Table 2. Quantitative determinations of DNA and RNA of rat liver

	Rats 10 months (nontreated)	28 months (nontreated)	28 months (treated)	p *a*	*b*	*c*
DNA (mg/g) (6)	2.731 ± 0.200	1.992 ± 0.138	2.957 ± 0.669	< 0.001	NS	< 0.01
RNA (mg/g) (6)	8.309 ± 0.728	8.027 ± 0.749	9.629 ± 1.579	NS	NS	NS

Mean values ± SD. Statistical significant, Student's t-test. *a* Differences between 28 months and 10 months nontreated rats; *b* differences between 28 months treated and 10 months nontreated rats; *c* differences between 28 months treated and nontreated rats. NS: Nonsignificant differences. The number of animals is given in parentheses.

treated or treated with biotin, and from the untreated adult rats, was carried out spectrophotometrically according to the Walker's method[39]. This is based on the variation of absorbance at 300 nm shown by DNA and DNH when the pH is increased from 9 to 12.5: such variations are due to the different ionization, correlated with the variation of the pH, of the cytosine and guanine groups as concerns the DNA, and of the phenolic groups of the tyrosine as concerns the histone proteins. The figure shows the pattern of the DNH and DNA extinction variations when pH increases from 9 to 12. The data, in accordance with previously published results[40,41], demonstrate in the DNH complex of the old untreated rats, as compared to the adult ones, a significant decrease of the electrostatic interactions between histone proteins and DNA; the data also demonstrate a significant increase of the concentration of the histone proteins bound to DNA by electrostatic interactions, which can be overcomed by chloroform-isoamylic alcohol deproteinization in both low and high ionic strength medium in the absence or in the presence 1 M $NaClO_4$. Such interactions become normal following biotin treatment.

All the results obtained show that biotin administration causes in the blood of old rats (28 months) an increase in ATP, glucose, triglycerides, alkaline phosphatase and a significant decrease in cholesterol and acid phosphatase; moreover the biotin administration increases in the old rat liver the amount of DNA and of the electrostatic interactions between DNA and histones: the values in the old rats treated with biotin come within the values shown by adult rats. This regulatory activity of biotin on the biochemical pathways is probably related to ATP-synthesis, which in old rats is decreased.

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The alcohol syndromes: The intrarecombigenic effect of acetaldehyde

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Summary. Sister chromatid exchange was studied in lymphocyte and fibroblast cultures. Alcohol caused no disturbance under normal conditions but an acetaldehyde level above 40 μM inhibited cell multiplication and elevated SCE considerably. A high acetaldehyde level is thought to elicit the fetal alcohol syndrome, a view supported by clinical and experimental observations.

It has been known for centuries that alcoholism has toxic consequences in the abuser and even in the offspring. In addition to alcoholic liver injury and delayed development of the fetus of alcoholic mothers, the close to 450 cases reported since 1968 leave no doubt about the existence of a fetal alcohol syndrome. 2 questions arise in this connexion: is the alcohol itself responsible for the syndrome; and why is only a fraction of the babies of addicts affected? We investigated these questions first in normal human lymphocyte cultures and in the cultured lymphocytes of alcohol-intoxicated subjects. Sister chromatid exchange (SCE) was studied applying 5-bromodeoxuridine treatment and 33258 Hoechst and Giemsa staining (FPG procedure)[1].

1. Ethanol added to the cultures at a final concentration of 0.5%, about the human lethal dose, did not increase SCE in comparison with alcohol-free controls.

2. From 7 alcohol addicts under the acute influence of alcohol, with blood levels ranging from 0.2 to 0.4%, lymphocytes were obtained and cultured in a 1:4 mixture of their own serum and TC 199 medium. In the 72 h cultures, the mitotic index was low, most of the cells were in the first metaphase, but there was no increase in SCE over the background. In subjects with a blood alcohol level under 0.2% even the cell cycle was normal. This result agreed with those[2,3] showing that alcohol does not prevent reproduction. At the same time, it might perhaps explain the lagging intrauterine development of the offspring of alcohol addicts.

Ethyl alcohol in the organism is metabolized in 2 steps. In the first it is oxidized by the hepatic enzyme alcohol dehydrogenase into acetaldehyde. In the second step, this compound is degraded by the ubiquitous enzyme aldehyde dehydrogenase. In further experiments, we have therefore studied the effect of acetaldehyde on the cultured lym-

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phocytes and fibroblasts of normal persons and then on lymphocytes of alcohol addicts under chronic disulfiram treatment who, 15–20 min before blood sampling, had been allowed to drink about 0.7 ml/kg of alcohol in the form of wine. Disulfiram is well-known to raise the blood acetaldehyde level and to cause thereby a characteristic vasodilatatory episode.

3. Acetaldehyde (analytical grade distilled twice immediately before use) added to the culture in an amount of 800 μM was toxic, killing the great majority of cells. At 400 μM it inhibited cell multiplication; after 72 h the majority of cells was M_1 and of these 12% showed labile chromosomal aberration. In the M_2 cells, SCE was increased 4–6fold over the background. An acetaldehyde level of 40 μM still affected cell progression and SCE was twice the control, while lower amounts had no adverse effect.

4. From patients under the influence of disulfiram and a minimum amount of alcohol, lymphocytes were obtained in the hypotensive period and cultured in a 1:4 mixture of their own serum and TC medium. After 72 h most cells were in the M_1 phase and SCE was elevated to about 3 times the control frequency. In normal humans, the elimination rate of alcohol is about 100 mg/kg/h irrespective of the amount consumed, and the blood acetaldehyde level does not exceed 25 to 30 μM except under the influence of disulfiram when it may attain levels well above 400 μM. Our results might therefore mean that alcohol causes no disturbance under normal conditions but may have grave consequences whenever the acetaldehyde level rises above 40 μM. This must be due to some low specific activity of aldehyde dehydrogenase. Such a defect was reported in animals during pregnancy[5], but may also be inherited as in some rat[6] and mouse[7] strains, or acquired, as it has been shown in alcoholics[8], and such a defect would presumably be responsible for the fetal alcohol syndrome.

The assumption is supported by the fact that practically all of the mothers of such babies had chronic liver disease[9], and especially by our observation of a family where the heavily drinking mother after having had 6 miscarriages gave birth to an afflicted child; during the early weeks of this pregnancy she was on disulfiram treatment but confessed to having occasionally consumed a little alcohol. Another female alcohol addict had a child with fetal alcohol syndrome; on consuming 0.4 g/kg of alcohol, her blood acetaldehyde level rose to 140 μM. In our experiments, fetal development was affected neither by considerable doses of alcohol nor by disulfiram, while their combination induced 60% fetal mortality.

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Depression of frog gustatory neural responses to quinine-HCl after adaptation of the tongue to various taste stimuli

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Summary. After the frog tongue was adapted to salt, sugar and bitter solutions, the amplitudes of initial phasic gustatory neural responses to quinine-HCl (Q-HCl) were depressed. However, adaptation to acid solutions did not affect the responses to Q-HCl.

It is well-known psychophysically and neurophysiologically that the gustatory response to a taste solution changes after adaptation to other taste solutions[2,3]. In the previous papers[4], we revealed that adapting the frog tongue to Q-HCl of a typical bitter compound enhances the amplitudes of gustatory neural responses to salts, acids and sugars, but generally depresses the neural responses to other bitter solutions. This study aims to investigate how the gustatory neural response to Q-HCl changes after adaptation to various solutions including those representing 4 taste qualities.

Materials and methods. Bullfrogs (*Rana catesbeiana*) anesthetized with urethane were used in the experiments. Whole glossopharyngeal nerve impulses recorded in situ were integrated with an electronic integrator having a time constant of 0.4 sec. Membrane potential changes of single taste cells elicited by chemical stimuli were recorded with 3 M KCl-filled glass microelectrodes having a resistance of 20–50 MΩ by inserting them into the taste disc of the fungiform papillae.

Taste solutions made up in deionized water were applied to the tongue surface using a semiautomatically controlled gustatory stimulator described previously[5]. The tongue was adapted to various taste stimuli for 10 sec, after which test Q-HCl was delivered successively. The flow rate of solutions was 0.78 ml/sec for nerve recording and 0.13 ml/sec for intracellular recording. The experiments were carried out at room temperature of 20–26 °C.

Results and discussion. An example of integrated gustatory neural responses is shown in figure 1. The record A represents the control response elicited by application of 1 mM Q-HCl immediately after adaptation of the

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