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EFFECTS OF DISULFIRAM ON MIXED FUNCTION OXIDASE SYSTEM AND TRACE ELEMENT CONCENTRATION IN THE LIVER OF RATS

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Abstract—Disulfiram (DSF), an inhibitor of chemically induced carcinogenesis, and its metabolite diethyldithiocarbamate (DDTC) have been investigated for their influence on trace element distribution and on certain enzymes of the drug metabolizing system in the livers of phenobarbital (PB) treated rats. Both substances diminished the PB induced enzyme response in liver microsomes, DDTC being more effective (−85%) than DSF (−60%). The copper, cobalt and zinc content of the livers of DSF treated animals were increased by factors of 6, 3 and 1.5 respectively as compared to controls, while DDTC treatment had no influence on liver trace element content. A correlation between enzyme inhibition and enhanced trace element uptake of the liver after DSF administration could not be observed. The change of trace element transport into the liver during DSF treatment is discussed.

Disulfiram (tetraethylthiuram disulfide, DSF)‡ exerts a strong influence on chemical carcinogenesis. Changes in organotropy of tumor induction after administration of nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA) [1] as well as a partial or complete blocking of chemically induced carcinogenesis have been reported [2–7].

Although the metabolic fate of DSF has been well investigated [8, 9, 10], its inhibitory action on chemical carcinogenesis is not clearly understood. It has been demonstrated that DSF reduces toxicity, DNA alkylation and the number of single strand breaks caused by NDMA and NDEA [11–13]. A scavenging effect of the SH groups of diethyldithiocarbamate (DDTC), an *in vivo* metabolite of DSF, upon the electrophilic metabolites of chemical carcinogens could not yet be proven [14]. It is more likely that DSF acts by blocking enzymes, which are essential for the activation of chemical carcinogens, because the inhibitory action of DSF on a wide variety of enzymes is well documented [4, 15–20].

Neal [21] reports an interaction of cytochrome P-450 (Cyt. P-450) containing monooxygenases with atomic sulfur released from thionosulfur compounds like DSF or DDTC. The metal complexing properties of DDTC and other chelating agents, however, also give rise to an inhibitory effect on enzymatic activities. Especially those enzymes which have copper ions incorporated in their structure or which need copper ions as cofactors for their enzymatic activity can be drastically inhibited by DDTC, bathocuproindisulfonate and in some cases EDTA and DSF. It is typical for such an inhibition of enzymatic activity, that a complete compensation can be achieved by the addition of suitable amounts of Cu-ions into the enzyme assay [22–26].

Our investigations were carried out to examine whether the well-documented decrease in activity of the mixed function oxidase system of the liver is accompanied by an effect on the concentrations of copper and other essential trace elements in the livers and other organs of DSF treated animals. Moreover, the influence of DSF on the trace element concentration is of interest, because chemical induction of liver tumors by NDEA is accompanied by a decrease of copper and zinc content in the liver, even at a pretumorous stage [27]. Foreign compounds applied over a long period of time are known to induce their own metabolism [28]. We, therefore, stimulated the microsomal enzymes of the liver with phenobarbital to obtain similar conditions as in long-term experiments with nitrosamines and DSF done at our institute several years ago [1].

MATERIALS AND METHODS

All reagents used were of analytical grade and purchased either from Fa. Boehringer, Mannheim, FRG (disodium salts of NADP, NADH, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase) or from Fa. Merck, Darmstadt, FRG (tetraethylthiuram disulfide, diethyldithiocarbamate, aniline hydrochloride, folinciocalteus reagent, aminopyrine, sodium dithionite and sodium phenobarbital).

Animals. Male Sprague–Dawley rats weighing 200–250g were obtained from Süddeutsche Versuchstieranstalt, Tuttlingen, FRG and were kept in

‡ Abbreviations: AH, aniline-4-hydroxylase; AP, aminopyrine demethylase; Cyt. P-450, cytochrome P-450; DDTC, diethyldithiocarbamic acid; DSF, disulfiram (tetraethylthiuram disulfide); MFO, mixed function oxidase; NDEA, nitrosodiethylamine; NDMA, nitrosodimethylamine; PB, phenobarbital.

Macrolon cages. They were allowed free access to Altromin rat diet and received water *ad libitum*.

Route of administration. DSF, finely powdered, was suspended in a 4% starch solution in water (1 g DSF/10 ml) and was given by means of a gastric tube. DDTC was dissolved in 0.9% saline and also applied orally. Control animals received starch suspension only. In cases where induction of enzyme activity by phenobarbital (PB) is described, all animals of the experiment received a 0.1% solution of phenobarbital as drinking water, starting 4 days before DSF or DDTC treatment.

Animal experiments. DSF or DDTC treatment was carried out during 10 days with a daily administration of DSF or DDTC on days 1–4 and 7–10. A recovering period of 2 days was chosen in accordance with the chronic experiment with DSF and NDEA [1]. 18 hr before killing of the animals food was withdrawn. Two hr or 24 hr after the last DSF or DDTC administration animals were killed by heart puncture under slight ether anethesia. Ether anesthesia was demonstrated to have no influence on enzymatic activities. Serum was isolated by a twofold centrifugation at 3000 rpm for 20 min. Livers and in some cases kidneys and spleens were removed and weighed. Samples for trace element determinations were taken with plastic scissors to avoid contamination with metals.

Homogenization of livers. All subsequent steps were carried out at 4°. Livers were homogenized (1 g liver + 4 vol. 0.05 M phosphate buffer, pH 7.4) at 800 rpm with 6 pestle strokes in 30 sec (Teflon/glass homogenizer Fa. Braun, Melsungen, FRG). The homogenate was centrifuged for 10 min at 9000 g. The supernatant was centrifuged for 60 min at 100,000 *g* in a Beckman LB 50B ultracentrifuge. The pellet was resuspended in 0.05 M phosphate buffer and again centrifuged for 60 min at 100,000 *g*. The final pellet was suspended in 0.05 M phosphate buffer to a volume of twice the liver weight (microsomal fraction) and was kept until use at −80°. Protein was determined according to the method of Lowry *et al.* [29] using bovine serum albumin as a standard.

Cytochrome P-450 and b_5. In the microsomal fraction, adjusted to 2 mg of protein per ml, the content of cytochrome P-450 and b_5 was determined simultaneously from the CO difference spectra of dithionite-reduced samples using an extinction coefficient of 91 mM/cm between 500 and 360 nm [30].

Drug metabolism. 4 mg microsomal protein were used in all enzyme assays. Determination of enzymatic activity of aminopyrine demethylase (AP) was carried out with the following cofactors: NADP (0.5 mM), glucose-6-phosphate-dehydrogenase (5 units), glucose-6-phosphate (5.0 mM), $MgCl_2$ (20 mM) and semicarbazide-HCl (10 mM) in a total volume of 6 ml phosphate buffer (pH 7.4, 0.05 M). Formaldehyde released from the substrate (aminopyrine 0.5–2.0 mM) was quantified according to the method of Nash [31]. Aniline (1.25 mM) was used to determine aniline-4-hydroxylase (AH) activity. The rate of *p*-aminophenol formation was measured photometrically [32]. Cofactors for hydroxylase determination were NADP (0.12 mM), glucose-6-phosphate-dehydrogenase (5 units), glucose-6-phosphate (2.5 mM), $MgCl_2$ (6.37 mM), and nicotinamide (12.5 mM) in a total volume of 4 ml of 0.1 M phosphate buffer, pH 7.4.

Rates of formaldehyde and *p*-aminophenol production at substrate concentrations between 0.5 and 2.0 mM were linear for 15 min (aminopyrine demethylase) and for 20 min (aniline hydroxylase). DDTC and DSF did not interfere with the photometrical measurement of *p*-aminophenol and formaldehyde.

Trace element determinations were carried out by means of neutron activation analysis.

From each organ usually two samples were taken and freeze dried. The first sample was irradiated for 1.5–2.5 hr in the TRIGA Mark II reactor at the German Cancer Research Center with a neutron flux of 5×10^{12} n·sec/cm^2. For determination of ^{56}Mn and ^{42}K samples were measured one hour after irradiation on a 70 cm^3 Ge/Li detector coupled with a computerized multichannel analyzer. Subsequent determination of ^{64}Cu and ^{69m}Zn was achieved by radiochemical separation of both nuclides after wet washing of the irradiated samples by a mixture of H_2SO_4/H_2O_2. While ^{64}Cu was separated from the acid solution by isotopic exchange on a CuS column, ^{69m}Zn was absorbed as chlorocomplex onto a small anion exchanger (Bio-Rad AG 1 × 8 mesh 100–200) [33].

To determine ^{60}Co, ^{59}Fe, ^{75}Se, ^{86}Rb and ^{65}Zn, the second sample together with suitable amounts of standard solutions was irradiated for about 60 hr at the same neutron flux as stated above. After a cooling period of 25 days samples were measured in a 100 cm^3 Ge/Li well type detector without chemical treatment. The separate determinations of Zn as ^{65}Zn and ^{69m}Zn served as controls. Differences in both determinations never exceeded 5%.

RESULTS

Lineweaver–Burke plots for the inhibition of AP by DSF are shown in Fig. 1. The K_i values for the three different DSF concentrations are all in the 95% confidence limits of PB-treated animals: 0.65 (0.51–0.79). The differences between the V_{max} values of all groups are highly significant. The inhibition of AP by DSF in microsomes of rats treated with PB

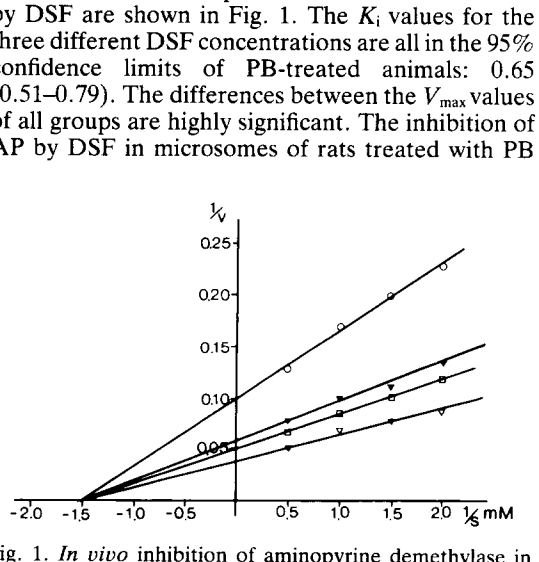


Fig. 1. *In vivo* inhibition of aminopyrine demethylase in liver microsomes of rats treated with DSF and PB. Animals were killed 2 hr after the last DSF application. ▽ PB; □ DSF 50 mg/kg + PB; ▼ DSF 150 mg/kg + PB; ○ DSF 500 mg/kg + PB.

Table 1. Effect of DSF and PB on Cyt. P-450, Cyt. b_5, aminopyrine demethylase, aniline hydroxylase and trace element content of the liver†

	Control	Control + PB	DSF 500 mg/kg + PB	DSF 150 mg/kg + PB	DSF 50 mg/kg + PB
Increase in body weight (g)§ during experiment	27.6 ± 6.3	20.6 ± 6.5	9.5 ± 12.3	18.5 ± 7.1	25.0 ± 7.5
Liver weight (g) (whole organ)§	7.9 ± 0.34	9.0 ± 0.78	10.4 ± 1.1	11.7 ± 1.0	10.4 ± 1.1
Liver weight (g) (per 100 g body weight)§	3.3 ± 0.13	3.9 ± 0.32	5.3 ± 0.23	5.0 ± 0.38	4.7 ± 0.4
Protein (mg/g liver microsomes)§	9.5 ± 1.2	25.5 ± 3.1	21.0 ± 2.8	25.7 ± 2.4	27.8 ± 3.3
Aminopyrine demethylase‖ (nmol HCHO/mg protein/min)	3.84 ± 0.24	13.6 ± 0.42	5.5 ± 0.22	9.6 ± 0.43	11.2 ± 0.53
Aniline hydroxylase‖ (nmol *p*-aminophenol/mg protein/min)	0.98 ± 0.02	1.87 ± 0.01	0.61 ± 0.12	1.27 ± 0.03	1.37 ± 0.02
Cytochrome P-450 (nmol/mg protein)‖	0.80 ± 0.06	2.60 ± 0.18	1.0 ± 0.06	1.82 ± 0.05	2.19 ± 0.09
Cytochrome b_5 (nmol/mg protein)‖	0.49 ± 0.02	0.89 ± 0.03	0.76 ± 0.04	0.83 ± 0.04	0.82 ± 0.02
Mn (μg/g wet liver)§	2.50 ± 0.58	2.43 ± 1.30	3.06 ± 0.47	3.40 ± 0.61	2.97 ± 0.91
Cu (μg/g wet liver)§	4.98 ± 0.38	4.64 ± 0.25	25.5* ± 10.7	6.65* ± 1.0	5.61 ± 1.76
Zn (μg/g wet liver)§	34.2* ± 1.5	29.3 ± 1.6	41.4* ± 6.0	37.1* ± 2.4	35.1* ± 1.7
Co (μg/g wet liver)§	0.017 ± 0.005	0.022 ± 0.004	0.077* ± 0.013	0.056* ± 0.011	0.052* ± 0.007
Rb (μg/g wet liver)§	5.75 ± 0.37	5.78 ± 0.40	5.85 ± 0.42	5.25 ± 0.27	5.65 ± 0.60
Fe (μg/g wet liver)§	119 ± 24	118 ± 15	136 ± 51	121 ± 15	130 ± 24
K (μg/g wet liver)§	4960 ± 870	5810 ± 2420	3470 ± 320	3550 ± 210	4770 ± 2200
Se (μg/g wet liver)§	0.77 ± 0.1	0.65 ± 0.03	0.71 ± 0.08	0.66 ± 0.03	0.68 ± 0.07
Liver wet weight/dry weight	3.36 ± 0.07	3.25 ± 0.04	3.47 ± 0.09	3.39 ± 0.1	3.32 ± 0.07

* Significantly different from controls + PB ($P < 0.001$, single variance analysis).
† Animals were killed 2hr after the last DSF intake.
‡ Average weight of animals before start of the experiment 204.0 ± 13.7g ($n = 30$).
§ Mean ± S.D. from 6 animals.
‖ Mean ± S.D. from 6 determinations in microsomes of a pooled liver homogenate from 6 animals.

Table 2. Effect of DSF and DDTC on aminopyrine demethylase and trace element concentrations in the livers of phenobarbital-treated rats†

	Control + PB	DSF 500 mg/kg + PB	DDTC 250 mg/kg + PB
Liver weight (g/100 g)‡	5.5 ± 0.7	5.4 ± 0.6	5.2 ± 0.5
Protein (mg/g liver microsomes)‡	27.7 ± 3.2	22.9 ± 2.4	16.6 ± 2.0
Aminopyrine demethylase (nmol HCHO/mg protein/min)§	9.9 ± 0.6	5.3 ± 0.8	1.5 ± 0.3
Trace elements in the liver‡			
Mn (μg/g wet weight)	3.26 ± 0.92	2.84 ± 0.54	3.43 ± 0.49
Cu (μg/g wet weight)	3.3 ± 0.56	28.2* ± 10.8	4.02 ± 0.44
Zn (μg/g wet weight)	22.7 ± 2.6	37.7* ± 5.4	26.0 ± 2.6

* Significantly different from controls + PB ($P < 0.001$, single variance analysis).
† Animals were killed 2 hr after the last DSF or DDTC intake.
‡ Mean ± S.D. from 6 animals.
§ Mean ± S.D. from 6 determinations in microsomes of a pooled liver homogenate from 6 animals.

Table 3(a). Effect of DSF on Cyt. P-450, Cyt. b_5, aminopyrine demethylase and aniline hydroxylase and on trace elements in liver, kidney, spleen and serum 2 hr after the last DSF intake*†

	Control + PB	DSF 500 mg/kg + PB	DSF 50 mg/kg + PB
Increase in body weight (g)‡ during experiment	35.2 ± 6.6	−16.6 ± 15.0	32.8 ± 17.0
Liver weight (whole organ) (g)	12.4 ± 1.6	11.6 ± 1.6	12.4 ± 1.3
Liver weight (g/100 g body weight)	4.73 ± 0.36	5.41 ± 0.34	4.96 ± 0.18
Protein (mg/g liver microsomes)	30.4 ± 4.7	20.9 ± 1.8	27.6 ± 4.3
Aminopyrine demethylase‡ (nmol HCHO/mg protein/min)	13.0 ± 1.57	6.76 ± 0.82	10.7 ± 1.08
Aniline hydroxylase‡ (nmol/mg protein/min)	1.88 ± 0.11	0.70 ± 0.06	1.40 ± 0.17
Cytochrome P-450‡	2.18 ± 0.32	1.06 ± 0.06	2.09 ± 0.17
Cytochrome b_5‡	0.73 ± 0.07	0.89 ± 0.05	0.82 ± 0.06
(a) Trace element content in the liver			
Co (μg/g wet weight)	0.046 ± 0.018	0.198 ± 0.017	0.148 ± 0.051
Cu (μg/g wet weight)	4.15 ± 0.22	24.3 ± 10.1	6.0 ± 2.12
Zn (μg/g wet weight)	24.9 ± 1.0	44.3 ± 2.3	34.7 ± 4.9
Wet weight/dry weight	3.29 ± 0.06	3.37 ± 0.06	3.22 ± 0.09
(b) Trace element content in the kidney			
Co (μg/g wet weight)	0.117 ± 0.016	0.168 ± 0.023	0.138 ± 0.010
Cu (μg/g wet weight)	4.25 ± 0.84	3.67 ± 0.30	3.77 ± 0.38
Zn (μg/g wet weight)	20.3 ± 1.54	20.9 ± 1.0	19.5 ± 0.9
Wet weight/dry weight	4.07 ± 0.09	3.84 ± 0.17	3.81 ± 0.27
(c) Trace element content in the spleen			
Co (ng/g wet weight)	9.22 ± 4.51	17.4 ± 4.39	13.6 ± 2.28
Cu (μg/g wet weight)	1.14 ± 0.04	1.29 ± 0.07	1.21 ± 0.05
Zn (μg/g wet weight)	18.0 ± 0.67	19.7 ± 0.66	17.9 ± 0.46
Fe (μg/g wet weight)	152 ± 21.7	495 ± 144	195 ± 28
Wet weight/dry weight	4.40 ± 0.07	4.28 ± 0.08	4.36 ± 0.05
(d) Trace element content in serum			
Co (ng/g wet weight)	2.51 ± 0.27	4.31 ± 0.53	3.28 ± 0.25
Cu (μg/g wet weight)	1.46 ± 0.16	1.36 ± 0.25	1.13 ± 0.26
Zn (μg/g wet weight)	1.33 ± 0.29	2.38 ± 0.63	2.03 ± 0.18
Wet weight/dry weight	13.15 ± 0.25	13.54 ± 0.66	13.23 ± 0.52

* Trace elements not listed in this table but in Table 1 showed no significant differences after DSF treatment.
† All data from this table represent means ± S.D. from 6 animals.
‡ Average weight of animals before start of experiment 226 ± 16 g ($n = 18$).

Table 3(b). Effect of DSF on Cyt. P-450, Cyt. b_5, aminopyrine demethylase and aniline hydroxylase and on trace elements in liver, kidney, spleen and serum 24 hr after the last DSF intake*†

	Control + PB	DSF 500 mg/kg + PB	DSF 50 mg/kg + PB
Increase in body weight (g)‡ during experiment	36.3 ± 18.0	−6.8 ± 6.6	42.7 ± 17.0
Liver weight (whole organ) (g)	11.75 ± 0.4	12.41 ± 0.8	13.6 ± 0.6
Liver weight (g/100 g body weight)	4.8 ± 0.39	5.8 ± 0.4	5.2 ± 0.22
Protein (mg/g liver microsomes)	32.1 ± 4.2	25.9 ± 5.7	28.3 ± 2.6§
Aminopyrine demethylase (nmol HCHO/mg protein/min)	11.06 ± 1.47	5.32 ± 0.60	10.60 ± 1.24§
Aniline hydroxylase (nmol/mg protein/min)	1.53 ± 0.19	0.73 ± 0.08	1.27 ± 0.24§
Cytochrome P-450	2.0 ± 0.17	0.92 ± 0.10	2.03 ± 0.21§
Cytochrome b_5	0.68 ± 0.03	0.79 ± 0.07	0.78 ± 0.06
(a) Trace element content in the liver			
Co (μg/g wet weight)	0.051 ± 0.009	0.243 ± 0.034	0.118 ± 0.023§
Cu (μg/g wet weight)	4.29 ± 0.15	40.3 ± 6.9	8.30 ± 1.0§‖
Zn (μg/g wet weight)	29.0 ± 5.0	42.6 ± 4.0	27.8 ± 2.1§
Wet weight/dry weight	3.25 ± 0.09	3.40 ± 0.04	3.53 ± 0.4
(b) Trace element content in the kidney			
Co (μg/g wet weight)	0.131 ± 0.07	0.141 ± 0.015	0.126 ± 0.014§
Cu (μg/g wet weight)	4.12 ± 0.31	4.03 ± 0.16	4.46 ± 0.63
Zn (μg/g wet weight)	19.5 ± 1.56	19.4 ± 1.64	18.8 ± 0.91
Wet weight/dry weight	4.15 ± 0.1	3.83 ± 0.13	4.25 ± 0.5
(c) Trace element content in the spleen			
Co (ng/g wet weight)	8.24 ± 1.97	23.74 ± 3.72	15.87 ± 3.29§
Cu (μg/g wet weight)	1.20 ± 0.09	1.44 ± 0.22	1.24 ± 0.04
Zn (μg/g wet weight)	18.37 ± 0.84	20.21 ± 0.93	18.26 ± 0.31
Fe (μg/g wet weight)	170 ± 24.5	549 ± 208	185 ± 20.5§
Wet weight/dry weight	4.40 ± 0.17	4.19 ± 0.14	4.37 ± 0.04
(d) Trace element content in serum			
Co (ng/g wet weight)	2.64 ± 0.55	4.74 ± 0.71	4.3 ± 0.57
Cu (μg/g wet weight)	1.33 ± 0.31	1.01 ± 0.03	1.16 ± 0.29
Zn (μg/g wet weight)	1.58 ± 0.16	2.65 ± 0.25	1.43 ± 0.13
Wet weight/dry weight	12.9 ± 0.25	12.95 ± 0.21	13.1 ± 0.35

* † For explanation see Table 3(a).

‡ Average weight of animals before start of the experiment 221 ± 13 g (n = 18).

§ and ‖ indicate the results of a two way variance analysis of data from Tables 3(a) and (b). Only results with P ≦ 0.001 are represented.

§ Indicates differences in enzyme activity or trace element concentration between the three differently treated groups.

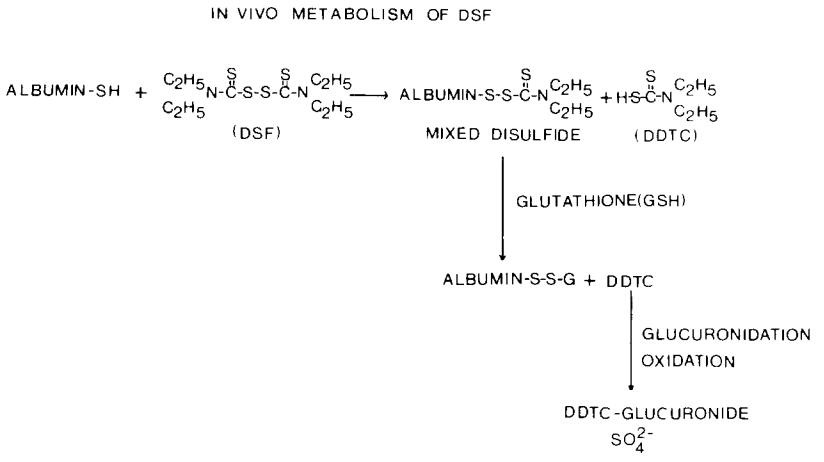
‖ Indicates a time response in enzyme activity or trace element concentration of the two groups killed at different time intervals after the last DSF intake. The third parameter of the two way variance analysis, "difference in time response", could not be observed in the experiments with a significance of P = 0.001.

and DSF can, therefore, be described as non-competitive. If we assume an even distribution of DSF over the whole tissue of the animal the concentration of the lowest dose of DSF is 0.168 mM with a K_i of 0.65 mM for AP.

Table 1 shows the influence of PB and of different doses of DSF concomitantly administered with PB, on the mixed function oxidase system (MFO) and on trace elements in the liver. Animals were killed 2 hr after the last DSF administration. PB brings about a 2–3 fold enhancement of the protein content and MFO activity in the liver microsomes, while DSF inhibits PB stimulation of the MFO system in a dose related way. The exception is Cyt. b_5 which is not affected by any DSF dosage.

PB treatment has no influence on the trace element content of the livers, while DSF administration gives rise to a pronounced increase of Cu, Co and Zn content in the same organ. No correlation, however, is seen between the amount of accumulation of any of these metals either with the administered DSF dosage or with the observed decrease in enzymatic activity. In contrast to Cu, the Co and Zn concentrations are much less enhanced after a 500 mg/kg dosage; but a significant enhancement of both metals can also be seen at the lower dosages of DSF.

Table 2 represents the effects of DDTC (250 mg/kg) on AP, protein and trace element content of the liver. The demethylase activity is much more suppressed by DDTC than by DSF. No influ-



Scheme 1.

ence on Cu or Zn content of the liver could be observed. To investigate the persistence of trace element accumulation and enzyme inhibition in the liver after an 8-day DSF treatment, animals were killed 2 hr, Table 3(a), and 24 hr, Table 3(b), after the last DSF intake. In addition, trace element contents in kidney, spleen and serum were measured to examine whether the accumulation in the liver is paralleled by a decrease in other organs, or if enhanced uptake of metals from the diet is responsible for the enrichment.

Tables 3(a) and 3(b) show that the inhibition of AP, AH and Cyt. P-450 is the same for animals killed 2 hr or 24 hr after the last DSF dose. Also, the Co and Zn content of the livers of DSF treated animals remains nearly constant during the time interval investigated. Only the amount of copper increased by an average factor of two, when animals are killed after 24 hr. Irrespective of the dose and the time interval between the last DSF intake and the time of killing there is significant accumulation of Co in spleen, kidney and serum. Fe in spleen and Zn in serum also increase significantly but only after the 500 mg/kg dosage of DSF.

DISCUSSION

Our results indicate a direct dose related inhibition of PB stimulated Cyt. P-450 and of the Cyt. P-450 dependent enzymes AP and AH, after DDTC or DSF treatment. In contrast to the results of Chetty *et al.* [34], who reported a decreased Cyt. P-450 content in the liver microsomes after enhanced dietary uptake of $CoCl_2$ (Co^{2+} competes with Fe^{2+} for the same binding sites in the cytochrome molecule), our results do not indicate that enhanced concentrations of Co and other trace elements in the liver have any effect on the activity of the enzymes mentioned above. These different results are no contradiction, because the chemical forms in which Co is deposited in the liver are not necessarily the same.

No correlation between enhanced liver cobalt after DSF treatment and enzymatic inhibition could be observed. Moreover, a pronounced stimulation and inhibition of enzymatic activity after PB or DDTC treatment, respectively, was not connected with any change in the trace element concentrations of the liver. It seems more likely that, as proposed by Neal [21], inhibition of enzymatic activity after DSF and DDTC treatment is caused by formation of atomic sulfur, which reacts with cysteinic SH groups of Cyt. P-450. The resulting hydrodisulfides might be the reason for the loss of Cyt. P-450 (detectable as its carbon monoxide complex) and, therefore, a lower activity of Cyt. P-450 dependent enzymes.

Increased trace element content was not only observed in the livers of DSF treated rats, but also in serum, kidneys and spleen. This indicates an enhanced uptake of metals from the diet and little mobilization of trace elements from other organs. The additional increase of Cu in the livers of animals killed 24 hr compared to those killed 2 hr after the last DSF intake must also be affected by the diet, since animals killed 2 hr after the end of treatment were fasted during the last DSF administration, while others had free access to food.

Accumulation of essential trace elements Cu, Co and Zn in the liver could only be observed after DSF treatment, whereas DDTC did not influence the elements investigated. The latter finding is in accordance with results of Aaseth *et al.* [35, 36] who found no longer-lasting accumulation of the radiotracers of Zn and Cu in the livers of mice after administration of DDTC.

According to Strömme [8, 9], the *in vivo* metabolism of DSF (Scheme 1) leads to appreciable amounts of mixed disulfides with the SH groups of plasma proteins. This is especially true for the albumin fraction, which carries nearly all of the reactive SH groups of plasma proteins, while formation of mixed disulfides with liver proteins could not be detected. The same author reports no formation of mixed disulfides with serum proteins after administration of DDTC. This may be the reason for the different effect of DSF and DDTC on the trace element uptake of the liver. Albumin is the main transport protein for most of the essential trace elements in serum. Ionic copper for instance which accounts only for 5% of total serum copper (95% of serum copper is tightly bound to ceruloplasmin and is not exchangeable *in vivo*) is loosely bound to albumin for transport in serum. Copper transport

between serum and tissues is mediated by copper amino-acid complexes which are in equilibrium with copper bound to albumins [37, 38].

Because of the higher stability constant of the Cu–DDTC complex compared to the amino acid and albumin complexes, DDTC reacts with ionic copper in serum by chelating the metal atom. DSF, however, is able to change the structure of the albumin in a Cu–albumin complex by intermediate formation of mixed disulfides. DSF itself failed to form stable, into $CHCl_3$ extractable complexes with Cu, Co or Zn.

Since the enhanced trace element uptake of the liver during DSF treatment seems not be involved in the inhibition of enzymatic activity (at least of the enzymes investigated by us), a connection between increased trace element content and the protective action of DSF against nitrosamine induced liver tumors should be considered.

The severe loss of Cu and Zn in liver cytosol of NDEA treated mice (30–40% even at a pretumorous stage) [27] may influence the subsequent development of tumors, because a Zn and Cu deficiency in mammalians leads to an overall impairment of biochemical functions [39]. This loss will be more than compensated by the concomitant administration of DSF. Moreover, Cu added to the diet, either as an inorganic salt or as a metal chelate, seems to be involved in cytostatic processes. While Yamane [40] showed a suppressive effect of cupric acetate on chemical carcinogenesis in the livers of amino-azodye treated rats, Cu-chelates with 3-ethyoxy-2-oxobutyraldehyde-bis-thio semicarbazone or 3,5-diisopropylsalicylate were demonstrated to possess a marked antitumor effect [41, 42]. Cu compounds act as O_2^--scavengers [43] and seem to be able to compensate the decrease of superoxide dismutase activity observed in many tumor systems [44].

Therefore, the protective action of DSF against nitrosamine induced carcinogenesis in the liver is possibly related to the enhanced trace element concentration, especially that of Cu, in the liver.

REFERENCES

1. D. Schmähl, F. W. Krüger, M. Habs and B. Diehl, *Z. Krebsforsch.* **85**, 271 (1976).
2. L. W. Wattenberg, *J. natn. Cancer Inst.* **54**, 1005, (1975).
3. C. C. Irving, A. J. Tice and W. M. Murphy, *Cancer Res.* **39**, 3040 (1979).
4. D. Malejka-Giganti, R. C. McIver and R. E. Rydell, *J. natn. Cancer Inst.* **64**, 1471 (1980).
5. L. W. Wattenberg, *J. natn. Cancer Inst.* **52**, 1583 (1974).
6. L. W. Wattenberg, *J. natn. Cancer Inst.* **60**, 11 (1978).
7. S. Fiala, E. C. Trout, H. Ostrander and A. E. Fiala, *J. natn. Cancer Inst.* **64**, 267 (1980).
8. H. Strömme, *Biochem. Pharmac.* **14**, 381 (1965).
9. H. J. Strömme, *Biochem. Pharmac.* **14**, 393 (1965).
10. M. D. Faiman, D. E. Dodd and R. E. Hanzlik, *Res. Comm. Chem. Path. Pharm.* **21**, 543 (1978).
11. D. Hadjolov, N. Frank and D. Schmähl, *Z. Krebsforsch.* **90**, 107 (1977).
12. D. Schmähl and F. W. Krüger, in *Topics in Chemical Carcinogenesis* (Eds. W. Nakahara, S. Takayama, T. Sugimura and S. Odashima). University of Tokyo Press (1972).
13. N. Frank, D. Hadjolov, B. Bertram and M. Wiessler, *J. Cancer Res. Clin. Oncol.* **97**, 209 (1980).
14. F. W. Krüger, M. Bodenmüller and B. Bertram, unpublished results.
15. R. A. Deitrich and V. G. Erwin, *Molec. Pharmac.* **7** 301 (1971).
16. M. Goldstein and B. Anagnoste, E. Lauber and M. R. McKeregham, *Life Sci.* **3**, 763 (1964).
17. K. P. Du Bois, A. B. Raymund and B. E. Hiltbrink, *Toxicol. appl. Pharmac.* **3**, 236 (1961).
18. M. Lang, M. Marselos and R. Törrönen, *Chem. Biol. Interactions* **15**, 267 (1976).
19. M. A. Zemaitis and F. E. Greene, *Biochem. Pharmac.* **25**, 1355 (1976).
20. B. Stripp, F. E. Greene and J. Gillette, *J. Pharmac. exp. Ther.* **170**, 347 (1969).
21. R. A. Neal, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis* (Ed. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien). Academic Press, New York (1980).
22. T. Skotland and T. Ljones, *Eur. J. Biochem.* **94**, 145 (1979).
23. R. E. Heikkila, F. S. Carbat and G. Cohen, *J. biol. Chem.* **251**, 2182 (1976).
24. T. Skotland, L. Petersson, D. Backström, T. Ljones, T. Flatmark and A. Ehrenberg, *Eur. J. Biochem.* **103**, 5 (1980).
25. E. Lengfelder, *Z. Krebsforsch.* **34c**, 1292 (1979).
26. S. Friedman and D. Kaufmann, *J. biol. Chem.* **120**, 4763 (1965).
27. D. A. Brown, K. W. Chatel, A. Y. Chan and B. Knight, *Chem. Biol. Interactions* **32**, 13 (1980).
28. F. Kratz, in *Progress in Drug Research* (Ed. E. Jucker). Birckhäuser, Stuttgart (1973).
29. O. H. Lowry, N. A. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
30. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
31. T. Nash, *Biochem. J.* **55**, 416 (1953).
32. B. N. LaDu, H. G. Mandel and E. L. Way, *Fundamentals of Drug Metabolism and Drug Disposition.* Williams & Wilkins, Baltimore (1971).
33. J. Schuhmacher, W. Maier-Borst and H. Hauser, *J. Radianal. Chem.* **37**, 503 (1977).
34. K. N. Chetty, L. Drummond, D. S. V. Subba Rao and D. Desaiah, *Drug Chem. Toxicology* **2**, 375 (1979).
35. J. Aaseth, N. Søli and F. Øystein, *Acta Pharm. Toxicol.* **45**, 41 (1979).
36. J. Alexander and J. Aaseth, *Biochem. Pharmac.* **29**, 2129 (1980).
37. B. Sarkar and T. P. Kruck, in *The Biochemistry of Copper* (Eds. J. Peisach, P. Aisen, W. E. Blumberg), p. 183. Academic Press, New York (1966).
38. L. Pickart, H. Freedman, W. J. Loker *et al.*, *Nature, Lond.* **288**, 715 (1980).
39. *Trace Metals in Health and Disease* (Ed. N. Kharasch), Raven Press, New York (1979).
40. Y. Yamane, *J. Pharmacobio-Dynamics* **3**, 19 (1980).
41. D. H. Petering and H. G. Petering, *Handbk. exp. Pharmac.* **38**, 841 (1975).
42. S. W. C. Leuthauser, L. W. Oberley, T. D. Oberley, J. R. J. Sorensen and K. Ramakrishna, *J. natn. Cancer Inst.* **66**, 1077 (1981).
43. L. R. De Alvare, K. Goda and T. Kimura, *Biochem. Biophys. Res. Commun.* **69**, 687 (1976).
44. L. W. Oberley and G. R. Buettner, *Cancer Res.* **39**, 1141 (1979).