Effects of Alcohol Ingestion on Adrenergic Nerve Endings of Rat Atrioventricular Valves

Autonomic dysfunction may occur in patients with peripheral neuropathy secondary to chronic alcoholism[1–3]. Recently NOVAK and VICTOR[4] described 4 patients with alcoholic neuropathy, in whom degenerative changes of the vagus and sympathetic nerves were demonstrated. However the effects of chronic alcohol ingestion on the intrinsic innervation of the heart is not known. The purpose of this report is to describe the effects of prolonged alcohol consumption on the adrenergic nerve endings of rat atrioventricular valves.

Materials and methods. 12 Wistar strain male rats (body weight 130–150 g) were allowed to drink only white rum (40% alcohol) for 4 months. 6 animals from the same stock were kept as controls and given no alcohol. All animals were fed on a balanced commercial rat food ad libitum. After the 4-month-period the rats were killed by exanguination in ether anesthesia. The daily amount of white rum consumed during the experiment was 4.9 ml/100 g body weight.

Tissues from both control and experimental groups were processed in exactly the same fashion. The hearts were quickly removed and as soon as the ventricular chambers had been opened the atrioventricular valves were dissected with the help of a stereomicroscope. To obtain consistent and duplicable results, the heart valves were excised 10 to 15 min after sacrifice.

Three control and 6 experimental animals were processed to demonstrate catecholamines. In accordance with FALCK-HILLARP's method[5–10], the excised valves were rinsed in cold calcium-free Tyrode solution for 2 min, stretched and mounted whole on glass slides and allowed to dry in the air at room temperature for 15 min. In our laboratory the relative humidity of the air was maintained at approximately 50%, and this proved satisfactory for drying the valves and did not produce any serious changes in the morphology of the adrenergic nerves as revealed by the fluorescence method. The tissue was then treated with formaldehyde gas of optimal humidity (70%) generated from paraformaldehyde at 80 °C for 1 h, mounted with non-fluorescent immersion oil and examined with a Zeiss fluorescence microscope.

The remaining animals were processed according to the method of CHAMPY, modified by MAILLET[11]. After rinsing, the valves were fixed in 2% osmium tetroxide and 3% zinc iodide solutions, mixed in a ratio of 1:4, immediately before use. The valves were fixed during 24 h at room temperature, rinsed, dehydrated, cleared in xylene and mounted in Histoclad.

Results. Fluorescence microscopy of control atrioventricular valves processed by the formaldehyde condensation method of FALCK and HILLARP shows dense networks of adrenergic nerves. The endings are varicose, exhibiting an intense greenish-yellow fluorescence. This appearance was very similar and can be compared to those described by others investigators[9,12–15].

In Figure 1 adrenergic nerve endings forming a dense network with intensely fluorescent varicosities can be seen in a control atrioventricular valve. A marked reduction in numbers of catecholamine-containing nerve fibres could be demonstrated in alcoholic rats. The terminals were more sparse than normal, although the intensity was about normal.

Figure 2 shows a diminution of the density of fluorescence fibres, a decrease of the fluor-intensity in a part of the present fibres, and fluorescence thickenings, which seem to be like retraction-balls during a posttraumatical degeneration or an accumulation of transmitter in the proximal fibrestump.

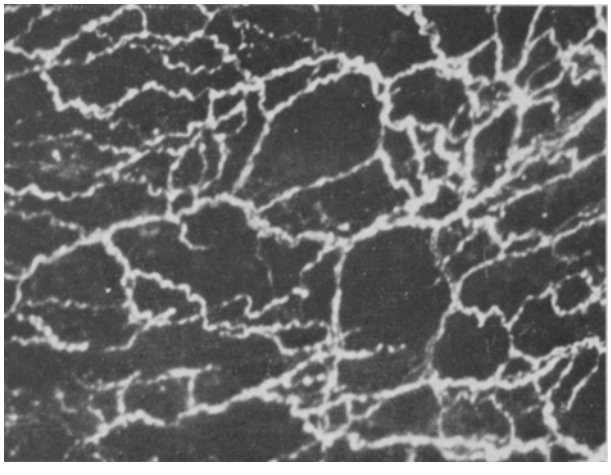

Fig. 1. Atrioventricular valve of a control rat. Whole-mounted stretch preparation. Fluorescence micrograph. ×250.

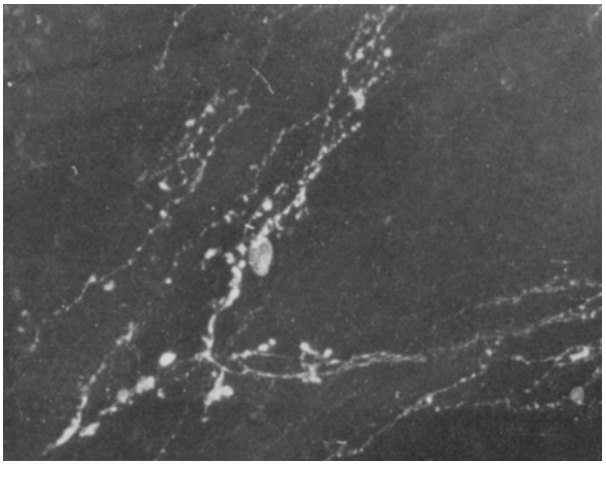

Fig. 2. Atrioventricular valve of an alcoholic rat. Whole-mounted stretch preparation. Fluorescence micrograph. ×250.

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Light microscopy of specimens fixed by means of the osmium-iodide technique shows an extensive distribution of nerve fibres in control atrioventricular valves. These fibres form networks with fine meshs similar to those previously described[9,16].

Figure 3 shows the autonomic baseplexus, fixed by means of the osmium-iodide technique, distributed in a control atrioventricular valve.

Degenerative lesions of osmiophilic nerve terminals occur in rats given alcohol for 4 months. The valves displayed less numerous nerve structures with definite alterations. They appear as a few thick fibres without network arrangement, frequently reduced to an amorphous debris of osmiophilic material. This aspect is revealed in Figure 4.

Discussion. This study has shown that prolonged consumption of white rum (40% alcohol) elicits severe degenerative lesions in the adrenergic nerve plexuses of rat atrioventricular valves.

The distinct loss of catecholamine fluorescence in adrenergic nerves following long-term ingestion of alcohol closely resembles the disappearance of fluorescent nerve fibres seen after postganglionic denervation[5,8] and after treatment with 6-hydroxydopamine[17,18]. The CHAMPY-MAILLET method gives results very similar to the fluorescent technique, and it seems likely that the same nerve fibres are being demonstrated by both techniques. The severe quantitative and qualitative changes of osmiophilic nerve fibres observed in atrioventricular valves of alcoholic rats are comparable with the degenerative lesions found in human mitral and tricuspid valves in the late phase of chronic Chagas' disease[19].

There is evidence of a causal relationship between alcohol consumption and heart disease in man[20–24]. The pathogenesis of alcohol-induced cardiac lesions, however, has not been clearly established. Although there is evidence to suggest that alcohol has a direct toxic effect on the myocardium, it is difficult to differentiate the roles played by alcohol per se, the alcohol metabolites, and the associated metabolic disorders accompanying chronic alcohol intake.

It has been demonstrated that alcohol ingestion produces alterations in the catecholamine metabolism and an increase in the urinary excretion of catecholamines[25–27]. These effects would result in disturbances of the sympathetic nervous system activity in chronic alcoholics, which are frequently observed[28]. POST and SUN[29] have pointed out that chronic alcohol administration leads to a considerable increase of the catecholamine concentration in different regions of the rat brain. Our present morphological observations speak for a change of the catecholamine levels in the myocardium of the chronic alcoholic, and it can be speculated that this might play a role in the production of anatomical and functional alterations in alcoholic cardiomyopathy.

Further experiments to investigate the myocardium catecholamine content following chronic alcohol consumption are proceeding.

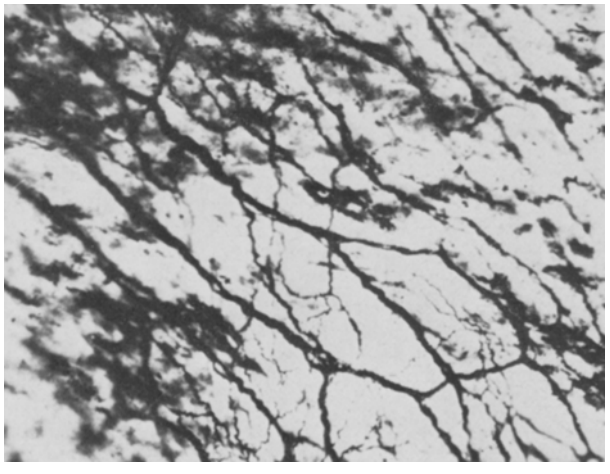

Fig. 3. Atrioventricular valve of a control heart. Whole-mounted. CHAMPY-MAILLET technique. ×250.

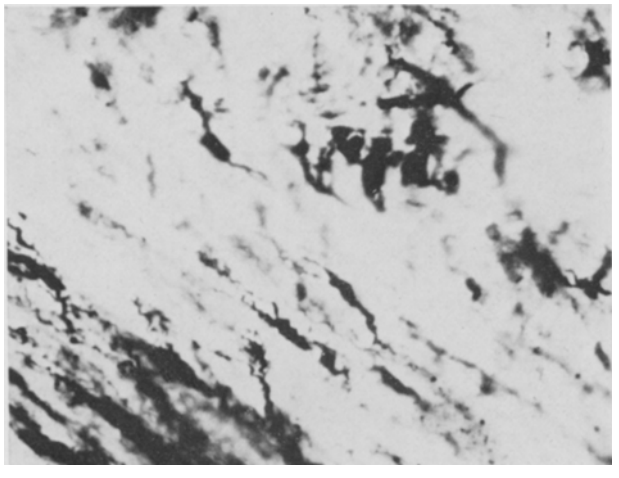

Fig. 4. Atrioventricular valve of an alcoholic heart. Whole-mounted. CHAMPY-MAILLET technique. ×250.

Zusammenfassung. Bei Ratten, die während 4 Monaten ad libitum 40%igen Zuckerrohrschnaps getrunken hatten, wurden tiefgreifende Degenerationserscheinungen des adrenergischen Nervenplexus der Atrioventrikularklappen festgestellt.

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[30] At the time of this investigation J.C.M.S. was holder of a fellowship of the Conselho Nacional de Pesquisas.