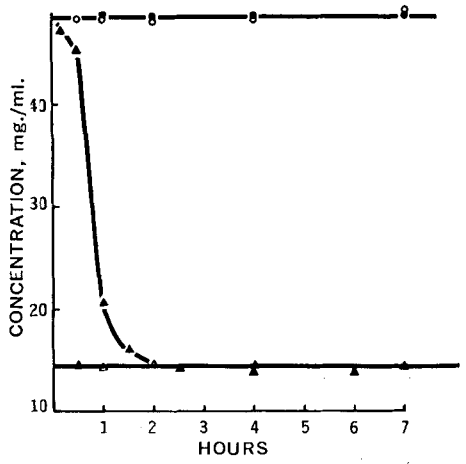

Figure 4—*Influence of seeding anhydrous Wy-4508 with 1 and 10% dihydrate crystals on the solubility in distilled water at 10°. Key:* ○, *anhydrous;* ●, *seeded with 1% dihydrate;* ▲, *seeded with 10% dihydrate; and* △, *dihydrate.*

utilized, there was no evidence of conversion of the more soluble anhydrous form to the less soluble dihydrate species as would be expected strictly from thermodynamic consideration. Undoubtedly, this may be due to steric factors involved in the association of the water molecules in the crystal system and to the relatively high water solubility of this particular amphoteric penicillin. Even at lower temperatures (10°), seeding of the anhydrous form with 1% dihydrate crystals did not result in a rapid conversion of the anhydrous form to the less soluble species. However, when the seed was incorporated at the 10% level, a relatively rapid and complete conversion of the anhydrous to the dihydrate form was observed as shown by the decrease in solubility. These data are summarized in Fig. 4.

SUMMARY

The solubility of the anhydrous and dihydrate forms of Wy-4508 in distilled water has been determined over a temperature range of 7–60°. From the solubility data, the transition temperature for this crystal system was estimated to be 61°. The anhydrous form was found to be significantly more water soluble than the dihydrate at all temperatures below the transition temperature. In addition, the solubility of the anhydrous crystal was shown to be inversely related to temperature (negative heat of solution). The thermodynamic values for the anhydrous–dihydrate Wy-4508 crystal system have been calculated.

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Effects of Disulfiram on Growth, Longevity, and Reproduction of the Albino Rat

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Abstract □ Chronic oral ingestion of disulfiram at concentrations of 1:1000 and 1:2000 in powdered food retards the growth and reproductive capacity of the albino rat; a concentration of 1:1000 also decreases longevity. Enhancement of diet with ascorbic acid has no antidotal action on the growth and reproductive effects. Chronic disulfiram feeding appears to have no significant effects on whole animal oxygen consumption, blood counts, tissue xanthine oxidase activity, and liver molybdenum content. Calcium deposition in the cerebellum was absent.

Keyphrases □ Disulfiram—chronic feeding studies, rats □ Blood cells, number—disulfiram effect □ Reproduction, longevity, body weight—disulfiram effects □ Oxygen consumption—disulfiram effect □ Ascorbic acid effect—disulfiram activity

While single oral doses and chronic feeding of disulfiram in the absence of ethanol are considered relatively nontoxic (1–3), chronic feeding of rats on diets containing disulfiram (1:400–1:10,000) has been reported to retard growth and increase mortality (4). Muscular incoordination was seen in rats chronically fed the 1:400 and 1:1000 diets by the age of 2 years, and microscopic examination of the cerebellum and basal ganglia revealed calcified masses in rats fed 1:400 disulfiram. In addition to its generally accepted inhibitory effect on liver aldehyde oxidase, disulfiram has been reported to have some antithyroid capacity (5). Disulfiram can inhibit the oxygen uptake of rat liver homogenates by apparently acting as a competitive hydrogen acceptor (6). Ascorbic acid overcomes this inhibition and is reported to be effective intravenously in man (7). The metabolic breakdown of disulfiram into diethyldithiocarbamate, an effective chelator, has also been noted (8).

After preliminary work in this laboratory, it was considered important to document the effects of disulfiram in the young rat during the most rapid phases of growth and to see whether longevity and reproductive capacity would be affected.

Table I—Effects of Disulfiram in the Diet on Body Weight of the Albino Rat[a]

Run	Sex (Number)	Age, Days	Days on Diet	Mean Wt., g. (±*SE*)	Mean Wt., g. (±*SE*)	Significance from Control, *p*	Mean Wt., g. (±*SE*)	Significance from Control, *p*
				Control Diet	**1:2000 Disulfiram**		**1:1000 Disulfiram**	
I	M (20)	30	0	55 (1.3)	56 (2.2)	>0.50	56 (1.7)	>0.50
		60	30	148 (3.3)	129 (4.6)	<0.01	115 (4.0)	<0.001
		90	60	191 (5.7)	169 (6.0)	<0.01	157 (5.0)	<0.001
		120	90	223 (4.9)	195 (4.3)	<0.001	185 (5.4)	<0.001
I	F (22)	30	0	52 (1.8)	51 (2.0)	>0.50	52 (1.9)	>0.50
		60	30	116 (2.6)	103 (4.0)	<0.01	90 (3.1)	<0.001
		90	60	139 (3.1)	127 (3.3)	<0.01	115 (2.9)	<0.001
		120	90	151 (2.8)	141 (3.1)	<0.02	129 (2.6)	<0.001
				Control Diet	**1:8000 Disulfiram**		**1:4000 Disulfiram**	
II	M (10)	30	0	49 (3.6)	50 (3.2)	>0.50	48 (3.2)	>0.50
		60	30	147 (4.2)	158 (5.2)	>0.10	148 (5.7)	>0.50
		90	60	218 (7.9)	225 (5.2)	>0.40	214 (5.0)	>0.50
II	F (10)	30	0	47 (2.8)	45 (2.2)	>0.50	46 (3.0)	>0.50
		60	30	114 (2.4)	116 (3.1)	>0.50	109 (3.3)	>0.20
		90	60	139 (4.7)	140 (3.4)	>0.50	133 (3.8)	>0.30

[a] The disulfiram was mechanically admixed with Purina powdered laboratory chow.

EXPERIMENTAL

All rats were albinos, bred originally from Wistar stock in this laboratory, and were maintained in animal quarters (26°) on unrestricted Purina powdered laboratory chow and tap water. Test diets containing 1:1000, 1:2000, 1:4000, and 1:8000 parts of disulfiram[1] per kg. of Purina powdered laboratory chow were prepared with an electric mixer. When ascorbic acid was added to the drinking water, the solution was prepared daily with tap water. From food consumption data, the daily intake of 1:2000 disulfiram in very young rats was equivalent to about 10 times the dosage of an adult man (0.5 g./day or 7 mg./kg.), while the intake for an adult rat was about 5 times the human dose.

RESULTS AND DISCUSSION

Growth Studies—Young rats were weaned after 30 days, segregated by sex, and randomly divided into three groups. Diets were instituted as shown in the first run of Table I. A significant growth lag was apparent after 30 days of feeding (13 and 22% for males and 11 and 22% for females fed 1:2000 and 1:1000 disulfiram, respectively). This lag was maintained up through 90 days of feeding (13 and 17% for males and 7 and 15% for females consuming 1:2000 and 1:1000, respectively). These results compare favorably with a 10 and 21% reduction, respectively, obtained in a preliminary study with only 10 males per diet. A second run, conducted in the same manner but feeding 1:4000 and 1:8000 disulfiram in the diet (Table I, Run 2), indicated that there was no significant retardation of growth with these concentrations. In another experiment, when feeding of male rats was begun at the age of 85 days and continued for 105 days, there was no significant difference in body weight between the controls and the rats fed 1:6750, 1:4500, 1:3000, and 1:2000 disulfiram (12 rats/test group).

Blood Counts—At the age of 175–185 days, red and white blood cell counts, as well as leucocyte differentials, were determined for another group of male and female rats fed continuously with either control, 1:2000, or 1:1000 disulfiram diets since the age of 30 days (10 males and 10 females/group). Mean red counts for the males were: 10.8, 10.5, and 9.6 million/cu. mm., and for the females: 10.2, 9.5, and 9.8 million/cu. mm., respectively. Mean white cell counts for males were: 13,360, 15,810, and 14,640/cu. mm., and for females: 12,570, 12,030, and 14,870/cu. mm., respectively. These figures, as well as the differential counts, all fell within the normal ranges (9), indicating no important differences between the groups that might be due to a chelation effect. Body weight differences were equivalent to those reported in the previous section.

Oxygen Consumption—At the age of 360–400 days, groups of male rats fed continuously either control, 1:2000, or 1:1000 disulfiram diets since the age of 30 days (9–10 animals/group) were placed in a closed system similar to that described by Maclagan and Sheahan (10) for the estimation of ml. oxygen utilized/kg./hr. at standard conditions. Mean oxygen consumption was not significantly different ($p > 0.50$) for the three populations (815, 793, and 846 ml./kg./hr. S.T.P., respectively). Apparently, these concentrations of disulfiram in the diet had not significantly altered thyroid function.

Table II—Effects of Disulfiram in the Diet on Longevity of the Male Albino Rat[a]

	Number/ Group	Mean Life Span, Days (±*SE*)	Significance from Control, *p*
Control diet	9	944 (33)	—
1:2000 Disulfiram	10	896 (37)	>0.30
1:1000 Disulfiram	10	744 (70)	<0.02

[a] Separated from mothers at the age of 30 days, and test diets begun *ad libitum*.

Effects on Longevity and Body Weight—Utilizing the techniques previously described, three groups of males were maintained on either control, 1:2000, or 1:1000 disulfiram diets until death. As shown in Table II, longevity was significantly reduced only in the animals receiving 1:1000 disulfiram. As in the preceding experiments, no signs of motor incoordination (4) were noted, although a general tendency for poor grooming was seen with the disulfiram-fed animals. The body weight of the rats receiving disulfiram in their food was consistently less than that of the control animals. As in previous experiments, there were no significant differences in the daily food intake between the groups. As with all old rats, a 10–20% incidence of tumors was seen in each of the three groups, but no specific tissue susceptibility was noted. Histologic examinations of the cerebella of two control rats, two on the 1:1000 disulfiram diets, and five on the 1:2000 disulfiram diets showed no calcification.[2] The age at the time of examination ranged from 2.68–2.97, 2.43–2.71, and 2.51–2.71 years, respectively. The age in all instances exceeded the 2-year maximum reported by Fitzhugh *et al.* (4), who found calcium deposition with a diet concentration of 1:400 of disulfiram.

Vitamin Enhancement of Diet—While the rat appears to have some intrinsic capacity to synthesize ascorbic acid (9), an experiment was performed to determine if inclusion of ascorbic acid in the drinking water would reverse or significantly mitigate the disulfiram-induced growth lag. As shown in Table III, a significant

[1] Supplies of disulfiram (Antabuse) were donated by Ayerst, McKenna and Harrison, Ltd.

[2] Histopathologic examinations were performed courtesy of Dr. Charles A. Mebus, D.V.M., Department of Veterinary Science, University of Nebraska.

Table III—Effects of Disulfiram Alone and with Ascorbic Acid on Growth of the Albino Rat

Sex (Number)	Age, Days	Days on Diet	Control Diet, Mean Wt., g. (±*SE*)	1:2000 Disulfiram: Mean Wt., g. (±*SE*)	1:2000 Disulfiram: Significance from Control, *p*	1:1000 Disulfiram, Tap Water: Mean Wt., g. (±*SE*)	1:1000 Disulfiram, Tap Water: Significance from Control, *p*	1:1000 Disulfiram, 0.5% Ascorbic Acid: Mean Wt., g. (±*SE*)	1:1000 Disulfiram, 0.5% Ascorbic Acid: Significance from Control, *p*	Significance between Tests, *p*
M (7)	30	0	56(2.5)	56(3.6)	>0.50	56(3.0)	>0.50	54(5.6)	>0.50	>0.50
	60	30	149(7.4)	131(8.1)	>0.10	122(6.2)	<0.02	85(4.8)	<0.001	<0.001
	90	60	193(12.3)	175(11.5)	>0.30	161(10.1)	>0.05	148(6.7)	<0.01	>0.20
	120	90	222(6.1)	192(4.6)	<0.01	184(9.8)	<0.01	200(7.4)	<0.05	>0.20
F (11)	30	0	48(2.9)	48(3.8)	>0.50	49(2.8)	>0.50	50(3.4)	>0.50	>0.50
	60	30	111(3.2)	97(6.3)	>0.05	82(2.8)	<0.001	76(3.3)	<0.001	>0.10
	90	60	131(3.7)	121(6.2)	>0.10	110(2.7)	<0.001	109(3.4)	<0.001	>0.50
	120	90	146(4.3)	135(5.3)	>0.10	126(2.0)	<0.001	129(2.5)	<0.01	>0.30

Table IV—Effects of Disulfiram Alone and with Ascorbic Acid on First- and Second-Generation Albino Rats

Generation	Sex	Age, Days	Days on Diet	Control Diet: No.	Control Diet: g. (±*SE*)	1:2000 Disulfiram: No.	1:2000 Disulfiram: g. (±*SE*)	1:2000 Disulfiram: Significance, *p*	1:1000 Disulfiram, Tap Water: No.	1:1000 Disulfiram, Tap Water: g. (±*SE*)	1:1000 Disulfiram, Tap Water: Significance, *p*	1:1000 Disulfiram, 0.5% Ascorbic Acid: No.	1:1000 Disulfiram, 0.5% Ascorbic Acid: g. (±*SE*)	1:1000 Disulfiram, 0.5% Ascorbic Acid: Significance, *p*	Significance between Tests, *p*
I	M	30	0	20	44(3.1)	12	41(3.1)	>0.50	9	37(3.0)	>0.10	6	37(1.6)	>0.05	>0.50
		60	30	20	146(5.1)	12	109(5.4)	<0.001	9	100(8.5)	<0.001	6	97(3.1)	<0.001	>0.50
		90	60	20	219(6.0)	12	163(5.6)	<0.001	9	144(7.5)	<0.001	6	133(5.6)	<0.001	>0.20
		120	90	20	260(6.8)	12	201(6.0)	<0.001	9	196(10.3)	<0.001	6	161(4.9)	<0.001	<0.01
II	M	30	0	10[a]	49(3.6)	6	45(3.6)	>0.40	0	0(—)	—	1	65(—)	—	—
I	F	30	0	18	42(0.8)	11	37(2.3)	>0.05	13	38(2.4)	>0.05	4	37(1.9)	>0.05	>0.50
		60	30	18	119(1.9)	11	93(1.7)	<0.001	13	77(4.2)	<0.001	4	92(3.7)	<0.001	<0.02
		90	60	18	153(2.1)	11	135(3.6)	<0.001	13	107(3.7)	<0.001	4	110(3.7)	<0.001	>0.50
II	F	30	0	12[a]	47(2.8)	12	47(2.5)	>0.50	1	40(—)	—	1	52(—)	—	—

[a] Only a representative random sample.

retardation of growth was generally seen after 90 days on the diets (14, 17, and 10% for males, and 8, 14, and 12% for females fed 1:2000 disulfiram, 1:1000 disulfiram, and 1:1000 disulfiram plus ascorbic acid in the drinking water, respectively). With the exception of the males on diet for 30 days, there was no significant difference in weight between animals with and without ascorbic acid on the 1:1000 diet. This exception was unexpected since it appeared to indicate that ascorbic acid might actually enhance disulfiram-induced growth retardation. However, this effect was not seen in the comparable female groups. A separate experiment was then set up to ascertain whether ascorbic acid in the drinking water of rats receiving only the control diet would retard growth or reduce fluid intake. No such effects were seen. In the experiment described in the next section, no consistent ascorbic acid retardation was seen, so this exception in Table III must be discounted as being presently unexplainable.

First- and Second-Generation Studies—In a preliminary study, 1:1000 disulfiram in the diet appeared to have a definite depressant effect on the reproductive capacity of rats, while 1:2000 disulfiram was without effect. At 130–135 days of age, the males and females of the preceding study (Table III) were mated while continuing to receive their assigned diets. The paired rats remained together until the female showed signs of pregnancy or reached the age of 228 days. The young from this mating were separated from their mothers at 30 days of age and placed on the same diet as their parents. These offspring are termed "first generation" in Table IV. First-generation males and females were then mated at the age of 90–95 days, and the young from this union were weighed at 30 days of age and tabulated as "second generation" in Table IV.

There was no significant effect ($p > 0.05$) between treatments as to the body weight of the first generation at the age of 30 days. However, after the diet was in effect for 30 days (60 days of age), the disulfiram-induced retardation of growth was seen (25, 32, and 34% for males, and 22, 35, and 23% for females fed 1:2000 disulfiram, 1:1000 disulfiram, and 1:1000 disulfiram plus ascorbic acid in the drinking water, respectively). There was one isolated instance of apparent ascorbic acid antagonism of disulfiram (females, 30 days of feeding), and one single instance of apparent ascorbic acid

Table V—Xanthine Oxidase Activity Obtained in the Presence (MB) and Absence (NMB) of Methylene Blue

Treatment, No. of Rats[a]	Mean Wt., g. (±*SE*)	Mean Oxygen Consumption, cu. mm./20 min./283 mg. Tissue (±*SE*): Liver NMB	Liver MB	Lung NMB	Lung MB	Intestine NMB	Intestine MB	Spleen NMB	Spleen MB	Kidney NMB	Kidney MB	Mean Liver Molybdenum, μg./g. Dry, Wt.
Control (7)	221(7.0)	18(3.7)	29(4.6)	13(1.2)	19(1.3)	33(3.5)	58(6.0)	17	17	4	8	2.21
1:2000 Disulfiram (8)	172(5.5)	19(3.3)	29(4.0)	8(0.7)	11(0.6)	36(4.2)	50(5.3)	12	15	3	9	2.64
Significance from control, *p*	<0.001	>0.50	>0.50	<0.01	<0.001	>0.50	>0.30					
1:1000 Disulfiram (4)	172(13.4)	21(2.4)	30(3.0)	8(1.0)	12(0.9)	32(3.7)	42(7.5)	10	14	6	12	2.05
Significance from control, *p*	<0.01	>0.50	>0.50	<0.02	<0.01	>0.50	>0.10					

[a] One determination at 37° per animal, except in the cases of kidney and spleen tissue where samples from two animals were pooled per determination.

Table VI—Effects of Disulfiram Alone and with Ascorbic Acid on the Reproduction of the Albino Rat

Treatment	Generation	Litters/ Pairs Mated	Mean Litter Size	Eaten by Mother, %	30-Day Survival, %
Control diet	I	11/12	6.7	2.7	89.2
Control diet	II	11/12	8.2	4.4	72.2
1:2000 Disulfiram	I	9/13	4.9	2.3	81.8
1:2000 Disulfiram	II	9/11	5.8	36.5	34.6
1:1000 Disulfiram	I	8/16	5.5	6.8	54.5
1:1000 Disulfiram	II	5/6	5.6	96.4	3.6
1:1000 Disulfiram[a]	I	5/7	6.4	6.2	31.2
1:1000 Disulfiram[a]	II	2/4	4.5	77.8	22.2

[a] Diet was supplemented with 0.5% ascorbic acid in the drinking water.

synergism with disulfiram (males, 90 days of feeding). Weight comparisons of second-generation animals were hampered by the antiproductive effects of 1:1000 disulfiram; however, there was no significant difference in body weight between second-generation controls and those from parents receiving 1:2000 disulfiram. It would appear in this experiment that disulfiram was not excreted or concentrated to any significant extent in the mother's milk, since offspring reaching 30 days of age did not exhibit significant retardation of growth. Ascorbic acid appears to have no predictable antagonistic effects against disulfiram.

Biochemical Studies—First-generation males (Table IV) were sacrificed and tissue xanthine oxidase activity measured in the presence and absence of methylene blue (11). Methylene blue has the capacity to overcome the disulfiram-induced inhibition of xanthine oxidase almost completely (12). Uric acid–allantoin–creatinine values for urine were determined prior to sacrifice in a manner similar to that of Bass and Place (13). As shown in Table V, lung xanthine oxidase activity was depressed in rats receiving disulfiram; however, this phenomenon is not easily explained since it was also seen with the tissue incubated with methylene blue. With this exception, chronic administration of disulfiram appears to have little to no effect on xanthine oxidase activity. This is in agreement with the finding (14) that xanthine oxidase apparently has little to do with acetaldehyde metabolism *in vivo*. There were no significant differences in the excretory uric acid/creatinine and allantoin/creatinine ratios (15) for the control and test diets. Since both aldehyde oxidase and xanthine oxidase are molybdenum dependent (16), it is of interest to note that the molybdenum content (17) was unaffected by chronic disulfiram feeding.

Effects on Reproduction—Animals were fed control and test diets and mated in a manner similar to that described for the rats in Table IV. The experiment, as summarized in Table VI, indicates that two factors are contributing to the antireproductive effect of disulfiram. By pooling the ratios (litters/pairs mated) for the first and second generations, a 92% incidence of successful conception for rats receiving the control diet is found. This can be contrasted with the 75 and 61% incidence seen for the pooled 1:2000 disulfiram generations and the pooled 1:1000 disulfiram (including those receiving ascorbic acid) generations. However, beyond this anticonceptive effect, one notes that the survival incidence of the young rats born from disulfiram-fed mothers is much less than the controls. Since malformations were not noted at birth and since those surviving 30 days did not have their growth stunted (Table IV), as could be caused by disulfiram excretion in milk, the cause of infant mortality may be due to decreased milk secretion by the mother. This could be a secondary result of chelation. Disturbances of lactation are often interrelated with cannibalism in the albino rat (9).

SUMMARY

Chronic feeding of 1:1000 and 1:2000 disulfiram in the diet retarded the growth of weanling albino rats, while concentrations of 1:4000 and 1:8000 were without effect. Continued feeding of the active concentrations limited the reproductive capacity of young adults by having an anticonceptive effect, as well as by decreasing the survival incidence of the offspring. This latter effect may be due to decreased lactation and secondary cannibalism. Since Lish (18) did not note any significant alterations in the rat estrus cycle it is possible that the mechanism of disulfiram's anticonceptive action involves inhibition of spermatogenesis. In this context, it is interesting to note that the experimental agents of the bis(dichloroacetyl)diamine type, such as *N,N'*-bis(dichloroacetyl)-1,8-octanediamine (WIN-18446) which inhibits spermatogenesis in the rat (19) and man (20), produce pronounced disulfiramlike reactions if the subject also consumes ethanol (21).

Chronic feeding of 1:1000 disulfiram decreases the longevity of rats without any significant accompanying gross symptomatology. This concentration is considered equivalent to about 10 (adult rats) to 20 (young rats) times the average human dose (500 mg./day or 7 mg./kg.). Decreased longevity is probably not correlated with blood dyscrasias.

Effects of disulfiram on growth and reproduction were resistant to supplemental feeding with ascorbic acid and also appeared not to be due to a thiouracil-like effect on the thyroid gland. Tissue xanthine oxidase activity was unaffected as was liver molybdenum by feeding 1:1000 and 1:2000 disulfiram.

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