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Implantation of Disulfiram in Rats[1]

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HELLSTRÖM, E., O. TOTTMAR AND R. FRIED. *Implantation of disulfiram in rats.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 73–82, 1980.—The biochemical and pharmacological effects of disulfiram implantation were studied in rats. Sterile disulfiram pellets (1000 mg/kg) were implanted subcutaneously. Groups of 5 rats were killed after 3, 7, 14, 28 and 56 days. The release of disulfiram during the first week corresponded to a daily dose of 12–16 mg/kg and during the following period to 5–8 mg/kg. The activity of the low-K_m aldehyde dehydrogenase in liver and brain, the carboxylesterase activity in liver and the dopamine-β-hydroxylase activity in heart were significantly decreased by approximately 45, 35, 20 and 35% respectively at all periods tested. The rate of ethanol elimination, the activity of monoamine oxidase in the brain, and the content of cytochrome P-450 in the liver were unaffected. The level of norepinephrine in the brain was slightly decreased after 14 days. The acetaldehyde level in blood after ethanol injection (1.0 g/kg) was 55–60 μM in the disulfiram group and 25–30 μM in the control group. Ethanol administration caused a slightly decreased blood pressure and increased respiratory rate 14 days after implantation but not after 28 days.

Disulfiram Antabuse® Implantation Ethanol Aldehyde dehydrogenase
Dopamine-β-hydroxylase Acetaldehyde

DISULFIRAM therapy is based on the alcoholics' fear of the unpleasant reaction, the disulfiram-ethanol reaction (DER), elicited after ethanol ingestion (for reviews, see [12, 15, 17, 21]). One of the major problems involved in the disulfiram treatment is the patient's compliance. In an attempt to avoid this problem, Marie [33] in 1955 introduced the method of subcutaneous implantation of disulfiram. Since then, the interest in this method has increased in several European countries and Canada [12,55]. The clinical reports are, in general, positive—patients with disulfiram implants show prolonged periods of abstinence. However, the therapeutic value of the implantation method is widely debated. It has been argued that the deterrent effects observed are due to psychological rather than pharmacological factors, and most reports seem to indicate that the typical symptoms of the DER are not elicited in patients with implants [24, 27, 30–32]. However, Wilson *et al.* [57] recently claimed that patients with implants do experience a mild form of the DER but only after several days of drinking, while sham-operated controls reported no reaction, which suggested a true pharmacological effect and not a placebo effect. The pertinent question is whether the amount of disulfiram released from the implantation site is sufficiently high to cause the ethanol-sensitizing effect normally found after oral administration of disulfiram. The dose of disulfiram implanted (1 g) corresponds to the usual oral dose given during four days [55].

Although the mechanism underlying the DER is not fully understood, it is believed that the main factors responsible for the reaction are the increased acetaldehyde level in blood and the low neuronal content of norepinephrine, caused by inhibition of aldehyde dehydrogenase (ALDH) and dopamine-β-hydroxylase (DBH), respectively [12, 17, 53]. These two enzymes, as well as the blood level of acetaldehyde and the cardiovascular response after an ethanol challenge, are therefore the appropriate markers for studies on the pharmacological effects of disulfiram implants. To our knowledge, no such studies have been performed either in humans or in experimental animals.

In a pilot study, Fried [12] found in experiments on rats that very little disulfiram was released from the implantation site. Since none of the markers mentioned above were studied in these experiments, the present investigation was carried out to study in more detail the biochemical effects of disulfiram implants in rats.

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METHOD

Materials

Sterile tablets of disulfiram (ESPERAL), weighing 100 mg, were supplied by Laboratory Solac, Toulouse, France. The tablets consist of pure disulfiram without any vehicle (R. Fried, unpublished results). ^{14}C-tyramine (specific activity about 50 mCi/mmol) was obtained from the Radiochemical Center, Amersham, England. Dowex 50 W, X-4, (200–400 mesh) was obtained from Bio-Rad Lab., Richmond, CA, USA. All other chemicals used were obtained from Sigma Biochemical Co., St. Louis, MO, USA, and E. Merck AG, Darmstadt, W. Germany.

Animals

Female Sprague-Dawley rats (Anticimex, Sollentuna, Sweden), weighing 200–220 g, were kept in individual cages and had free access to food (R-3 diet obtained from Astra Ewos, Sollentuna, Sweden) and tap water. The rats were weighed once a week throughout the study.

Implantation of Disulfiram

Two disulfiram tablets (2×100 mg) were implanted subcutaneously in sixty-three rats, in the back of the neck, inaccessible to the mouth or paws. The animals were anaesthetized with methohexital sodium (BRIETAL®, 50 mg/kg, IP). The hair was removed, and the skin was washed with a 70% solution of alcohol. A longitudinal incision of 1 cm was made between the scalpulae, and the skin was separated from underlying connective tissue. The tablets were inserted subcutaneously and the incision was closed with 3–4 silk sutures. Fifty-five control animals were sham-operated, and the implantation site was manipulated with blunt tweezers. At periodic intervals after implantation (7–84 days), the rats were sacrificed, and the tablets were removed, blotted dry and kept in open air at room temperature for at least 24 hr before weighing.

Recording of Blood Pressure, Heart Rate and Respiratory Rate

Rats were anaesthetized with hexobarbital sodium (EVIPAN®, 150 mg/kg, IP). Blood pressure was measured with a Statham P 23b transducer connected to one of the carotid arteries by a polythene catheter. Heart rate was measured by the use of a tachograph (built in this laboratory) connected to the blood-pressure unit. The respiratory rate was counted visually following the ventilation movements of the chest. Ethanol was injected when a stable base line had been obtained (after 20–30 min), and the blood pressure was then recorded continuously during 30 min. Respiratory rate and heart rate were recorded every 10 min. The body temperature was kept at 37.0–37.5°C (rectal temperature) by placing the animals on an automatically regulated warming-pad. Responses have been calculated from the changes in the initial resting mean blood pressure, heart rate and respiratory rate respectively.

Determination of Ethanol and Acetaldehyde in Blood

Blood samples of 0.1 ml were taken from the tip of the tail. Ethanol concentration was determined enzymatically with yeast alcohol dehydrogenase as described by Krebs *et al.* [20]. Acetaldehyde was determined fluorimetrically with the use of a partially purified preparation of ALDH (isolated from rat liver mitochondria) according to Tottmar *et al.* [51]. No corrections have been made for the non-enzymatic formation of acetaldehyde from ethanol in blood extracts [9, 45, 51].

Tissue Preparation and Enzyme Assays

^{14}C-tyramine was injected intravenously in anaesthetized rats (hexobarbital sodium, 150 mg/kg, IP). The rats were killed after 30 min and livers, brains and hearts were quickly removed. The livers were homogenized in a medium (pH 7.2) containing 0.25 M sucrose, 0.5 mM EDTA and 5 mM Tris-HCl. The microsomal fraction was isolated from a 10% (w/v) liver homogenate in a 0.25 M sucrose medium according to Tottmar *et al.* [48]. The brains were divided by a mid-sagittal section, and one half of the brain was homogenized in ice-cold saline (0.9%). The other half of the brain was frozen in liquid nitrogen, stored at −70°C, and was later used for determination of norepinephrine and dopamine. The hearts were frozen in liquid nitrogen.

Dopamine-β-hydroxylase (DBH) activity in the heart *in vivo* was measured as the rate of formation of ^{14}C-octopamine (^{14}C-OA) from injected ^{14}C-tyramine (^{14}C-TA) as previously described [52]. The dose of ^{14}C-tyramine given was 14 μg/kg (4 μCi/kg).

The activity of the low-K_m ALDH in liver homogenates was determined spectrophotometrically by measuring the reduction of NAD^+ at 340 nm with acetaldehyde (25 μM) as the substrate [48,50]. The activity of ALDH in brain was measured as follows: Samples of brain homogenates (10%, w/v) were solubilized with sodium deoxycholate (0.5%, w/v final concentration) shortly before addition to the reaction mixture. The incubation mixture was prepared in 10 ml serum vials and contained 40 mM sodium pyrophosphate buffer (pH 8.8), 0.5 mM NAD^+ and 0.5 ml of the sample in a total volume of 2.6 ml. The mixture was preincubated in a water bath at 25°C for 5 min with shaking. The vials were tightly stoppered, and acetaldehyde was added through the rubber stoppers using a Hamilton syringe. The final concentration of acetaldehyde was 20 μM. The reaction was stopped after 15 min by adding 0.5 ml of 16% (w/v) perchloric acid through the rubber stoppers. The mixture was transferred into 10 ml polystyrene centrifuge tubes and centrifuged at 1000×G for 10 min at 4°C. The supernatant was neutralized with 1 ml of 0.85 M K_2CO_3, and the precipitate was removed by centrifugation as above. The acetaldehyde concentration was determined fluorimetrically with the use of aldehyde dehydrogenase [51]. Tissue blanks and water blanks were treated with perchloric acid before the addition of acetaldehyde. The ALDH-activity was calculated as the rate of disappearance of acetaldehyde. The concentration of acetaldehyde in tissue blanks was similar to that found in water blanks, showing that no or very little acetaldehyde was lost through tissue binding. The activity was linear for at least 25 min and proportional to the amount of sample added.

Alcohol dehydrogenase (ADH) activity in liver homogenates was assayed essentially as described by Büttner [5] by following the oxidation of NADH spectrophotometrically with acetaldehyde (5 mM) as the substrate.

Monoamine oxidase (MAO) was measured by the method of Krajl [19]. Brain homogenates (10% w/v) were solubilized with Triton X-100 (1%, w/v) and centrifuged at 1000×G for 10 min. 0.1 ml of the supernatant was used for the assay. The

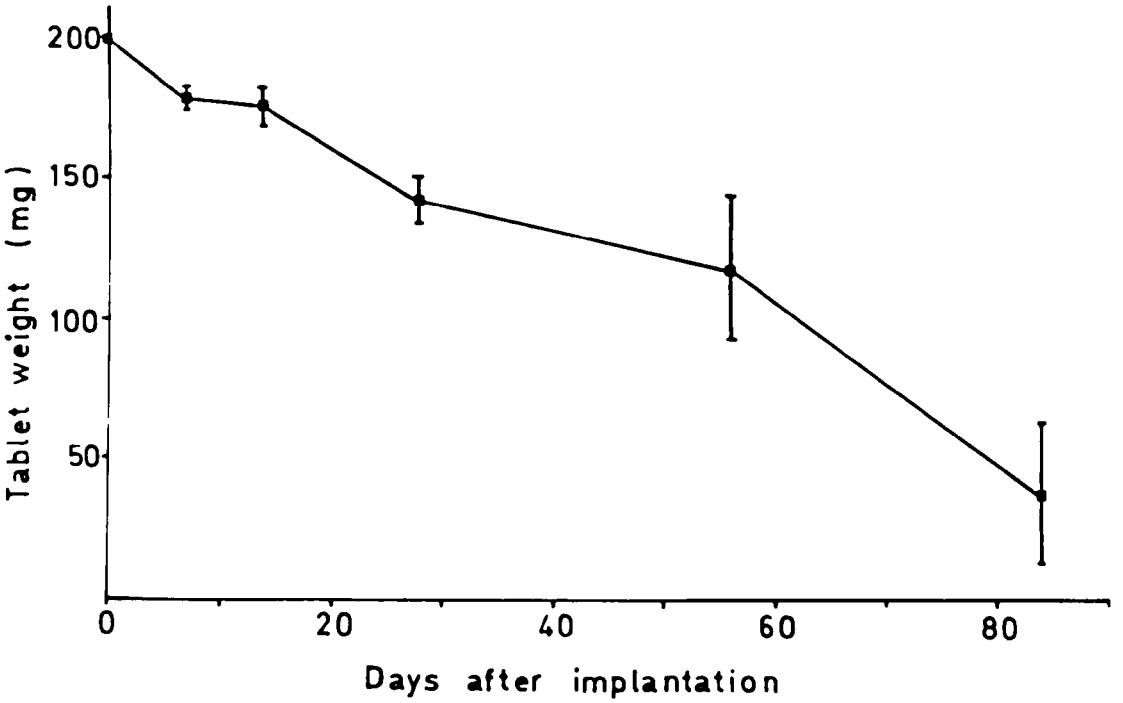

FIG. 1. Release of disulfiram from implanted tablets. The initial weight was 2×100 mg. The results are given as means ± S.D. from 5 animals.

activity was measured fluorimetrically by following the formation of 4-hydroxyquinoline from kynuramine at 37°C in 0.3 M phosphate buffer (pH 7.4).

Carboxylesterase activity in liver homogenates (10% w/v) was determined by using a colorimetric assay with indophenyl-acetate as the substrate [58]. The content of cytochrome P-450 in liver microsomes was assayed as described by Omura and Sato [38].

The activities of ALDH, DBH and cytochrome P-450 were measured within 4 hrs after killing the rats. Samples of brain and liver homogenates were stored at −70°C and used for determination of the other enzyme activities 2–4 weeks later. Protein was determined by the method of Lowry *et al.* [26] with bovine serum albumin as a standard.

Determination of Norepinephrine (NE) and Dopamine (DA)

Brains were extracted with perchloric acid as previously described [52]. The extracts were passed through Dowex 50 W columns (Dowex 50 W, X-4, 200–400 mesh, resin bed 3.7 mm diameter and 85 mm long in Na^+-form) and NE and DA were eluted according to the method of Atack and Magnusson [4]. The fluorimetric determination of NE and DA were performed as described by Kehr *et al.* [16] and Atack [3], respectively.

Statistical analyses were performed by the Wilcoxon test for two independent samples.

RESULTS

General Effects of Disulfiram Implantation

Implantation of disulfiram did not affect the growth rate of the rats during the following 8 weeks. No side effects such as sedation or gross behavioral changes were observed. In some rats inflammation developed at the implantation site during the first two weeks, probably due to chemical irritation or to a foreign-body reaction. Breakdown of the wound and extrusion of one or both tablets occurred in 27 of 63 rats (43%). This complication took place 3–4 weeks after the operation. In sham-operated controls, no signs of inflammation were observed.

Release of Disulfiram

In approximately 30% of the rats, one or both tablets were found to be encapsulated in connective tissue. This encapsulation could be observed already during the first week. The encapsulation did not affect the absorption of disulfiram. About 11% of the implanted disulfiram (initial weight 200 mg) was released during the first week (Fig. 1). The percentage of disulfiram released during 14, 28 and 56 days were 13, 29 and 41%, respectively. Values from animals in which only one tablet could be found have not been included, with the exception of the value of the 84th day, when only one rat was left in which both tablets could be recovered. The weight of these two tablets had decreased by 81%. In five other rats, having only one tablet after 84 days, the release of disulfiram was approximately 81% (Fig. 1).

Effects on the ALDH-Activity in the Liver and the Brain

The activity of the low K_m-ALDH in the liver was significantly decreased (32–54%) at all periods tested ($p<0.01$). The inhibition was already observed after 3 days and was most pronounced 14 days after the implantation (Fig. 2).

A similar pattern was obtained when the ALDH-activity

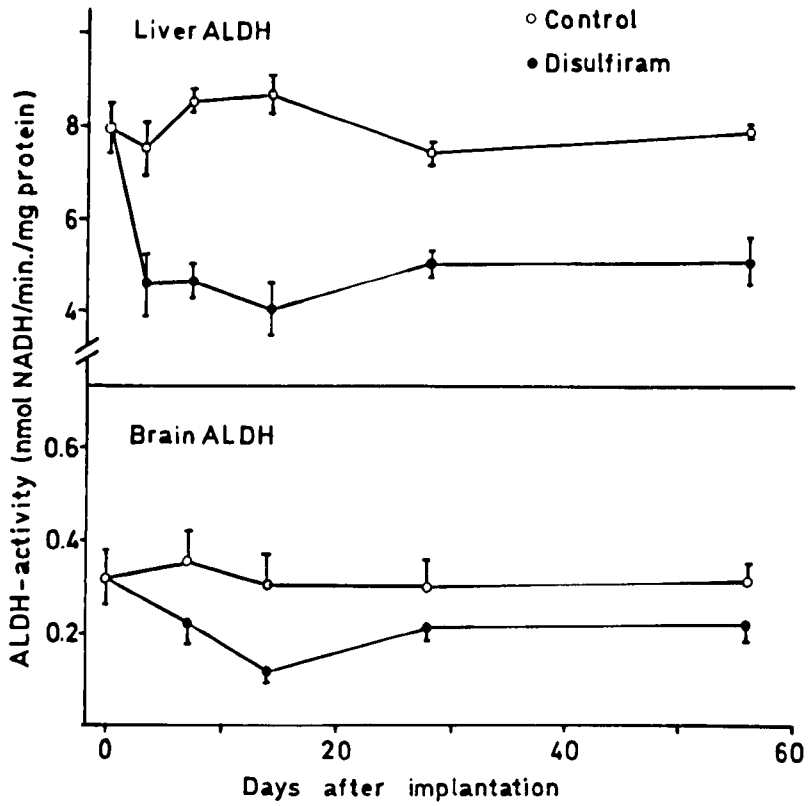

FIG. 2. Effects of disulfiram on the low K_m-aldehyde dehydrogenase (ALDH) activity in the liver and the brain. The points are the means ± S.D. from 5 animals.

in the brain was measured (Fig. 2). The activity of the low K_m-ALDH in disulfiram treated rats was significantly lower than control values ($p<0.01$) during the whole period. As in the liver, the greatest inhibition of brain ALDH occurred after 14 days.

In separate experiments, disulfiram was administered orally during one week at a daily dose of 14 or 30 mg/kg. The lower dose corresponded to the daily amount of disulfiram released from the implants. After seven days, the inhibition of the low-K_m ALDH in the liver was 28% and 54% ($p<0.01$, n=5) in rats given the low and high dose, respectively. In rats with implants, the inhibition was 38% after the corresponding period (Fig. 1).

Rate of Ethanol Elimination and Activity of Liver ADH

Forty days after implantation, ethanol (1.5 g/kg) was given intraperitoneally to 5 sham-operated and 5 disulfiram implanted rats. Blood samples were taken from the tip of the tail every 30 minutes during 5 hours. No difference in the rate of ethanol elimination was seen between the two groups (Fig. 3). The blood ethanol concentration was about 20 mM 60 minutes after ethanol administration. The blood acetaldehyde levels were significantly higher ($p<0.05$) in the disulfiram-treated group at 30, 60 and 120 minutes. The highest concentration of acetaldehyde (43 ± 16 μM) was found after 60 minutes and was approximately two times higher than the control level (19 ± 7 μM).

The activity of ADH in liver homogenates was measured in two other groups of rats 28 and 56 days after implantation. As shown in Table 1, the activity of ADH was not affected by disulfiram.

Effects on DBH-Activity in the Heart and the Levels of Norepinephrine (NE) and Dopamine (DA) in the Brain

In disulfiram treated rats, the yield of ^{14}C-OA was markedly decreased ($p<0.01$) at all periods tested (Fig. 4A), and the retention of ^{14}C-TA was slightly increased (not shown). At 14 days after implantation, the NE-content in the brains of disulfiram-treated rats was significantly lower ($p<0.01$) than that in the corresponding control rats (Fig. 4B). However, the difference observed was mainly caused by an increased NE-content in the control group, whereas the amount in the disulfiram-treated rats was rather similar to that observed at the other periods tested. The levels of DA were not significantly different from control levels at 14 days (0.86 ± 0.14 and 0.87 ± 0.11 μg/g brain, respectively) nor at the other days of observation.

Effects on Carboxylesterase and MAO Activities and Cytochrome P-450

The activity of carboxylesterase in liver homogenates from disulfiram treated rats was significantly lower (15–20%,

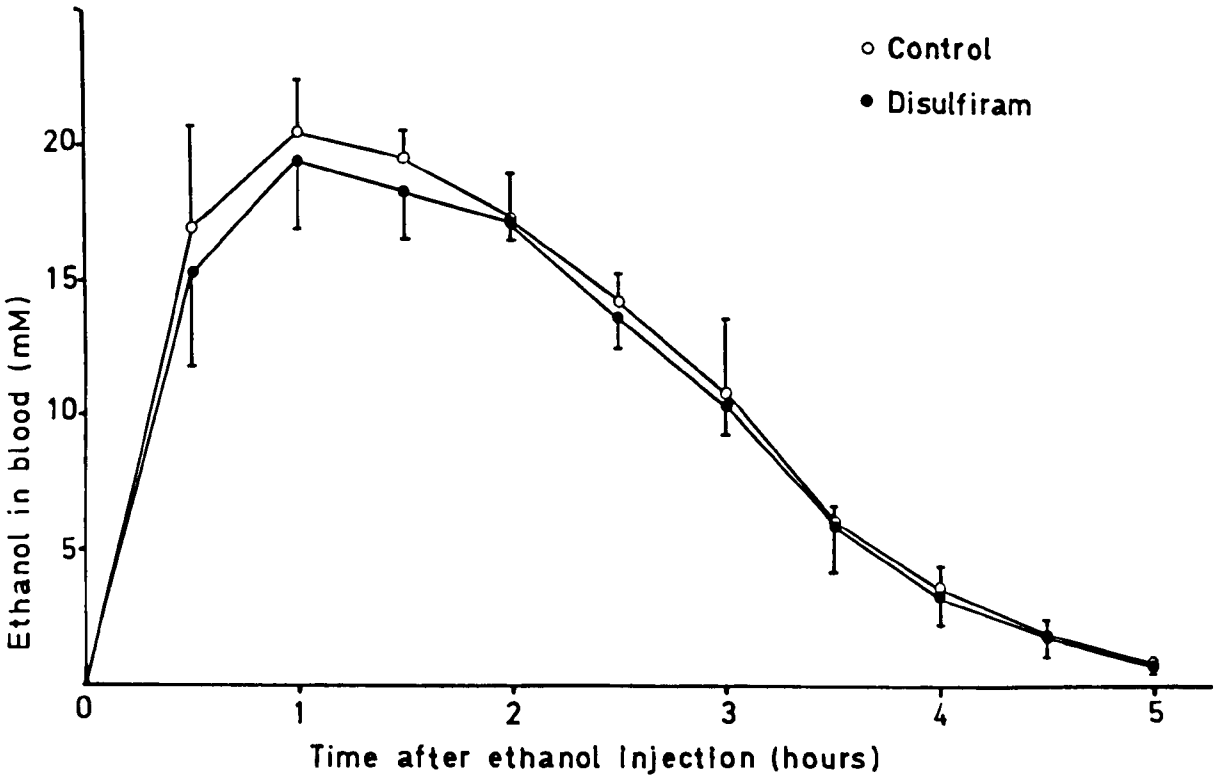

FIG. 3. Ethanol levels in peripheral blood from disulfiram-treated rats and control rats. Ethanol (1.5 g/kg) was given intraperitoneally 40 days after implantation. Each point represent the mean ± S.D. from 5 experiments.

$p<0.01$) than the control activity at all the periods tested (Table 1).

The effect of disulfiram on cytochrome P-450 in liver microsomes was studied at 28 and 56 days after implantation. There were no significant differences in cytochrome P-450 levels between controls and disulfiram-treated rats at these two periods (Table 1).

The activity of MAO in brain homogenates was not influenced by the disulfiram treatment (Table 1).

Blood Pressure Response after Ethanol Administration

The cardiovascular effects following ethanol administration were studied 14 and 28 days after implantation. Blood pressure, heart rate and respiratory rate were followed during 30 minutes after ethanol injection (1.0 g/kg, IP). No differences in the initial resting values of mean blood pressure, heart rate and respiratory rate were seen between the control group and the disulfiram group. The initial values

TABLE 1

EFFECTS OF DISULFIRAM ON MONOAMINE OXIDASE (MAO) ACTIVITY IN BRAIN, ALCOHOL DEHYDROGENASE (ADH)- AND CARBOXYL ESTERASE ACTIVITIES IN LIVER AND CYTOCHROME P-450 CONTENT IN LIVER MICROSOMES*

Experimental Group	Days	MAO (nmol/hr/mg Protein)	ADH (nmol/min/mg Protein)	Carboxyl Esterase (nmol/min/mg Protein)	Cyt. P-450 (nmol/mg Protein)
Control	7	30.2 ± 3.5	—	215 ± 11	—
	14	23.8 ± 1.7	—	234 ± 15	—
	28	26.8 ± 2.7	46.1 ± 2.5	189 ± 17	0.41 ± 0.03
	56	26.1 ± 2.9	47.5 ± 2.8	190 ± 9	0.47 ± 0.05
Disulfiram	7	30.8 ± 3.2	—	183 ± 11†	—
	14	25.0 ± 3.6	—	175 ± 7†	—
	28	26.9 ± 1.5	47.9 ± 3.0	143 ± 6†	0.43 ± 0.05
	56	27.1 ± 9.8	47.0 ± 3.8	165 ± 11†	0.44 ± 0.03

*The results are presented as the means ± S.D. from 5 animals. †Significantly different from control group ($p<0.01$).

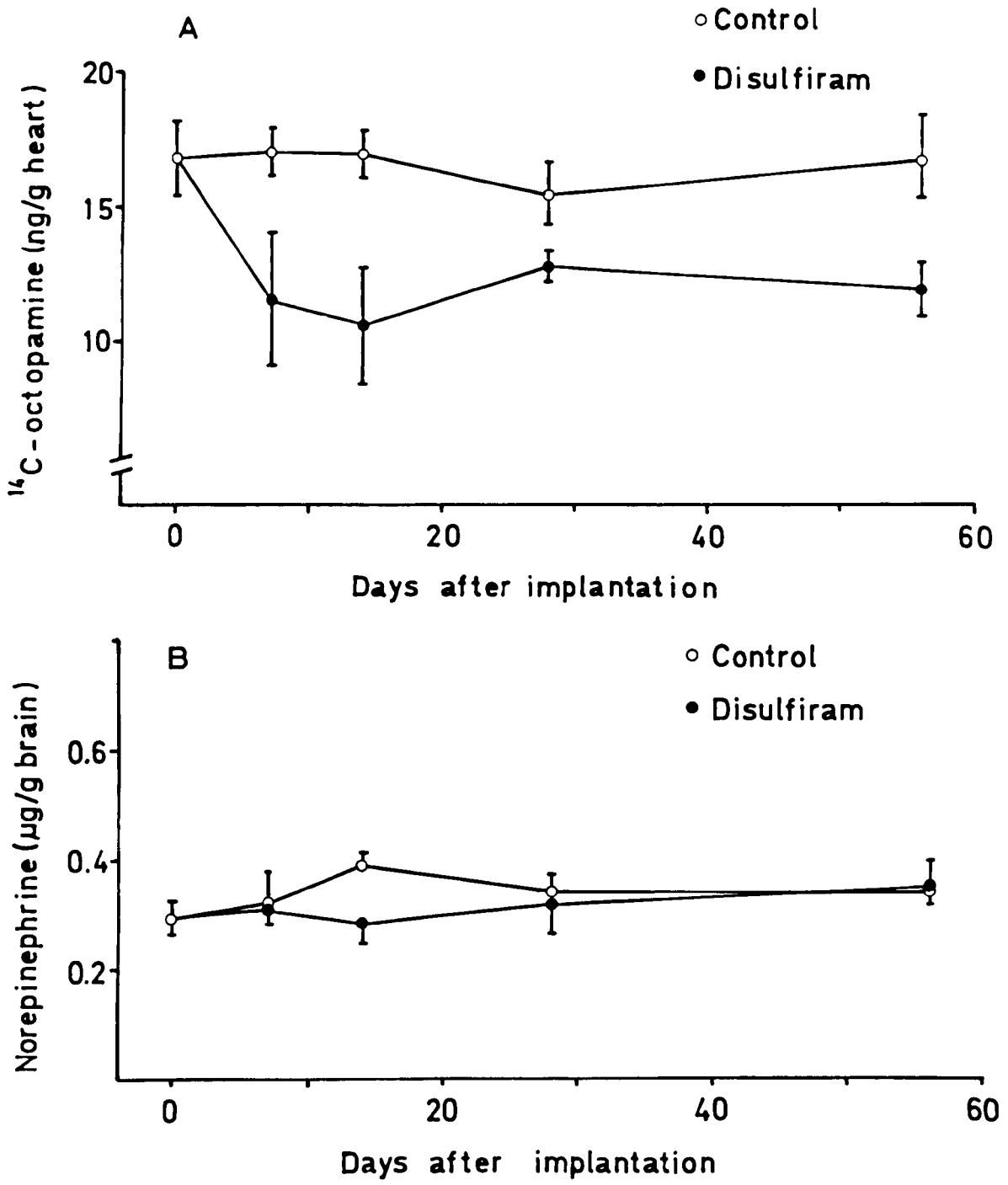

FIG. 4. Dopamine-β-hydroxylase activity in the heart (A) and norepinephrine levels in the brain (B) after implantation of disulfiram. The results are given as mean values ± S.D. from 4–5 animals.

TABLE 2

BLOOD PRESSURE RESPONSE, HEART RATE AND RESPIRATORY RATE AFTER ETHANOL ADMINISTRATION TO DISULFIRAM IMPLANTED RATS IN RELATION TO ACETALDEHYDE LEVELS IN PERIPHERAL BLOOD*

Experimental Group	Days	Change in Blood Pressure (mm Hg)	Change in Heart Rate (beats/min)	Change in Respiratory Rate (breaths/min)	Acetaldehyde Levels (μM)
Control	14	0 ± 2	↑20 ± 8	↑3 ± 1	34 ± 9
Disulfiram	14	↓7 ± 5†	↑24 ± 19	↑7 ± 3†	65 ± 21†
Control	28	↑1 ± 2	↑16 ± 8	↑2 ± 4	25 ± 9
Disulfiram	28	↓3 ± 2	↑22 ± 3	↑3 ± 2	54 ± 11†

*The experiments were performed on anaesthetized rats. The results are expressed as the changes observed 30 min after ethanol injection (1.0 g/kg, intraperitoneally). The values are the means ± S.D. of 5 experiments in each group. An increase or decrease in the values are indicated by arrows, (↑) and (↓) respectively.

†Significantly different from control group ($p<0.05$).

were as follows: 70 ± 6 mmHg, 378 ± 21 beats/min and 70 ± 3 breaths/min (mean ± S.D., N=20).

In most experiments, a slight increase in blood pressure and heart rate were seen in control rats after the ethanol injection (Table 2). However, in 4 of 5 rats with disulfiram tablets implanted for 14 days, a slight but significant fall in blood pressure and an increased respiratory rate were observed.

No significant effects on blood pressure, heart rate and respiratory rate were found in rats having disulfiram implanted for 28 days. Blood acetaldehyde levels in the disulfiram groups were 55–65 μM as compared to 25–35 μM in the control groups ($p<0.05$). The ethanol concentration in blood was 15–20 mM after 30 minutes in all groups.

DISCUSSION

Disulfiram has been used in alcohol therapy for about thirty years. Although much information has accumulated during this period, surprisingly little is known about its pharmacodynamics and disposition kinetics [6, 12, 18, 21]. The lack of basic information probably constitutes the main course of controversy about the therapeutic value of disulfiram implantation in alcoholics.

The pharmacogenesis of the DER is not yet understood. Disulfiram is a potent inhibitor of ALDH [8, 18, 50]. The inhibiton of this enzyme causes an increased acetaldehyde level in the body during ethanol oxidation, and this effect is considered as the prime cause of the DER [12, 18, 53]. However, disulfiram is a rather unspecific inhibitor, and several other factors in addition to acetaldehyde may be responsible for the reaction. As will be discussed below, disulfiram affects the synthesis of NE, and this effect is considered an important factor underlying the cardiovascular symptoms of the DER [53], as well as being responsible for many of the side-effects associated with disulfiram treatment [10, 28, 29, 42].

Another enzyme being inhibited by disulfiram, at least *in vitro*, is MAO [44]. Ethanol potentiates the disulfiram-induced inhibiton of rat-liver MAO *in vitro*, and it was suggested that this synergistic effect of disulfiram and ethanol could be involved in the DER [44].

Disulfiram inhibits the metabolism of many drugs [15, 37, 46, 54, 58, 59]. Although this effect is probably not involved in the DER, it is an important factor to consider in disulfiram therapy, since the alcoholic patients usually are given other drugs concomitantly.

The biochemical effects of disulfiram mentioned above were used in the present study on rats as suitable criteria for the bioavailability and pharmacological effects of implanted disulfiram.

In previous studies on rats, large doses of disulfiram (150–600 mg/kg) have been administered orally or intraperitoneally in order to produce well-defined biochemical effects. Thus, in order to obtain measurable effects, it was thought necessary to implant a rather large dose of disulfiram (1000 mg/kg). This dose is about seventy times higher than that implanted in humans.

Disulfiram was released at a fairly constant rate from the implantation site (approximately 2 mg/day/rat), and the dose implanted should thus have lasted for at least 100 days, if a linear disappearance rate is assumed. This is in contrast to the results reported by Fried [12], who found very little release of disulfiram over a period of one month. This slow release was attributed to the encapsulation of the tablets which occurred within a few days after implantation. In the present study, however, the absorption of disulfiram did not seem to be affected by encapsulation. It is possible that differences in surgical methods used for implantation can account for these different results.

The marked inhibition of the low-K_m ALDH in the liver and the brain was rather unexpected considering the small amounts of disulfiram released. However, the results obtained in rats given the corresponding daily amount of disulfiram as single, oral doses during one week indicate that the bioavailability of disulfiram for inhibition of the low-K_m ALDH is similar in rats with implants and in rats given disulfiram orally.

The activity of the high-K_m ALDH in the liver and the brain was not measured. However, previous studies in this laboratory have shown that the high-K_m ALDH in the liver [49,50] and the brain (unpublished results) is largely unaffected by disulfiram *in vivo*.

The increased acetaldehyde level in blood after ethanol administration to rats with disulfiram implants was consistent with the inhibition of the low-K_m ALDH, since the activity of this enzyme is closely related to the acetaldehyde level [25, 49, 50].

Previous studies have shown that the rate of ethanol elimination is decreased in rats given disulfiram [49,50]. This effect has been ascribed to the increased acetaldehyde level and the equilibrium characteristics of the ADH-catalyzed reaction, which favour the backward reaction, i.e. the reduction of acetaldehyde to ethanol [49,50]. In the rats with disulfiram implants, the acetaldehyde level was apparently too low to cause any observable decrease in the rate of ethanol oxidation. ADH in the liver was unaffected in rats with disulfiram implants, which is consistent with previous reports [23,50].

DBH, which is responsible for the conversion of DA to NE in the nerve terminals, is inhibited both *in vitro* and *in vivo* by diethyldithiocarbamate, the first metabolite of disulfiram [13,34]. Previous studies on rats have shown that disulfiram treatment causes a decreased content of NE and a slightly increased content of DA in the brain, heart and several other organs [14,35]. In the present study, a significant inhibiton of DBH in the heart was found. Whether this inhibition caused a decreased synthesis of NE is not known, since the NE content in the heart was not measured. The NE content in the brain was largly unaffected except for a slight decrease 14 days after implantation. However, this decrease may be artifactual, because the change observed was mainly caused by an increased NE content in the brains of the control rats. It is possible that the results obtained for the brain are also representative for the heart, since NE synthesis in the heart and the brain appears to be affected to the same extent by disulfiram, at least in rats [52]. This slight effect on NE synthesis is consistent with the fact that DBH is not the rate-limiting enzyme in the synthesis of NE from tyrosine [36]. Much larger doses of disulfiram than those released from the implants are evidently needed to cause an effective inhibition of NE synthesis.

The role of DBH in the DER has recently been questioned. Lake *et al.* [22] found no significant changes in plasma DBH and an increased plasma level of NE in humans taking disulfiram orally. In a similar study by Rogers *et al.* [42], no apparent changes in the response of the peripheral adrenergic system were found, although the urinary excretion data were consistent with decreased NE synthesis. Furthermore, studies on disulfiram-treated rats indicate that a DER can be elicited at increased acetaldehyde levels despite any inhibition of DBH [52]. Thus, the role of DBH in the DER might have been overestimated in previous studies where rather large doses have been given to experimental animals.

It is possible, however, that the inhibition of DBH in the brain explains some of adverse reactions associated with disulfiram therapy (tiredness, drowziness, headache, psychotic reactions [10, 28, 29]. Recent reports indicate that patients with low plasma activity of DBH are more susceptible to these side-effects, and that plasma DBH could be used as a marker for adverse reactions to disulfiram [10,29]. It has also been suggested that DBH inhibitors, including disulfiram, decrease the voluntary alcohol consumption in rats by supressing the positive reinforcing properties of ethanol [1, 2, 7]. In fact, it has been suggested that disulfiram may be of therapeutic value in its own right and not only as an alcohol-sensitizing drug [28].

The inhibition of the metabolism of drugs in both humans and experimental animals is well documented [15, 37, 46, 54, 58, 59]. Both acute and chronic administration of disulfiram to rats impaired the metabolism of ethylmorphine, decreased the activity of carboxylesterase and decreased the content of cytochrome P-450 in the liver [58,59]. Administration of disulfiram orally at a daily dose of 100 mg/kg during 12 days caused a 40% inhibition of carboxylesterase in the liver [58]. In the present study, the inhibition was about 25% at 14 and 28 days after the implantation. The content of cytochrome P-450 in the liver microsomes was unaffected after 28 and 56 days. However, it was reported that the content of cytochrome P-450 returned to control levels after 12 days of continued disulfiram therapy (100 mg/kg) [59]. Thus, an effect during the first two weeks in the present study cannot be excluded.

No effects on liver MAO were observed in rats with disulfiram implants. Similarly, in rats pretreated orally with disulfiram for 24 hr with a dose of 300 mg/kg, the MAO activity in the liver as well as in the brain was unaffected (unpublished results). In separate experiments it was confirmed that rat liver MAO is inhibited irreversibly by disulfiram *in vitro*, but that MAO was less susceptible to disulfiram inhibition than the low-K_m ALDH (E. Hellström, unpublished results). The lack of inhibition of MAO *in vivo* can probably be explained by the rapid metabolism and the low tissue concentration of disulfiram [17,47]. Whether or not ethanol affected MAO activity in the disulfiram-treated rats was not studied, but this possiblity should be investigated in further studies.

One of the most typical symptoms of the DER is hypotension [17, 40, 53]. In rats pretreated for 2–24 hr with low doses of disulfiram (9–27 mg/kg, IP), ethanol administration caused a pronounced hypotension [52]. In these rats, the acetaldehyde levels were similar, or even lower, than those found in the present study in rats with disulfiram implants. However, only slight changes in blood pressure and respiratory rate were observed 14 days after the implantation and no effects after 28 days. These results support the suggestion that the level of acetaldehyde is not solely decisive for the intensity of the DER, but that other factors, so far unknown, in combination with acetaldehyde, are responsible for the reaction [41, 43, 52]. It is possible, that these other factors are not affected to the same extent in rats with implants as in rats given the corresponding daily amount of disulfiram orally or intraperitoneally.

The dose of disulfiram implanted in humans (1000 mg) corresponds to the usual oral dose given during four days. The implanted tablets are believed to last for about 6 months [55]. Based on theoretical calculations, and assuming a daily release of 7 mg/day from an implant of 1000 mg, Kitson [18] concluded that insufficient amounts of disulfiram were released to cause any inhibition of ALDH in the liver and an increased acetaldehyde level after ethanol ingestion. If the classical explanation of the DER is accepted, this suggests that much larger doses of disulfiram than those presently used in humans would have to be implanted in order to produce an alcohol-sensitizing effect similar to that obtained after oral administration.

However, Wilson *et al.* [57] reported that the DER in patients with disulfiram implants differs from that observed after oral administration on three parameters: it has a slower onset, it is of longer duration, and it is less severe. Several days of drinking are often required before the reaction begins. According to Wilson *et al.*, this difference might explain, why the reaction has not been observed in most other studies. It was hypothesized that ethanol mobilizes lipid deposits of disulfiram, and that peripheral vasodilation increases the blood flow at the implantation site resulting in an increased absorption of disulfiram. Experimental evidence supporting these speculations is still lacking.

The challenging report by Wilson *et al.* [57] shows that it

might be premature to conclude that disulfiram implantation causes no biochemical or pharmacological effects sufficient to alter the physiological response to ethanol or the desire to drink. Further studies on the pharmacology and pharmacokinetics of disulfiram are needed before any firm conclusion can be drawn about the efficacy of disulfiram implantation.

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