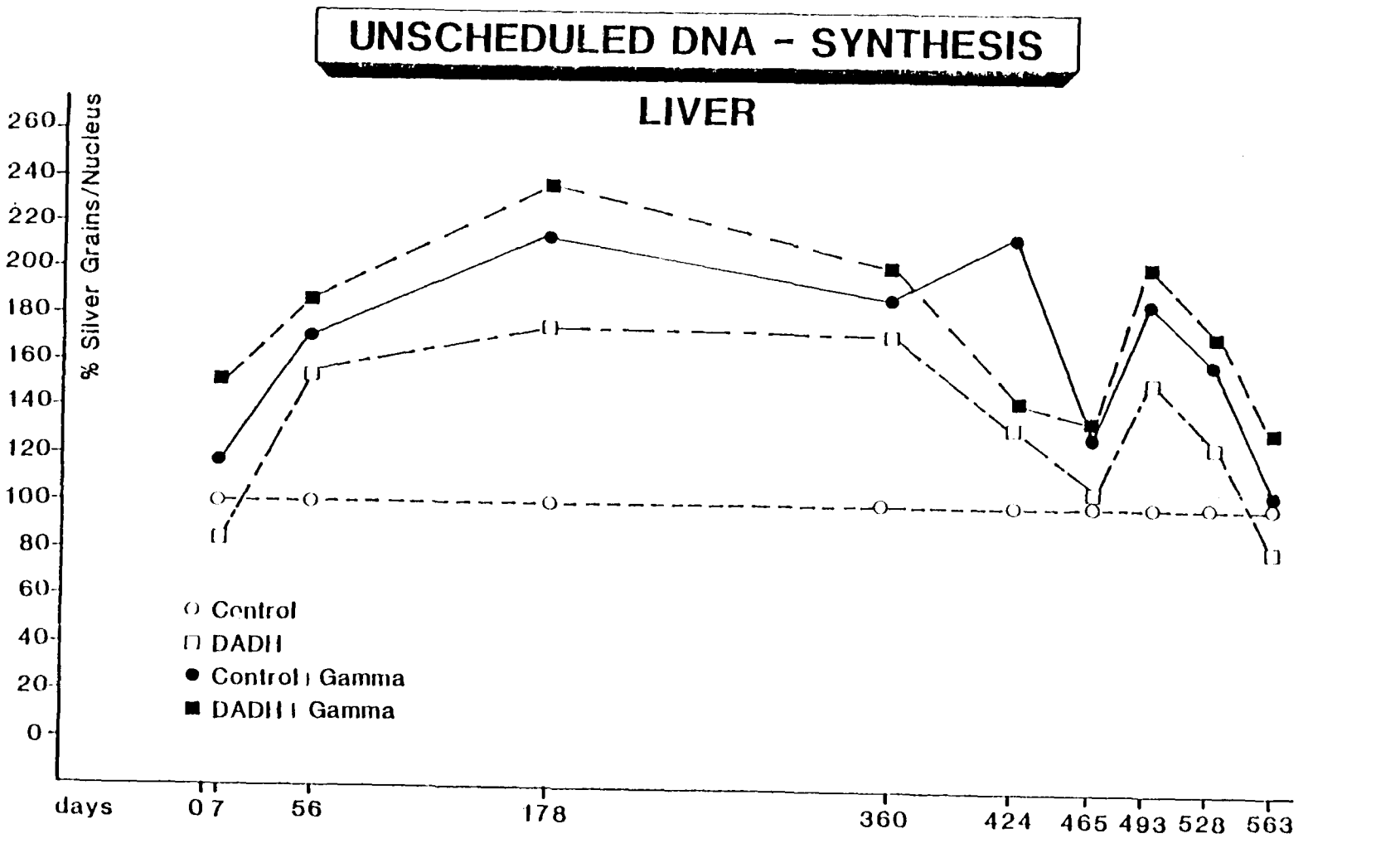


Figure 3



Seibersdorf

Table 1

MOUSE NR.	INDUCTION UDS	%
501	0.53	190
502	0.38	136
503	0.16	57
504	0.38	136
101	0.20	71
102	0.16	57
103	0.21	75
104	0.21	75
MEAN	0.28	100

MOUSE NR.	INDUCTION UDS	%
701	0.31	111
702	0.30	107
703	0.24	86
704	0.32	114
301	0.38	136
302	0.30	107
303	0.33	118
304	0.37	132
MEAN	0.32	114

MOUSE NR.	INDUCTION UDS	%
105	0.22	47
106	0.38	81
107	0.36	77
108	0.92	197
109	0.40	86
110	0.50	107
111	0.40	86
112	0.54	116
MEAN	0.47	100
305	0.36	77
306	0.74	159
307	0.24	51
308	0.22	47
309	0.20	43
310	0.48	103
311	0.38	81
312	0.50	107
MEAN	0.39	83

MOUSE NR.	INDUCTION UDS	%
505	0.76	163
506	0.82	176
507	0.66	141
508	0.42	90
509	0.26	55
510	0.46	98
511	0.40	86
512	0.56	120
MEAN	0.54	116
705	0.50	107
706	0.76	163
707	0.82	176
708	0.50	107
709	1.50	322
710	0.56	120
711	0.60	129
712	0.38	81
MEAN	0.70	151

Table 1, cont.

MOUSE NR.	INDUCTION UDS	%
113	0.50	56
114	1.42	159
115	0.46	51
116	0.64	71
117	1.18	132
118	0.96	107
119	1.40	157
120	0.56	62
MEAN	0.89	100
313	1.00	112
314	0.78	87
315	1.04	116
316	2.62	294
317	1.60	179
318	1.96	220
319	1.06	119
320	1.20	134
MEAN	1.41	158

MOUSE NR.	INDUCTION UDS	%
513	2.54	285
514	1.86	208
515	1.22	137
516	1.34	150
517	2.32	260
518	0.66	74
519	1.34	150
520	0.88	98
MEAN	1.52	170
713	1.62	182
714	1.74	195
715	1.48	166
716	1.84	206
717	1.80	202
718	1.32	148
719	2.12	238
720	1.24	139
MEAN	1.65	184

MOUSE NR.	INDUCTION UDS	%
121	0.58	163
122	0.24	67
123	0.32	89
124	0.26	73
125	0.38	106
126	0.40	112
127	****	**
128	0.31	87
MEAN	0.36	100
321	0.50	140
322	0.88	247
323	0.60	168
324	0.32	89
325	0.56	157
326	0.98	275
327	0.90	253
328	0.34	95
MEAN	0.64	178

MOUSE NR.	INDUCTION UDS	%
521	0.34	95
522	0.82	230
523	0.92	258
524	0.60	168
525	0.70	196
526	1.22	342
527	0.86	241
528	0.70	196
MEAN	0.77	216
721	0.34	95
722	0.74	208
723	0.54	151
724	1.06	297
725	1.04	292
726	0.54	151
727	1.30	365
728	1.22	342
MEAN	0.85	238

Table 1, cont.

MOUSE NR.	INDUCTION UDS	%
129	0.54	164
130	0.22	67
131	0.26	79
132	0.34	103
133	0.34	103
134	0.50	152
135	0.22	67
136	0.20	61
MEAN	0.33	100
329	0.52	158
330	0.46	140
331	0.76	232
332	0.80	244
333	0.68	207
334	0.60	183
335	0.44	134
336	0.40	122
MEAN	0.58	177

MOUSE NR.	INDUCTION UDS	%
529	0.68	207
530	0.32	97
531	0.66	201
532	0.80	244
533	0.60	183
534	0.34	103
535	0.68	207
536	0.76	232
MEAN	0.61	184
729	0.64	195
730	0.72	219
731	1.00	305
732	0.36	109
733	0.50	152
734	0.86	262
735	0.78	238
736	0.44	134
MEAN	0.66	202

MOUSE NR.	INDUCTION UDS	%
137	0.48	106
138	0.62	137
139	0.34	75
140	0.44	97
141	0.60	133
142	0.34	75
143	0.34	75
144	0.44	97
MEAN	0.45	100
337	0.56	124
338	0.58	128
339	0.42	93
340	0.54	120
341	0.52	115
342	0.82	182
343	0.66	146
344	0.82	182
MEAN	0.62	136

MOUSE NR.	INDUCTION UDS	%
537	0.82	182
538	0.76	168
539	1.42	315
540	0.70	155
541	0.94	208
542	0.90	200
543	0.98	217
544	1.24	275
MEAN	0.97	215
737	0.48	106
738	0.52	115
739	0.80	177
740	0.88	195
741	0.60	133
742	0.90	200
743	0.58	128
744	0.32	71
MEAN	0.64	141

Table 1, cont.

MOUSE NR.	INDUCTION UDS	%
145	1.10	119
146	0.90	98
147	1.12	122
148	0.84	91
149	0.74	80
150	1.18	128
151	0.58	63
152	0.88	95
MEAN	0.92	100
345	1.08	117
346	1.24	135
347	1.04	113
348	0.80	87
349	1.22	132
350	0.82	89
351	0.54	58
352	0.80	87
MEAN	0.94	102

MOUSE NR.	INDUCTION UDS	%
545	1.94	211
546	1.20	130
547	1.06	115
548	1.18	128
549	0.92	100
550	0.84	91
551	1.28	139
552	1.08	117
MEAN	1.19	129
745	0.88	95
746	1.82	98
747	0.96	104
748	1.20	130
749	1.34	146
750	1.16	126
751	1.08	117
752	1.48	161
MEAN	1.24	135

MOUSE NR.	INDUCTION UDS	%
153	1.08	130
154	0.86	104
155	0.64	77
156	1.06	128
157	0.84	101
158	0.56	67
159	0.58	70
160	0.98	118
MEAN	0.83	100
353	1.44	174
354	1.36	164
355	1.18	143
356	1.12	135
357	0.90	109
358	2.20	266
359	1.06	128
360	1.12	135
MEAN	1.30	157

MOUSE NR.	INDUCTION UDS	%
553	2.64	320
554	0.86	104
555	1.74	210
556	1.72	208
557	1.38	167
558	1.70	206
559	0.96	116
560	1.24	150
MEAN	1.53	185
753	1.70	206
754	1.70	206
755	1.86	225
756	1.18	143
757	2.26	273
758	1.26	152
759	1.90	230
760	1.42	172
MEAN	1.66	201

Table 1, cont.

MOUSE NR.	INDUCTION UDS	%
161	0.82	132
162	0.44	70
163	0.52	83
164	0.74	119
165	0.36	58
166	0.58	93
167	0.52	83
168	0.98	158
MEAN	0.62	100
361	0.64	103
362	0.90	145
363	0.78	125
364	1.14	183
365	0.72	116
366	0.84	135
367	0.58	93
368	0.76	122
MEAN	0.80	128

MOUSE NR.	INDUCTION UDS	%
561	1.24	200
562	0.58	93
563	1.00	161
564	1.38	222
565	0.86	138
566	0.64	103
567	0.82	132
568	1.44	232
MEAN	1.00	160
761	1.16	187
762	0.96	154
763	1.46	235
764	1.14	183
765	0.88	141
766	1.28	206
767	1.02	164
768	0.80	129
MEAN	1.09	175

MOUSE NR.	INDUCTION UDS	%
169	1.84	134
170	0.66	48
171	0.94	68
172	1.02	74
173	2.14	155
174	1.94	141
175	1.58	115
176	0.86	62
MEAN	1.37	100
369	1.16	84
370	****	**
371	****	**
MEAN	1.16	84

MOUSE NR.	INDUCTION UDS	%
569	1.26	91
570	0.76	55
571	2.60	189
572	1.54	112
573	2.26	164
574	1.22	88
575	1.06	77
576	0.66	48
MEAN	1.42	103
769	1.86	135
770	1.58	115
771	2.08	151
MEAN	1.84	134

QUANTITATIVE DETERMINATION OF THE POLY(ADP-RIBOSE) LEVEL IN SPLEEN DNA LIVER CELLS

The data obtained from poly(ADP-ribose)-polymerase activity determinations in crude chromatin in the last 1/3 of the life span, when already an increase in lymphoma rate could be observed, showed the highest ADPribosylation of proteins in the DADH group followed by the γ-irradiated and the combined group. This relationship was nearly the same in spleen and liver cells. It was of interest for us whether poly(ADP-ribose)-synthesis and the level of poly(ADP-ribose) show a certain correlation.

For the following investigations we used a modified method of A. Hakam et al. for the quantitative determination of poly(ADP-ribose) of cells by high performance liquid chromatography.

To get a higher sensitivity, this method is based on the oxydation by sodiumperiodate of 2',3'cisdiol-groups of the ribose moieties in poly(ADP-ribose) to aldehyd groups, followed by the reduction by ^{3}H-borohydride to primary alcohol groups. For the separation into oligo- and poly(ADP-ribose) the stationary phase used was a reversed phase - silicagel, which was chemically modified, and the polar mobile phase was water, methanol and acetonitrile (gradient C). The detection system was an UV-detector coupled with a fraction collector. The radioactivity was measured in a liquid scintillation counter.

In fig. 1 and 2 the oligo and poly(ADP-ribose) levels in spleen and liver cells are shown. There is a certain similarity between the obtained poly(ADP-ribose)-synthesis values and the level in the cells. In liver cells, which are poorly dividing cells, poly(ADP-ribose) level is a little higher compared with the oligo(ADPribose)-fraction and positive with the DADH and γ-irradiated group of spleen cells. The γ + DADH group again showed the tendency to reach the level of the control groups. It means

that there is again a correlation with the number of lymphomas in this period of the life time of C57 bl mice.

Figure 1

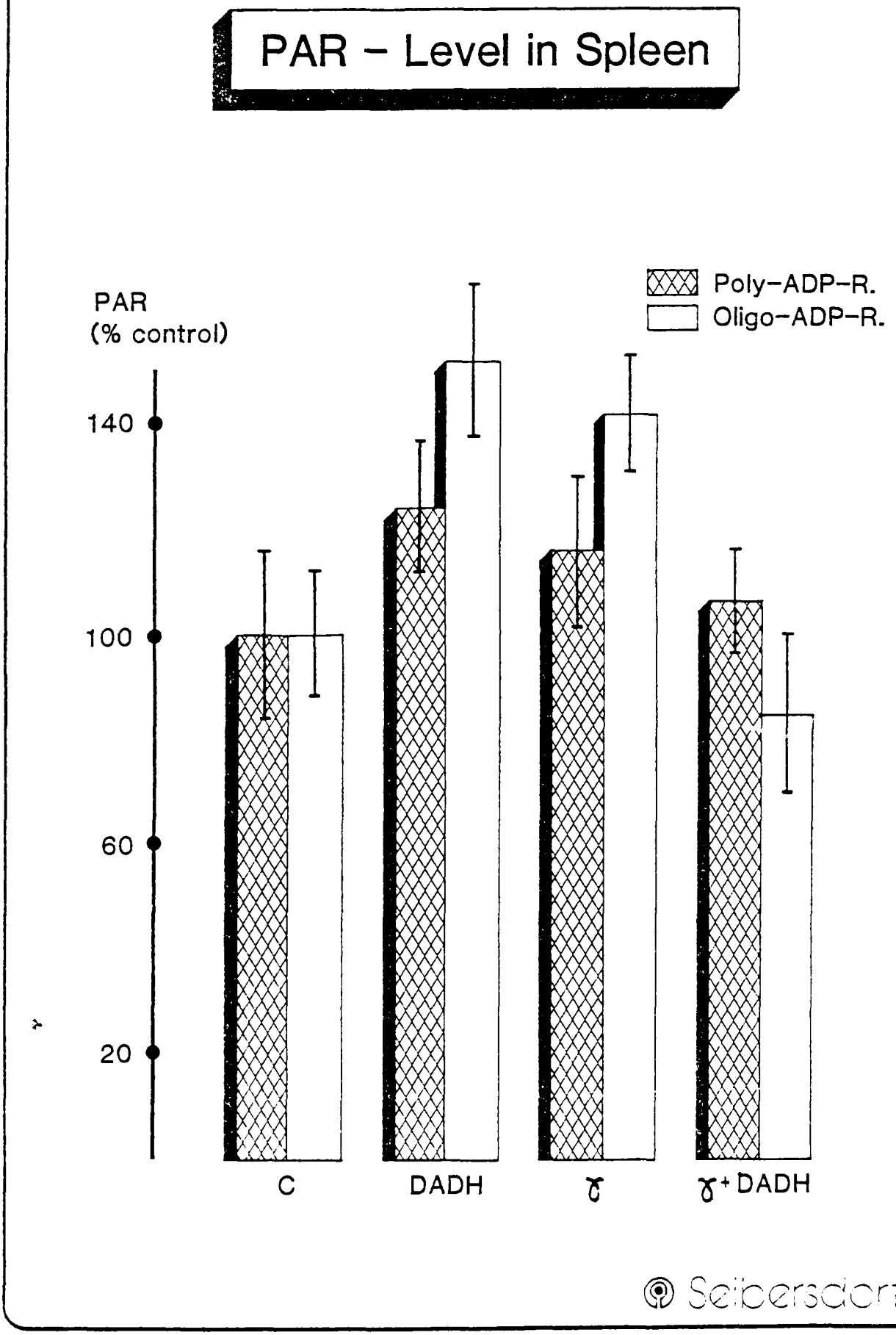
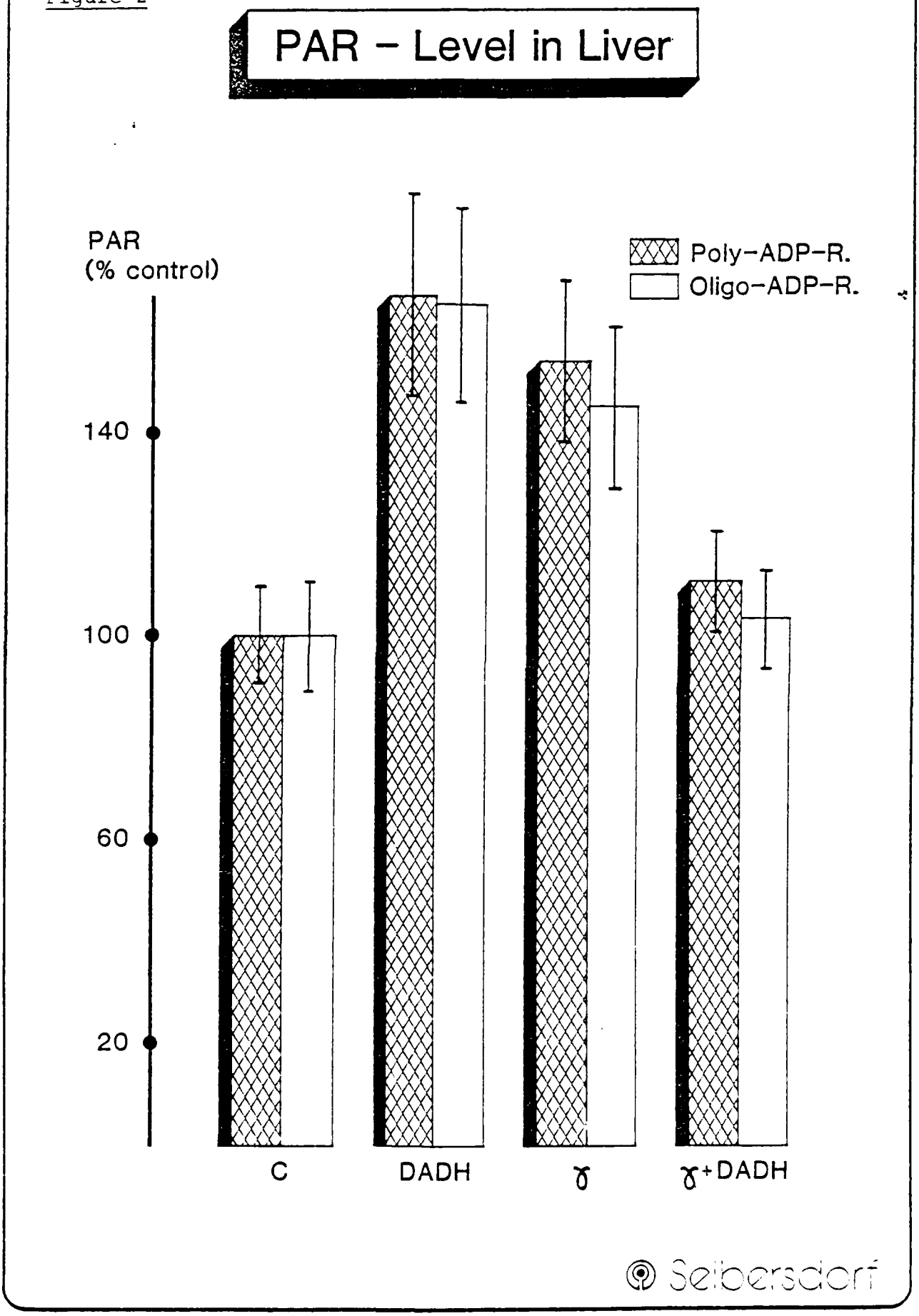

PAR – Level in Spleen
Poly-ADP-R.
Oligo-ADP-R.
PAR
(% control)
140
100
60
20
C
DADH
γ
γ + DADH
Seibersdorf

Figure 2


PAR – Level in Liver
PAR
(% control)
Poly-ADP-R.
Oligo-ADP-R.
140
100
60
20
C
DADH
γ
γ+DADH
Seibersdorf

SUMMARY OF ALL RESULTS

The most important results of the animal studies on the mode of action of the antitransformer DADH in short and long term experiments are the following:

1. Combined treatment of DADH and γ-irradiation generated a decreased incidence of malignant lymphoma compared to γ-irradiation alone.
2. DADH itself shows some carcinogenic properties.
3. In short term experiments DADH has an immunoprotective effect with respect to γ-irradaition
   a. earlier reconstitution of lymphocyte subsets,
   b. increase in natural killer cell activity.
4. Higher poly(ADP-ribose)-polymerase activity to a certain extent seems to control replicative DNA synthesis and specific DNA amplification determined by double minutes.
5. Spleen cells with loss of DNA repair increased remarkably with age. At the same time lymphoma incidence is increasing.
6. Nucleoid sedimentation studies showed an oversedimentation phenomenon rather than DNA breaks during short and long term experiments .
7. A certain correlation between basic UDS in spleen cells and the occurrance of lymphomas exists.
8. Basic UDS was highest in the combined (γ + DADH) group. But also after a single irradiation dose of 1 Gy basic UDS was elevated during the whole life time.
9. Poly(ADP-ribose)-polymerase activity parallels the poly(ADP-ribose) content in spleen and liver cells at the end of the life span of C57 bl mice.


DATE
FILMED
4 8