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The fetal alcohol syndrome: symptoms and pathogenesis

by

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The symptoms of the fetal alcohol syndrome and their frequency of appearance are described based on 41 reports in the literature and on own observations. Experimental evidence is presented proving the lack of cytotoxicity, mutagenicity and teratogenicity of alcohol itself and the intensive cytotoxicity, mutagenicity and teratogenicity of acetaldehyde. Responsibility for the fetal alcohol syndrome is ascribed to acetaldehyde at maternal blood concentrations surpassing 35 μM and it is suggested that the raised acetaldehyde level is due to an inherited or acquired defect of mitochondrial aldehyde dehydrogenase. Prospective mothers displaying acetaldehyde levels exceeding 30 μM after a drink should be advised against bearing a child.

The history of alcohol consumption dates back to the earliest periods of human civilization and the connection of parental alcoholism with frailness and liability to mental deficiency of the child has been known ever since biblical times. The question has often been discussed in the last 100 years when, with the stressful consequences of urbanisation, "social" drinking has become customary. Most of the warnings, however, appeared in the lay press or in publications of temperance leagues, while the majority of medical papers was restricted to statistics and vague descriptions of ill health and susceptibility to diseases of the offspring. The first report on a distinct pattern of malformations in 127 infants born to chronically alcoholic women appeared in French periodicals in 1968 [24, 25]. It seems to have raised barely any interest until light had been shed on the subject by Jones et al. who devoted a series of studies to the syndrome [17, 18, 19, 11]. Since then, more than 60 pertinent reports have been published and now the number of cases described in the international literature exceeds 500.

Opinions are divided concerning the very factor and the mechanism responsible for the syndrome. Most authors ascribe it to a direct effect of alcohol and bring it into relation with the maternal or fetal blood alcohol

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level. Others assume that the underlying cause is not the alcohol but some disturbance often associated with the addiction, especially calorie or protein malnutrition. In view of the satisfactory calorie intake of many alcoholic mothers, an indirect malnutrition has been suggested, due to the inhibition by alcohol of the utilization of calories required for growth. Finally, the possibility of thiamine and/or folate deficiency, nicotine abuse, or a deficiency of trace elements such as zinc or magnesium has also been raised. A further problem is why many of the heaviest addicts sometimes deliver a normal child when some social drinkers have an affected baby. In the present work, besides giving a survey of the syndrome, an attempt has been made to clarify the above questions experimentally.

The syndrome

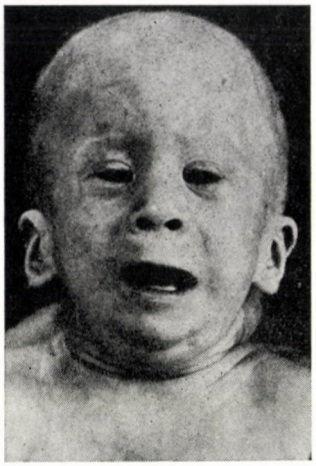
The infant

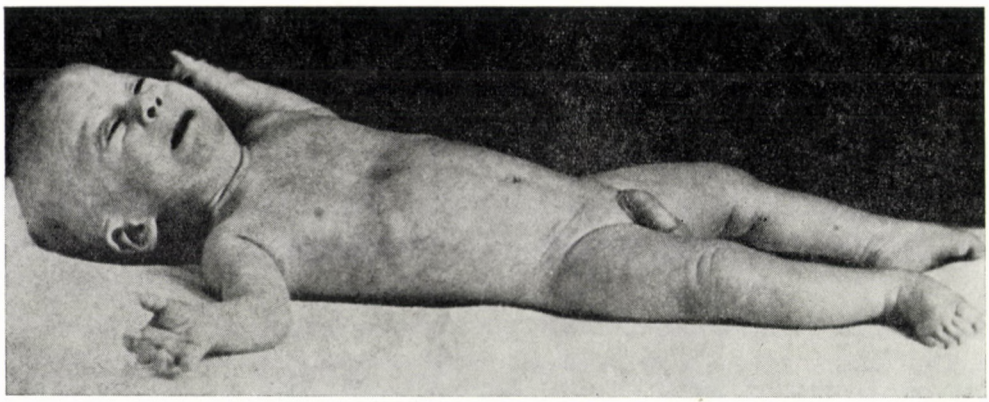
The main symptoms of the fetal alcohol syndrome are abnormalities of growth and performance and of craniofacial appearance, and anomalies of the limbs (Figs 1—5); these are often associated with heart defects and anomalous external genitalia. An attempt has been made to tabulate according to their frequency the characteristic signs reported in 41 papers (Table I). Among these, the most detailed survey is that by Majewski [27] compiled on grounds of 85 patients observed at the Tübingen (GFR) Department of Paediatrics. (The survey includes the material presented earlier by Bierich et al. [23].) Of the other teams, only 9 had a material of more than 10 patients, while 17 papers presented less than 10, and the rest dealt with single cases. Thus, the total number of patients considered in Table I, including 6 observed by us, amounts to 367. Many authors contented themselves with enumerating the main signs and failed to mention whether some symptoms were present or lacking; these have been neglected at evaluation of the frequency of the sign at issue.

The delay in intrauterine growth and mental development is an obligatory sign of the alcohol syndrome. In fact, we believe that, in spite of the characteristic facial appearance, a normal birth weight and due mental development exclude an alcoholic affection. Changes in hormonal factors also appear to do so, as hypothalamic-pituitary function is normal in these patients [39, 44].

Abortion and stillbirth are well known to be frequent with alcoholic mothers, especially with those having a long history of heavy drinking. Among their live-born offspring, females are in considerable excess; this has been suggested to reflect increased wastage of male fetuses to the effect of alcohol [38]. Preterm delivery is also frequent; according to a careful study [34] its proportion is 20% against 8% born to abstinent mothers.

The newborns with alcohol syndrome are small for dates and the lag in weight development is more expressed than that in length. Mean birth weight of 102 term infants mentioned in dif-





FIGS. 1 & 2. 3-month-old child born at term with fetal alcohol syndrome. Birth weight, 1590 g; length, 40 cm, head circumference, 28 cm. Epicanthic folds, small palpebral fissures, abnormal external ear, funnel chest, camptodactily, deep palmar creases, heart defect, mental backwardness. Mother regularly consumes alcohol since the age of 16 years and was treated with liver disease before pregnancy. Case of Prof. H. R. Wiedemann (Kiel), with kind permission of the author

ferent reports was 2210 g and mean length, less than 46 cm; both values are under the 3rd percentile. Head circumference at birth was about 2 SD below the mean. For this, multiple anomalies of brain development are responsible; common findings are leptomeningeal, glial heterotopia with agenesis of the corpus callosum and in a few cases of the brainstem and cerebellum, and internal hydrocephalus [7]. These findings fully explain the E.E.G. changes observed [13]. The baby is hyperactive, irritable, jittery. Some authors suggested that this was the result of alcohol withdrawal but as

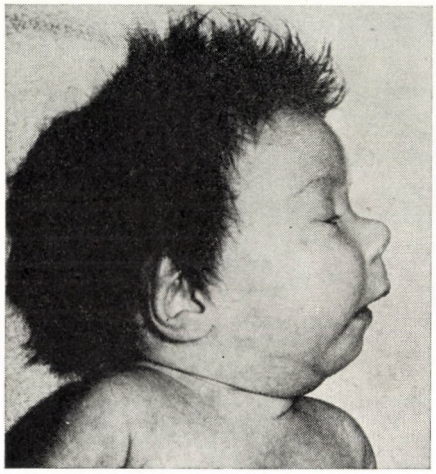


FIG. 3

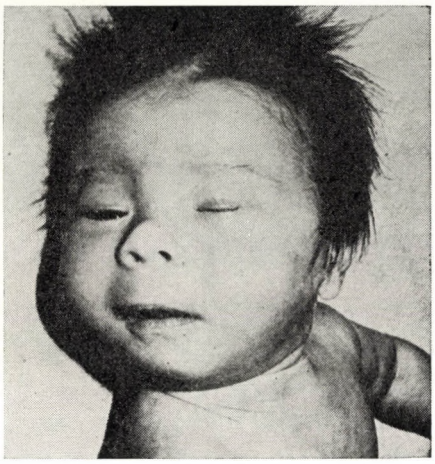


FIG. 4

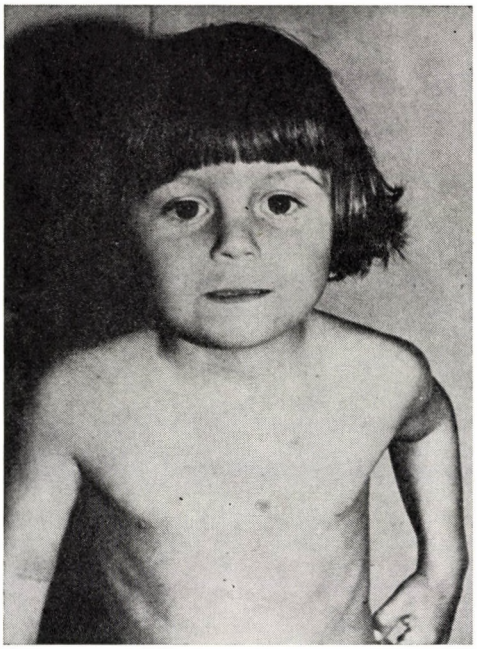


FIG. 5

FIGS. 3 & 4. 5-month-old child born at term with fetal alcohol syndrome. Weight at birth, 1970 g; at 6 weeks, 2500 g; at 5 months, 3200 g. Epicanthic folds, ptosis of eyelids, blepharophimosis, Gothic palate, heart defect, hypermotility. Mother alcohol addict since 3 years; one year before delivery, liver biopsy had revealed a fatty liver and cirrhosis. Case of Prof. J. R. Bierich (Tübingen), with kind permission of the author
FIG. 5. 4-year-old female child with fetal alcohol syndrome. Moderate epicanthic folds, thin vermilion border, retrogenia, mental backwardness. Case of Dr. F. Majewski (Tübingen), with kind permission of the author

TABLE I

Symptoms of the fetal alcohol syndrome and their percentual frequency according to 41 reports in the literature

Abnormal growth and performance	
Prenatal growth deficiency	96
Postnatal growth deficiency	96
Microcephaly	89
Mental backwardness	88
Motor dysfunction	81
Craniofacial abnormalities	
Midfacial hypoplasia	73
Thin vermilion border	69
Epicanthic folds	69
Short palpebral fissures	61
Ptotic eyelids	36
Cleft (or gothic) palate	30
Strabismus	23
Limb anomalies	
Abnormal palmar creases	69
Joint anomalies	46
Other abnormalities	
Anomalous external genitalia	43
Heart (mostly septal) defect	36
Abnormal external ear	30
Camptodactily, clinodactily, small nails	25
Funnel chest	19

it persists for years or permanently, it must be ascribed to the cerebral anomaly.

The face is typical. The epicanthal folds, the antimongoloid slant and smallness of the eyes with short palpebral fissures and ptosis of the eyelids, the short upturned nose and the thin lips lend an unmistakable appearance to these patients.

The palmar creases are usually abnormal. Different patterns have been reported. Mostly the mid-palmar crease was rudimentary, the upper one absent or, in contrast, forming a deep furrow, and the 3rd, 4th and 5th interphalangeal creases were lacking. Camptodactily and clinodactily of the 5th finger and clinodactily of some toes are common.

The genitalia display minor abnormalities such as hypertrophy of the clitoris or cryptorchidism, but several patients had hypospadias and two girls pseudohermaphroditism.

Congenital heart defects have been observed in more than one third of the cases, but some authors report on a higher incidence. The rule is an atrial or, less often, a ventricular septal defect, but patent ductus arteriosus, Fallot tetralogy and pentalogy, interruption of the aortic arch, hypoplasia or aplasia of a pulmonary artery were also observed.

Perinatal mortality of the offspring of alcoholic mothers amounted to 17% among the patients of Jones et al. [18] while in some other reports a small increase over the usual rate and in the largest material hitherto surveyed [27] no increase whatever was observed. In the survivors mortality was slightly higher than in the average population but an increased susceptibility of these infants to infections and different diseases has been emphasized.

Chromosomal studies were normal in all the patients without exception. Clinical tests yielded non-informative or negative results for blood counts, coagulation factors, electrolytes, liver function, blood protein levels, blood sugar, thyroid hormones, and growth hormone.

As to the fate of infants with the fetal alcohol syndrome, they continue to develop poorly in length and weight, and also mentally. Although several authors reported on subsequent improvement of growth and somatic and mental performance, all carefully controlled studies show that the deficiencies persist and the I.Q. even becomes lower with age.

The mother

The age of mothers of children with the fetal alcohol syndrome ranged from 21 to 41 years. Few were younger than 25 years, and the great majority was between 31 and 34 years at birth of the affected child. Parity was high in these women and few of the affected children originated from the first pregnancy. Most were third children and as a rule the previous ones were normal. Five instances of dizygotic and two of monozygotic twins with the alcohol syndrome have been reported; in at least three instances the previous siblings were healthy.

As to liver disease in the mothers, data are astonishingly scarce in the records published. Among the 51 mothers whose history is mentioned in detail, at least 28 had been treated for liver disease before their pregnancy with the affected child. In 11 of the latter mothers liver biopsy revealed a fatty liver or cirrhosis and 5 mothers died with hepatic coma.

Of maternal nutrition no details are found in any of the reports. None refers to signs of malnutrition or vitamin deficiency of any kind, while several studies emphasize that no malnutrition has been observed in these women [35].

References to disulfiram (Antabuse^{R}) treatment are even more neglected. Only a single report [3] describes two instances where the mother had

been treated with the drug, one before pregnancy and the other during the first trimester. No mention is made of an eventual alcohol consumption during that treatment. Part of the patients namely continue to have a few drops of alcohol in secret when they have experienced that under the effect of disulfiram minute amounts offer full satisfaction. The importance of this will be seen later.

Pathogenesis

The metabolism of alcohol

The alcohol ingested is rapidly absorbed from the stomach and the intestines. The great majority dissolves in the body water and is oxidized by alcohol dehydrogenase, an enzyme located in the liver, kidneys and brain. Its cofactor is nicotinamide adenine nucleotide (NAD) which in the course of the process is reduced to NADH. As a result, acetaldehyde is formed. Two other pathways also exist, a catalase + peroxide enzyme system and the microsomal ethanol oxidizing system, but these do not seem to have a role in human metabolism.

I alcohol (C_2H_5OH) ⟶ acetaldehyde (CH_3CHO) ⟶ (alcohol dehydrogenase) ⟶ (NAD ⇌ NAHD)

The acetaldehyde formed is converted to acetyl CoA which in its turn is oxidized in the citric acid cycle. The first step is catalysed by the enzyme aldehyde dehydrogenase located mainly in the liver and the brain. Part of its activity is in the cytoplasm and part in the mitochondria and the microsomes. The cofactor is again NAD. As the removal of acetaldehyde occurs much faster than its formation from alcohol, the latter reaction limits the rate of elimination.

II. acetaldehyde ⟶ (aldehyde dehydrogenase) ⟶ (NAD ⇌ NADH) ⟶ acetyl CoA

In man the main site of alcohol metabolism is the liver; extrahepatic oxidation is only a fraction of the hepatic one. The total elimination rate in healthy adults is 90 to 130 mg/kg/hr (mean, 105 mg/kg/hr), independently of the amount consumed. Alcohol oxidation seems to depend on the race, being more rapid in Caucasians than in Eskimoes or Indians [9], and to a certain extent on the overall metabolic rate. It is very slightly affected by drugs, diets, and remains normal even in liver disease. Animal studies are conflicting in this respect but in humans no significant differences in alcohol elimination were noted between normal subjects and chronic alcoholics with liver disease [28], cirrhotic patients or in those with liver necrosis [41]. In spite of these changes being the common morphological alterations caused by alcoholism, alcohol dehydrogenase activity is not depressed in the affected hepatic areas [32].

The human placenta is permeable for alcohol [15] but the embryo has no alcohol dehydrogenase. In the fetus the enzyme is very weak and becomes

fully active at 5 years of age. Therefore, alcohol elimination is slow in preterm babies [10]. Doses of 0.4 to 0.95 g/kg infused into the umbilical vein were eliminated at a rate of 64 to 86 mg/kg/hr [47] and if at birth a mother and her baby had displayed identical blood alcohol levels, a few hours later the concentration was significantly higher in the baby.

Acetaldehyde is a very active lipid soluble material which binds easily to different substances. Its metabolization proceeds rapidly so that the amount formed from alcohol or injected intravenously disappears immediately from the blood. It passes across the decidua but is metabolized by the placenta so that after the third month no acetaldehyde is found in the fetus [21].

After alcohol consumption by a normal human subject, the blood acetaldehyde level is between 21 and 30 μM. The level is higher in a number of conditions. In a careful study [23] the mean blood concentration was significantly higher in alcoholic ($42.7 \pm \pm 1.2$ μM) than in nonalcoholic subjects (26.5 ± 1.5 μM). In addition, high levels were observed in rats during pregnancy [20] and in those fed certain diets [26]. Genetic differences also exist [22], clearly proving that for the high levels of acetaldehyde some inherited or acquired defect of one or the other form, or of some hitherto undefined isozyme, of aldehyde dehydrogenase must be responsible.

Inhibition of aldehyde dehydrogenase by disulfiram, a compound widely used in the treatment of alcohol addicts, produces the highest blood acetaldehyde levels after the ingestion of alcohol. They increase at least 5-fold, but concentrations as high as 0.8 to 1 mM, 30- to 40-fold of the usual, were also noted. In our material of 17 such patients, the mean blood acetaldehyde level was 280 μM.

The period of impact

All pertinent studies emphasize that alcohol drunk in excess by expectant mothers is a powerful cause of congenital malformations without, however, stating whether this was valid for the whole period of pregnancy or only part of it. A study investigating into the question [12] states that only drinking in the period immediately before conception showed a statistically significant association with the defects, while some authors [3, 11] assume that the greatest effect inducing fetal maldevelopment is exerted in the first trimester, whereas heavy alcohol consumption near term may have a greater effect on fetal nutrition and size.

Somatic and mental development may be affected by alcohol during the whole course of pregnancy. As to the other symptoms, some embryological data allow to estimate the period when the fetus is susceptible to the consequences of alcohol and/or acetaldehyde. Thus, for microcephaly the 4th to the 8th weeks are decisive. The palpebral fissure develops from the 4th to the 15th intrauterine weeks but its length will be determined by the

12th week. At this time terminates development of the lips, but the width of the vermilion border depends on the form of the crista and this is complete by the 6th week. Ossification of the mandible begins at 6 weeks and its shape practically persists after the 12th week. For the heart defects the period between the 4th and 6th weeks is of importance, it being at this time that the septum primum becomes attached to the ventral and dorsal endocardial cushions. For the other signs too, the same weeks are significant. All this would mean that for the full-blown syndrome to develop, maternal drinking between the 3rd and 8th, and especially in the 4th to the 6th, weeks is decisive. Alcohol consumption in the third and fourth month of pregnancy may, and very probably does, affect development, but the outcome will be different from the alcohol syndrome.

As regards the effect of alcohol on the unfertilized ovum, data are lacking. It is certain that in the graafian follicle the ovum is protected against, or is able to withstand, the usual levels of alcohol and/or acetaldehyde, or else, to eliminate an eventual injury. This clearly follows from the scores of observations of healthy infants delivered after an affected one had been born. It is not known whether the same is valid for the ovum in the fallopian tube, although it very probably is protected, partly by its pellucid zone and partly by its having practically no contact with blood and body fluids. The nidating cell after losing the pellucid zone is exposed to injuries without protection, although for a short time the resulting effect might still be eliminated. Subsequently, however, the embryo must bear the impact of the mutagenic or teratogenic agents acting on it.

Maternal alcohol treatment in and after the 32nd week of pregnancy does not induce alcohol syndrome in the fetus. Such treatment resulting in blood alcohol levels up to 1.83 g/l was widespread at a time with the purpose of preventing premature labour. The offspring showed no delay in postnatal somatic or mental development [10].

Evidence in support of the above claims was offered by the case of a woman observed by us. She has been drinking regularly between 100 and 200 ml of absolute alcohol a day since the age of 23 years in 1965. In 1970 and 1972 she had delivered two normal babies, then in 1975 she had one with a characteristic alcohol syndrome. In spite of medical advice she had neither stopped drinking nor reduced the customary intake. In 1976, a year after delivering the affected child, she again became pregnant but 10 days later she was arrested and jailed. In the prison she had no possibility whatever to obtain a drop of alcohol. After 6 months she was released and immediately resumed drinking. In spite of this, at due term she gave birth to a somewhat underweight but otherwise fully normal baby. Three weeks later she was given to drink 0.5 ml/kg of alcohol; by 30 minutes her blood acetaldehyde had risen to 140 μM (Fig. 6).

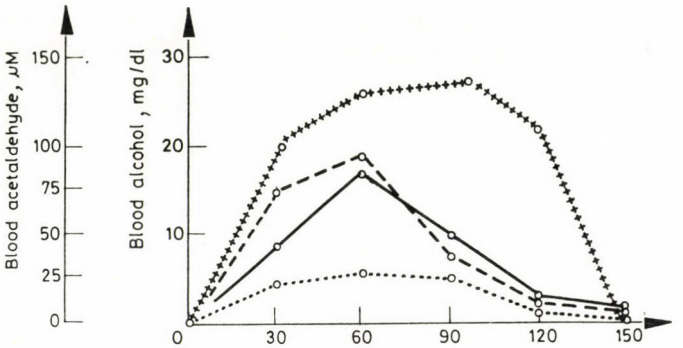


FIG. 6. Blood alcohol and acetaldehyde levels after drinking 0.4 g/kg of absolute alcohol in normal controls (mean of three subjects) and in a woman who had had a baby with fetal alcohol syndrome. ——— blood alcohol level in controls; . . . . . blood acetaldehyde level in controls; - - - - - - blood alcohol level in addict woman; + + + + blood acetaldehyde level in addict woman

The quantity consumed

Alcohol consumption by the mothers varied widely. The majority drank every day without exception, but some only on two or three occasions weakly. One woman allegedly never drank except on Saturdays when she regularly consumed one bottle of wine (1.45 ml/kg of absolute alcohol). The highest consumption reported was 4.9 ml/kg/day of absolute alcohol systematically for years, and several women confessed to having drunk about 3 ml/kg/day regularly for long periods.

Of course, these data cannot be relied upon. Few are the alcoholics who would not euphemize their addiction although just the heaviest drinkers with the longest histories often seem to be sincere, while this is rarely the case with repentant women, especially of a higher social standing and a higher education.

The same is true for the length of the drinking history. Still, few mothers said that they had been drinking for less than three years and regular alcohol consumption since childhood was no rarity. In a single case had the mother been drinking since less than a year. She drank 2 l of wine (3.3 ml/kg absolute alcohol) or more daily throughout the entire course of pregnancy. The child was born before term and displayed a typical alcohol syndrome. There is another report of a similarly short history of alcohol addiction but in this case the diagnosis was somewhat questionable.

Analysis of all serial studies failed to disclose any connexion between the occurrence and severity of the fetal syndrome and the frequency of drinking or the amount of alcohol consumed. For example, in a German report [27] the mothers of the slightly afflicted children drank a daily mean of 234 ml absolute alcohol, 100 ml more than

did the mothers of the gravest patients. All the other evaluable reports yielded similarly paradoxical results.

Experimental studies

Experiments were performed to establish the responsibility in the fetal alcohol syndrome of alcohol and/or acetaldehyde. Their effect on the mitotic cycle and DNA was studied by the sister chromatid exchange technique, mutagenicity on E. coli, and embryotoxicity and teratogenicity by investigating fetal resorption in rats.

Experimental material was obtained from 37 alcohol addicts under hospital treatment, and 8 healthy volunteers as controls.

Acetaldehyde was determined in blood samples taken under high purity argon in sample vials. After ultrasonic dispersion, 500 μl of blood was added in argon atmosphere to 500 μl of 3.2 mg/ml n-propanol in twice distilled water as internal standard. The Perkin-Elmer Model F-42 headspace gas chromatograph with hydrogen gas ionization detector was used with a 1 mV Honeywell Brown Electronic recorder. The column measured 1 mm in diameter and 500 mm in length and was packed with 80/120 mesh Chromosorb 102 (made by J. Manville). The operating conditions were, column temperature 120°C, detector temperature 160°C, injection port and needle temperature 150°C, flow rate of nitrogen high purity grade carrier gas, 30 ml/min, headspace equilibrium temperature was 40°C. Evaluation was made by a calibration curve plotted with a blood-water mixture containing 0.16 mg/ml gas chromatography grade n-propanol (C. Erba) as standard and 0.001 + 1 mM acetaldehyde (99.5% gas chromatography grade, Fluka A.G.).

Cell cycle and sister chromatid exchange (SCE)

were studied in Chinese hamster ovary (CHO) cell and human lymphocyte cultures, applying 5-bromodeoxuridine (BrdU) treatment and 33258 Hoechst and Giemsa staining (FPG) procedure [36].

(i) Alcohol added at 1 ml/dl concentration, about double the lethal human dose, had no effect whatever on CMO cells (Fig. 7).

(ii) Acetaldehyde (analytical grade, distilled twice immediately before use) added at 880 μM concentration killed 100% of CHO cells. The LD_{50} corresponded to 220 μM while 26.5 μM was ineffective (Fig. 8).

(iii) Acetaldehyde added in a dose of 400 μM to normal human lymphocytes was toxic and inhibited the multiplication of the surviving cells and had a clastogenic effect. After 72 hours the majority of cells was M_1 and of these, 12% displayed labile chro-

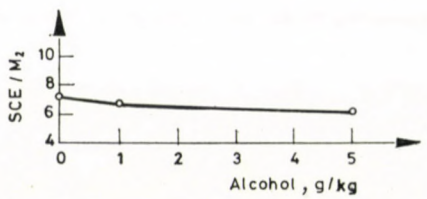


Fig. 7. Effect of alcohol on sister chromatid exchange frequency in CHO cells

mosomal aberrations. In the M_2 cells SCE was increased 4 to 6fold over the background. An acetaldehyde level of 40 μM still affected cell progression and SCE was double the control value, while lower amounts of acetaldehyde had no adverse effect (see Fig. 8).

(iv) Three alcohol addicts under chronic disulfiram treatment were made to drink about 0.2 ml/kg of alcohol in the form of wine. In some minutes when their blood acetaldehyde level had risen to above 100 μM, they developed a characteristic hypotensive episode. In the lymphocytes obtained during the episode, the distribution of M_1—M_2—M_3 cells was significantly altered in one and moderately in two patients, and SCE increased over the background in all the three. Such changes were absent in the same patients before they had drunk alcohol and in other disulfiram-treated addicts who had taken no alcohol (see Fig. 9).

(v) In 72-hour lymphocyte cultures of 7 subjects under the acute influence of alcohol, with blood levels ranging from 0.1 to 0.4%, the mitotic index was low, many of the cells were in the first metaphase, but there was no increase in SCE. In subjects with a blood alcohol level below 0.2% even the cell cycle was normal (see Fig. 10).

The above results, of which those enumerated under *(i)* and *(ii)* have been supported by a recent observation [33], seem clearly to show the harmlessness of alcohol and to prove the cytotoxicity and mutagenicity of acetaldehyde, a conclusion arrived at by us previously [45, 46].

Mutagenicity

of alcohol and acetaldehyde was then tested on Escherichia coli WP2 uvrA trp$^-$ cells incubated in hermetically stoppered test tubes at 0°C and then cultured on minimal agar supplemented with 0.25 μg/ml l-tryptophan. The number of colony-forming units was considered to reflect the frequency of mutated cells the tryptophan-synthetising capacity of which had been restored [16].

In these experiments, 1200 mg/kg of alcohol was ineffective while acetaldehyde at a concentration of 880 μM increased the spontaneous mutation frequency of the surviving cells 4.5 times at 31% lethality after 30 minutes exposition (see Table II). Variation of the exposition time between 5 and 30 minutes had no significant effect on the mutation frequency.

TABLE II

Mutagenic effect of 880 μM acetaldehyde on Escherichia coli WP2 uvrA trp$^-$ (Mean of 8 experiments each)

	Time minutes	Mutation frequency $\times 10^{-8}$
No acetaldehyde		6.3 ± 4.2
880 μM acetaldehyde	5	31.0
	10	28.0
	20	37.0
	30	28.3 ± 8.3

The difference was significant statistically (Student's t test).

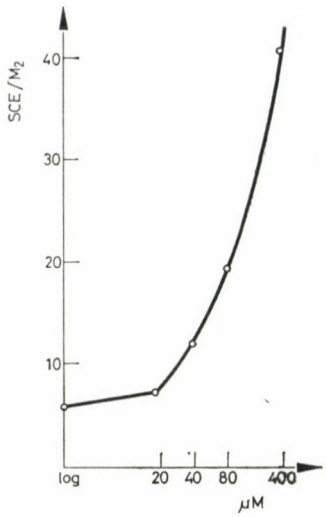


FIG. 8. Effect of acetaldehyde on sister chromatid exchange frequency in human lymphocytes. Ordinate, SCE in M_2 cells; abscissa, log acetaldehyde concentration

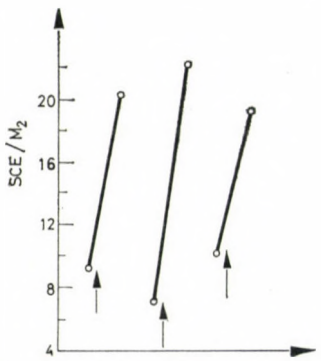


FIG. 9. Sister chromatid exchange frequency in M_2 lymphocytes of three subjects under disulfiram treatment, before and after drinking alcohol. Arrow: ingestion of 0.2 ml/kg of alcohol

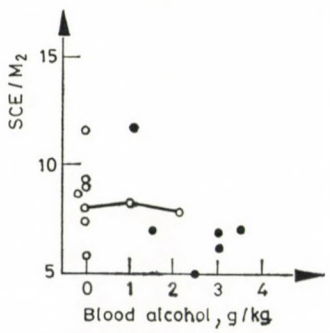


FIG. 10. Sister chromatid exchange frequency in M_1 lymphocytes of alcohol-intoxicated and control subjects. ● alcohol-intoxicated subjects; ○ control subjects. The three points connected with a line originate from the same person

Teratogenicity

was studied in virgin female Wistar/Riop rats weighing 150 to 200 g. They were caged with males of the same strain, weighing 250 to 300 g. The first day of pregnancy was determined by the presence of spermatozoa in the vaginal smear stained with 1% methylene blue. The pregnant rats were separated. On the 21st day of pregnancy, Caesarean section was performed and the fetuses were removed together with the uterine

horns. Live and dead fetuses and resorptions were counted in each horn. The offspring were weighed, one half was fixed in Bouin's solution and dissected according to a standard technique [48]. The rest was fixed in 96% alcohol and the skeleton was examined after KOH alizarin red S staining [42] and, when necessary, histological sections were prepared and examined after H + E staining.

Fetal mortality was calculated from the total implantation count while the rates of retardation and malformations were related to the live fetuses. Statistical significance was estimated by the x^2 test.

The effect of alcohol and disulfiram and their combination was tested in four groups of rats. The first group consisted of 82 untreated animals and served as control. The 6 rats in the second group were given by stomach tube 10 ml/kg of 40% ethanol daily from the 7th to the 16th days of pregnancy. The third group of 9 animals received by stomach tube 150 mg/kg of disulfiram suspended in 5 ml/kg of 1.25% methyl cellulose, on the same days. The 10 animals in the fourth group were given the same disulfiram treatment and 1.5 hours later 10 ml/kg of 40% ethanol in the same way on the same days.

The results (Table III and Fig. 11) showed, that while alcohol and disulfiram by themselves had no or a very slight effect on the rate of fetal resorption and on the weight development of living fetuses, the combined action of disulfiram and alcohol caused a strongly significant increase in fetal resorption and skeletal retardation and a strongly significant decrease in fetal weight. This combined action was ascribed to the high acetaldehyde level and was considered to prove the embryotoxic and teratogenic effect of the compound.

TABLE III

The effect of 40% alcohol, disulfiram and their combination on organogenesis in Wistar/Riop rats

	No. of pregnant females	No. of implantations	Resorptions per cent	Living fetuses	Weight per fetus, g	Skeletal retardation per cent
Controls	82	911	10.8	813	3.5	12.7
Ethanol 40%	6	74	12.2	65	3.3	14.7
Disulfiram	9	89	4.0	86	3.1	11.3
Disulfiram + Ethanol 40%	10	102	64.7*	36	2.5*	61.1*

* Difference strongly significant statistically ($p < 0.001$).

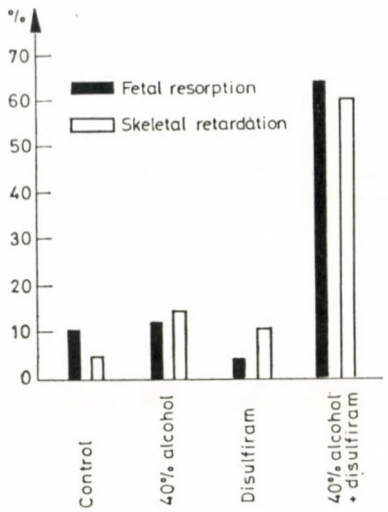


FIG 11. Teratogenicity test of alcohol, disulfiram and their combination. (Explanation see in text)

DISCUSSION

On the evidence of our experimental studies it is only the prenatal growth deficiency which may perhaps be traced back to the direct effect of alcohol on the rate of cell multiplication.

The rest of the symptoms cannot be ascribed to the alcohol itself, as not even double the lethal dose can injure the cells including their DNA. Nor can it be ascribed to the effect of acetaldehyde, provided its concentration does not exceed 30 μM, the peak level appearing in healthy people after alcohol consumption. Thus "mom's couple of drinks a day mean an abnormal baby" [31] is an excellent slogan, but as far as healthy mothers are concerned, its truth is questionable, at least in connexion with the fetal alcohol syndrome. And it is certainly questionable whether the incidence and gravity of the syndrome increase in proportion to the amount of alcohol consumed and especially to that consumed on one occasion: "women when they find out they're pregnant shouldn't celebrate with a bottle of champagne" [30]. The connexion has been assumed on empirical grounds and on the basis of some retrospective studies without calculations of statistical significance, while experiments in mice suggested that the occurrence of fetal malformations depended solely on the maternal peak blood alcohol levels [6].

The validity of this suggestion has been refuted not only by our experimental results but also by all the pertinent reports. These namely show that even the heaviest addicts often deliver unaffected children, whereas affected babies are sometimes born to mothers who drank infrequently and/or moderate quantities. The explanation lies with the mother. If she is

fully normal, her fetus will not be hurt by "binge drinking" or by a couple of drinks; the 30 ml of alcohol contained in them corresponds to 300 ml of wine and for instance in France and Italy many million pregnant women continue to take this amount with their midday and evening meals with comparative impunity. However, in some women little alcohol will suffice to damage the fetus. This will be the case if the second step of alcohol breakdown, the oxidation of acetaldehyde, is deranged, and its blood level increases above 40 μM. Such levels have been reported to be the rule in chronic alcohol addicts [23], due presumably to mitochondrial damage and a defect of specific aldehyde dehydrogenase activity caused by the acetaldehyde itself. While there is some evidence in support of the mitochondrial damage [4], data are lacking concerning the inheritance of such a defect in humans, although it is known to occur in certain rat [22] and mouse [40] strains.

The highest blood acetaldehyde levels, up to 1 mM, i.e. more than thirty times the usual, occur in subjects who drink while they are under the effect of disulfiram. In this case an amount of alcohol as little as 5 ml consumed in whatever form will suffice to exert a lethal effect on the fetus or its actually growing part. It is only natural that in this case the baby will be aborted, stillborn or born with somatic and mental defects. This was supported by our observation of a family where the heavily drinking mother after having had 6 miscarriages delivered an afflicted child; during the early weeks of this pregnancy she was on disulfiram treatment but occasionally consumed a little alcohol.

Thus, the responsibility for the fetal alcohol syndrome is ascribed to a higher than usual blood acetaldehyde level owing to a deficient functioning of some, most probably a mitochondrial, kind of maternal aldehyde dehydrogenase. Acetaldehyde, the intensive mutagenic and teratogenic property of which has been proved beyond doubt in our experiments, is believed to act by lesioning the fetus directly in the first two months of gestation and by affecting placental function during the further course of pregnancy. The direct effect would manifest itself with malformations while the placental lesion with a delay in fetal development.

Alcoholism being often associated with poor nutrition, smoking, thiamine and folate deficiency and a deficiency in trace metals such as magnesium and zinc, several authors assumed that these factors were implicated in the production of the fetal alcohol syndrome. As regards malnutrition, apart from the fact that no increased occurrence of malformations has been noted in the offspring of malnourished mothers [8], the eventual delay in growth and mental development of their children is rapidly caught up in postnatal life [43]. Neither is there any evidence of a teratogenic effect of smoking or of the other associated deficiencies, and all of the mothers mentioned in the studies displayed normal vitamin levels.

Concerning the role of alcohol addict fathers in the production of alcohol syndrome in their children, there has been no reliable evidence. A congress report suggesting this on the basis of clinical studies of 50 families and of experiments with human lymphocytes exposed to alcohol [1] has never been published, and the observation by the same author of dominant lethal mutation in male mice induced by alcohol [2] could not be reproduced by us and has recently been contradicted also by others [5, 29]. Except in that questioned study, chromosomal aberrations in male germ cells or in patients with the fetal alcohol syndrome have never been observed.

Our own studies unequivocally indicated the cytotoxic, mutagenic and teratogenic action of acetaldehyde. The higher than normal incidence of cancer in alcohol addicts has long been known; it remains to be studied whether this was the case with the fetal alcohol syndrome. To date, a single report has only been published on cancer in a 13-year-old patient with a history of fetal alcohol syndrome [14].

Since the height of the acetaldehyde level does not depend on the amount of drinks nor on their rate of elimination, it is erroneous to assume that the fetal alcohol syndrome could be prevented by setting an upper limit to the daily alcohol consumption of the mother. Once her capacity to metabolize acetaldehyde is defective, minute amounts of alcohol will already harm the child. There are two means to prevent the risk. One is a strict abstinence from any kind of alcoholic beverage during the whole course of pregnancy. This measure seems to promise an unharmed child even if the mother had had a previous one displaying the alcohol syndrome, or if her aldehyde oxidizing enzyme is not fully active. The abstinence, however, will in all probability be violated by many a woman and especially those who are regular drinkers. A more promising possibility would be a screening of perspective mothers for their blood acetaldehyde level after a drink. With a blood acetaldehyde level surpassing 30 μM, the woman must be strongly advised against bearing a child or, if she is pregnant, to have it aborted.

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