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Nicotine Effects on the Acid Mucopolysaccharide Content of Chick Embryo Cardiac Jelly*

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Summary. Histochemical studies were made on the developing chick embryo heart to determine the effects of nicotine on acid mucopolysaccharide content during the critical stages of the cardiac morphogenesis. Two-day old embryos were injected with the dosages of nicotine ranging from 1.5 to 3 mg per embryo. The embryos were studied on the 3rd and 4th days of incubation. For an evaluation of acid mucopolysaccharides, the staining procedures of Mowry (1958) and Saunders (1964) were employed.

It was found that nicotine decreases the content of acid mucopolysaccharide in the developing cardiac jelly. In the control embryos, the cardiac tissues picked up very intense coloration. At lower dosages (Group A; 1.5 mg/egg), the staining response of the treated heart tissues containing acid mucopolysaccharides was moderate as compared to the control embryos. At higher dosages (Group B; 3 mg/egg), the cardiac tissues were faintly colored as compared to the embryos of Group A. It is possible that the cardiac lesions previously reported in the chick embryos following the administration of nicotine (Gilani, 1971) are due to the reduction of the amount of acid mucopolysaccharides in the developing cardiac jelly—a susceptible period of the genesis of heart.

Key words: Cardiac jelly— Acid mucopolysaccharides — Cardiac tissues — Cardiac anomalies — Nicotine.

Introduction

The cardiac jelly is a sticky, pliable and acellular material between the developing endocardium and myocardium in the embryonic tubular heart (Davis, 1924). Its role in the genesis of heart is well emphasized in the literature (Bremer, 1932; Barry, 1948; Patten *et al.*, 1948; Jaffee, 1965). Johnston and Comar (1957) and Ortiz (1958) noted the presence of acid mucopolysaccharides in the cardiac jelly of chick embryos. Later, Gessner and Bostrom (1965) and Gessner *et al.* (1965) using histochemical and radioautographic techniques, concluded that a significant amount of sulfated acid mucopolysaccharides were present in chick embryo cardiac jelly. These results were confirmed in the studies made by Manasek (1970 b, c). By using autoradiographic methods, Overman and Beaudoin (1971) studied the effects of 6-aminonicotinamide, dietary induced folic acid deficiency, aminopterin and trypan blue on acid mucopolysaccharide synthesis in isolated embryonic rat hearts. They observed the inhibition of acid mucopolysaccharide synthesis and concluded that the synthesis of mucopolysaccharides in isolated embryonic rat hearts was susceptible to the action of the teratogens employed.

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The normal synthesis of mucopolysaccharides is vital for the normal development of the embryos. Disturbances of acid mucopolysaccharide metabolism may lead to congenital malformations (Larsson, 1962; Jacobs, 1964; Larsson and Bostrom, 1965; Mathews, 1967; Meyer, 1969). As a result of these studies, the present work was undertaken to investigate the effects of nicotine on the acid mucopolysaccharide content in cardiac jelly, and to explore some relationship with the cardiac anomalies observed in earlier studies in the chick embryos following the administration of nicotine (Gilani, 1971).

Materials and Methods

For this study, fertile chick eggs of White Leghorn stock were obtained from a commercial hatchery (Shamrock Farms, New Jersey). Nicotine HCL was injected into 2 day old embryos with the dosages of 1.5 mg/embryo (Group A) and 3 mg/embryo (Group B). The volume of fluid introduced was 0.1 ml per embryo. The controls received an equal amount of distilled water. Sixty embryos were used each for the Groups A, B and the controls. In all, 180 embryos were utilized for this study. After the administration of nicotine, all the embryos were returned to the chick incubator maintained at 38.5° C. The embryos were studied on the 3rd and 4th days of incubation. Experimental as well as control embryos were fixed in 10% neutral formalin or in Newcomers solution. They were then dehydrated, embedded in paraffin, and serially sectioned at 6 μ intervals in the cross and longitudinal planes.

For an evaluation of acid mucopolysaccharides, the staining procedures of Mowry (1958) and Saunders (1964) were employed.

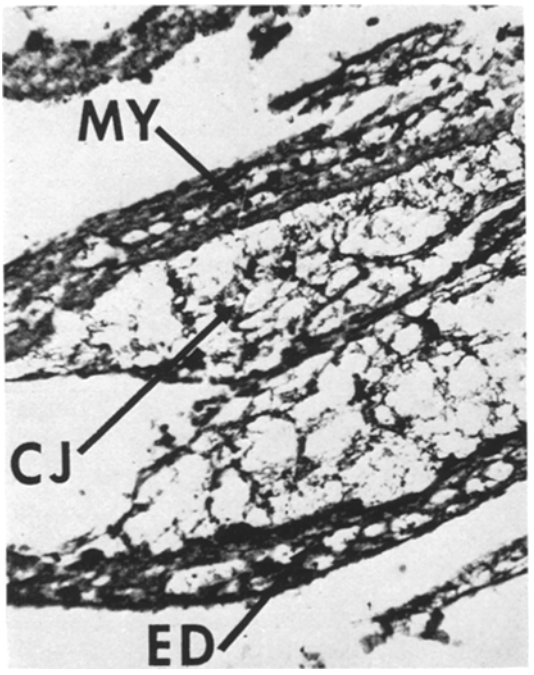

Fig. 1. Section through the heart of 4 day old chick embryo from the control group. Note the intensely stained material in the cardiac jelly (Mowry's 1958 colloidal iron stain, × 325). *CJ* Cardiac jelly, *ED* endocardium, *MY* myocardium

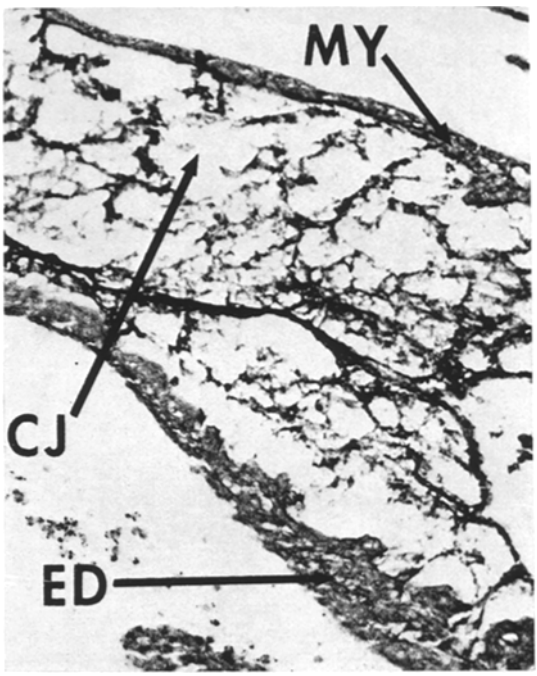

Fig. 2. Section through the heart of 4 day old chick embryo from Group A (1.5 mg/embryo). The content in the cardiac jelly are stained but the intensity is less than controls (Mowry's 1958 colloidal iron stain, ×325)

Results

Controls

In all the experimental series, the control sections showed an intensely stained material in the cardiac jelly (Fig. 1).

Group A (Treated with 1.5 mg/embryo)

In the embryo of this group, the space between the developing endocardium and myocardium was filled with the stainable material, and the cardiac jelly was moderately stained (Fig. 2) as compared to the controls (Fig. 1). The acid mucopolysaccharide content seem to be less affected by nicotine in this group.

No differences in the staining reaction were observed within the chick embryo hearts of the 3rd and 4th days of incubation.

Group B (Treated with 3 mg/embryo)

The intensity of the color decreased in the embryos of this group. Cardiac jelly content appeared to be faintly stained (Fig. 3). The acid mucopolysaccharide content showed marked decrease at this higher dosage (Fig. 4). Those embryos who had faintly stainable material in the cardiac jelly showed also faint coloration in the developing endocardium and myocardium. The acid mucopolysaccharide content appeared to be significantly affected by the nicotine in this group.

Within this group, the reactivity to stains was the same in the embryos observed at 3 and 4 days of incubation.

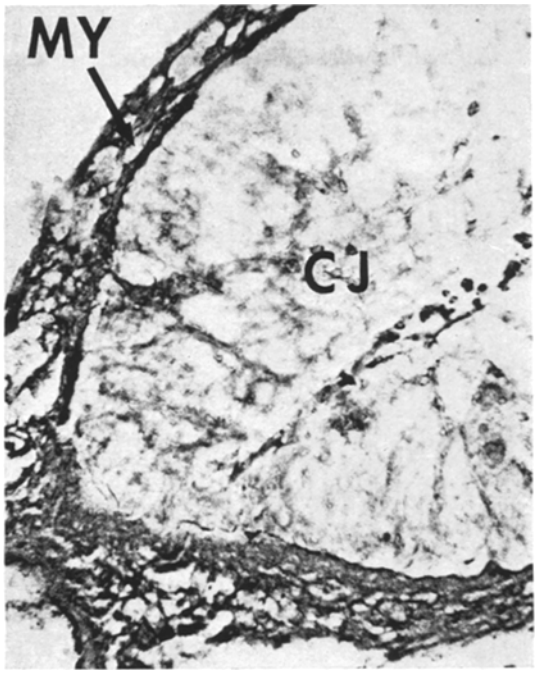

Fig. 3. Section through the heart of 4 day old chick embryo from Group B (3 mg/embryo). Note the material in the cardiac jelly has less coloration than the controls and the embryos from Group A (Mowry's 1958 colloidal iron stain, × 325)

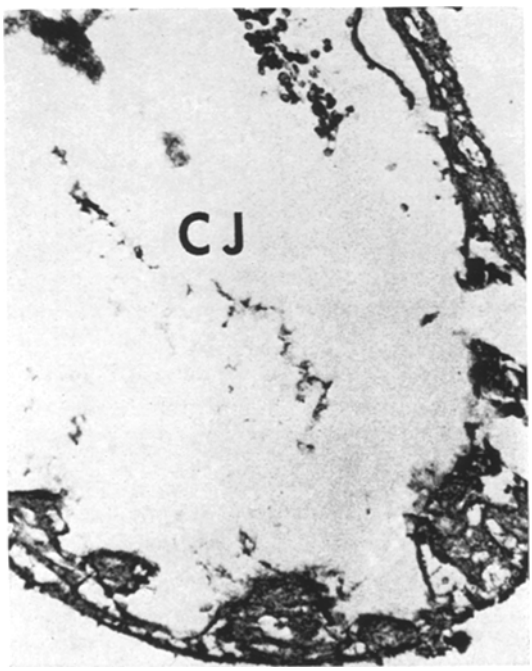

Fig. 4. Section through the heart of 4 day old chick embryo from Group B (3 mg/embryo). Note a decrease in the amount of stainable material in the cardiac jelly (Mowry's 1958 colloidal iron stain, ×325)

Discussion

These studies have shown that nicotine affects the content of acid mucopolysaccharides in the cardiac jelly during the critical stages of cardiac morphogenesis. The 6-aminonicotinamide has been shown by Overman and Beaudoin (1971) to interfere with acid mucopolysaccharide synthesis in the heart of rat embryos.

During the early phases of cardiogenesis, the cells of the endocardial cushion tissue, which are derived from the cardiac jelly, and which lead to formation of valves and septa (Van Mierop *et al.*, 1962) are metabolically very active. Since these cells are rich with acid mucopolysaccharide content (Gessner *et al.*, 1965), damage to these cells at the critical stage may lead to the reduction in the synthesis of acid mucopolysaccharides. A considerable cellular damage was observed in the cardiac tissues of the chick embryos following the administration of nicotine in the previous studies by Gilani (1971). From the results, it appears that normal content of acid mucopolysaccharides during the critical stage of cardiogenesis are very essential, and any interference at this stage, could lead to the cardiac anomalies. In the previous studies (Gilani, 1971), endocardial cushion defects such as valvular and ventricular septal defects were observed.

These experiments have indicated that nicotine affects the content of acid mucopolysaccharides at the critical stage of heart development, and this interference, probably leads to the endocardial cushion defects.

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