necessary conditions: 1. As an active center, an α-methylene cyclopentanone is present in their molecule. 2. Some hydroxy group(s) is present at the position suitable for contact with and binding with an enzyme containing a specific nucleophile. (The β-hydroxy group at the 14 position and/or the hydroxy group at the 7 position.) 3. A hydrogen bonding between the hydroxy group at the 6 position and the carbonyl group at the 15 position is present to enhance the electrophilicity of the carbon atom at the 17 position.

Addendum. After the manuscript was submitted, we learned that ARAI et al.[13] investigated the antitumor activity of enmein, its diacetate, and dihydroenmein, of which only the partial structures had been elucidated, and suggested the activity to be attributed to the exocyclic methylene group attached to 5-membered cyclic ketone on the basis of the results of their biological test. Our findings also support their preliminary experiments and generalize the concept.

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Heart Norepinephrine Concentration after Chronic Alcohol Ingestion in the Rat

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Summary. The effect of long-term alcohol ingestion on the norepinephrine concentration of the heart was investigated in rats. The alcoholic animals showed a highly significant increase in cardiac norepinephrine concentration as compared with the corresponding controls. It is further suggested that continued exposure to high levels of norepinephrine may play a role in the development of cardiomyopathy in chronic alcoholism.

There is considerable evidence of an association between excessive alcohol consumption and heart disease in man[1–7]. The pathogenesis of alcohol-induced cardiac lesions, however, has not been clearly established. JAMES and BEAR[8] have suggested that the chronic cardiac effects of alcohol are mediated through a metabolite, acetaldehyde, and due to chronic depletion of stored norepinephrine. On the other hand, ALEXANDER[9] has advanced the view that the development of alcoholic cardiomyopathy is consequence of an increase in the heart levels of catecholamine and serotonin. In a recent paper, POHORECKY[10] has reported that, following an acute dose of alcohol or 2 weeks of chronic alcohol intake, there is no change in the heart norepinephrine concentration. In the present investigation, a prolonged period of alcohol ingestion was used. The size of the hearts of alcoholic and control rats was studied and the catecholamine concentration determined.

Materials and methods. 39 5-week-old Wistar strain male rats were assigned randomly to 6 treatments, i.e., 6 rats were allowed to drink only alcohol for 4 weeks, 6 rats were fed isocalorically for 4 weeks, 9 rats were allowed to drink only alcohol for 12 weeks, 6 rats were fed isocalorically for 12 weeks, 6 rats were allowed to drink only alcohol for 24 weeks, and 6 rats were fed isocalorically for 24 weeks. A solution of 32% (v/v) ethyl alcohol in 25% (w/v) sucrose in water was given to rats in the three experimental groups[11], while rats in the three control groups were given no alcohol.

After 4 weeks on test, 6 experimental and 6 control rats were sacrificed by dislocation of the neck. Also after 12 and 24 weeks on test, the remaining animals were sacrificed in the same manner. The hearts were rapidly removed, cleaned, and weighed. Heart norepinephrine was separated and assayed by the method of ANTON and SAYRE[12,13]. The catecholamine values were expressed in μg free base/g of wet tissue weight.

Results. The general appearance of the experimental rats was essentially similar to that of the controls. However they weighed 20–25% less than the controls. In animals drinking alcohol, inebriation was a common

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Table I. Effect of chronic alcohol ingestion (4 weeks) on heart norepinephrine concentration and heart: body weight ratio.

	Control ($n = 6$)	Experimental ($n = 6$)	Deviation from control (%)	Statistical significance (p)
Norepinephrine (μg/g)	0.597 ± 0.056	0.611 ± 0.045	0	NS
Heart ratio (g/100 g)	0.363 ± 0.021	0.372 ± 0.042	0	NS

Values are means ± SEM (standard mean error). n = number of animals.

Table II. Effect of chronic alcohol ingestion (12 weeks) on heart norepinephrine concentration and heart: body weight ratio

	Control ($n = 6$)	Experimental ($n = 9$)	Deviation from control (%)	Statistical significance (p)
Norepinephrine (μg/g)	0.630 ± 0.16	0.925 ± 0.15	+ 47	< 0.01
Heart ratio (g/100 g)	0.263 ± 0.012	0.334 ± 0.031	+ 27	< 0.005

Values are means ± SEM. n = number of animals.

finding. The alcohol-fed rats consumed 24% of their calories as alcohol and the total amount of daily consumed calories was the same as in the control groups.

Tables I, II and III show the mean values ± SEM for norepinephrine concentration and heart ratio (heart weight: body weight) of the alcoholic groups as compared with the controls. The norepinephrine levels and heart ratios of rats on test for 4 weeks were not significantly different from controls. On the other hand, in rats which had received alcohol for 12 and 24 weeks, the heart norepinephrine concentration and the heart: body weight ratio showed a significant increase in the experimental animals as compared with the corresponding controls. In no case was a significant amount of epinephrine observed.

Discussion. In the present study, we have found that heart norepinephrine increases after a prolonged period (12 and 24 weeks) of alcohol intake. In addition, an increased heart weight: body weight ratio, indicating the presence of cardiac hypertrophy, could also be observed. Nevertheless, after 4 weeks on test, there was no change in norepinephrine concentration and heart ratio, which may indicate a delay in the effect of alcohol in the cardiac tissue. POHORECKY[10] has also found no change in the norepinephrine levels in the myocardium following 2 weeks of alcohol treatment.

The increase in norepinephrine concentration in the heart may suggest either an increase in catecholamine synthesis or a decreased rate of catabolism. SUN[14] has demonstrated that the re-uptake of norepinephrine by isolated synaptosomes is inhibited by alcohol, which may deprive the nerve terminals of their catecholamine content. As a consequence, the biosynthesis of catecholamines could be activated by a feed-back mechanism[15]. CORRODI et al.[16] have reported that alcohol enhances the release and synthesis of norepinephrine in the rat brain after acute administration. It would appear then that, with chronic alcohol ingestion, the rate of norepinephrine synthesis is increased. POST and SUN[17] have pointed out that chronic alcohol administration leads to considerable increase of the catecholamine concentration in different regions of the rat brain. Previous studies in our laboratory[18] have shown that prolonged consumption of alcohol elicits severe pathological changes in the adrenergic nerve plexuses of rat atrioventricular valves. This finding suggested a change of the catecholamine levels in the myocardium of the chronic alcoholic, which has been substantiated in the present investigation.

Catecholamines are known to play a role in the pathogenesis of myocardial lesions. It has been found that patients dying after treatment with norepinephrine, or those with pheochromocytoma, present evidence of cardiomyopathy similar to that produced by injecting norepinephrine in experimental animals[19–22]. This cardiomyopathy manifests by various pathological changes, including hypertrophy, hyalinization, vacuolization, edema, fatty degeneration, and increased granularity of myofibres, and interstitial fibrosis. Three types of pathogenetic mechanism have been recognized in the cardiovascular effects of norepinephrine and other sympathomimetic agents: metabolic changes, hemodynamic disturbances, and myogenic alterations related to a reduction in myocardial contractile force[21, 23]. These catecholamine-induced myocardial lesions resemble those seen in autopsy material from chronic alcoholic patients in whom the clinical diagnosis of alcoholic cardiomyopathy had been made[9, 24–26], or in experimental ani-

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Table III. Effect of chronic alcohol ingestion (24 weeks) on heart norepinephrine concentration and heart: body weight ratio

	Control ($n = 6$)	Experimental ($n = 6$)	Deviation from control (%)	Statistical significance (p)
Norepinephrine (μg/g)	0.652 ± 0.05	0.815 ± 0.13	+ 25	< 0.025
Heart ratio (g/100 g)	0.243 ± 0.011	0.286 ± 0.015	+ 18	< 0.05

Values are means + SEM. n = number of animals.

mals[27-29]. The pathological findings in all these studies are not specific and have also been observed in myocardial ischemia[30] as well as in deficiency in magnesium[31] and potassium[32]. Nevertheless, in view of our present results it can be speculated that continued exposure to high levels of norepinephrine might play a role in the development of cardiomyopathy in chronic alcoholism due to interference with both cardiac contractility and metabolism.

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Habituation to Iterative Photostimulation in the Palmar Skin Conductance Response of Mice, its Delay by Psychoanaleptics

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Summary. In mice, iterative photostimulation results in habituation, detected in the palmar skin conductance response. Psychoanaleptics delay this habituation in proportion to the dose administered.

On human subjects, it has been demonstrated[1] that repetition of stimuli produces decrements in the magnitude of the skin conductance response (SCR). Habituation has been detected in mice, where iterative photostimulation (IPS) provoked first a progressive decrease of the amplitude of the palmar SCR (PSCR), then its annulation[2]. This phenomenon – iterative photostimulation habituation (IPSH) – is delayed by psychoanaleptics[2,3].

Methods. Swiss Orl male mice (body weight 18 to 25 g) were randomized into batches of 10. The animals were vertically restrained in individual wire cylinders (diameter 2.5 cm, height 7.5 cm). A 100 W glow lamp located 10 cm above their heads was automatically switched on for 7 sec every 2 min (photostimulus: PS). The PSCR was recorded as the mice grasped by reflex the electrodes of a palmar skin conductance-meter which has been described elsewhere[4]. Following a scheme previously used for studying its magnitude[5], PSCR was recorded every 10 min and the amplitude calculated in relation to the corresponding initial reading.

Drugs were given i.p. or p.o. between the 1st and the 2nd PS. Each batch of mice was dosed in such a way as to achieve logarithmic increase of dosage over the whole experiment.

Results. Regression equations 'delay of habituation (1/100 h)/log dose (mg/kg)' in the Figure clearly show the delaying effects on IPSH of the psychostimulants tested: amphetamine sulf., dexamphetamine tart., caffeine and pyrisuccideanol dimal.

Discussion. Together with other autonomic and EEG responses, SCR is a part of the orienting reflex[6]. It readily habituates upon repetition of the stimuli. This phenomenon could occur in the visual pathways and

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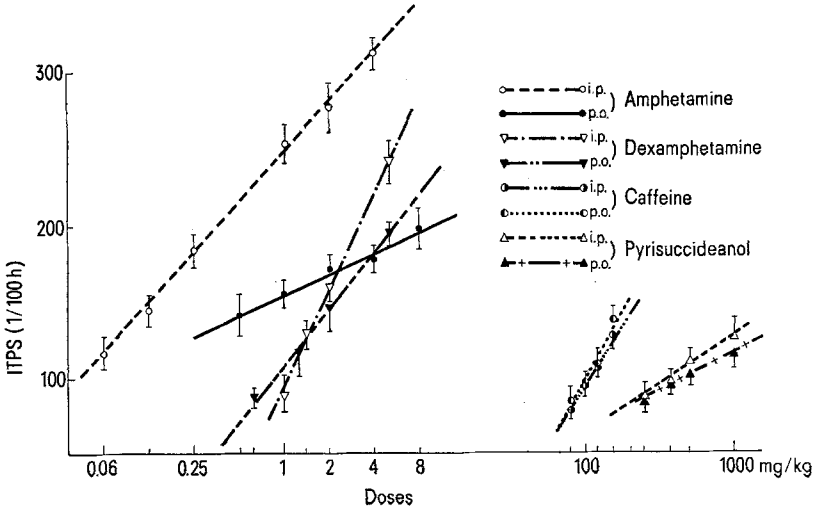

Dose-related inhibition by psychoanaleptics of habituation to iterative photostimulation in the palmar skin conductance response of mice.