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Endomyocardial fibrosis in rats treated with N-nitrosomorpholine

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Summary. Endomyocardial fibrosis was observed after long lag periods in male Sprague-Dawley rats treated for 1–14 weeks with the carcinogen N-nitrosomorpholine. The fibrosis developed predominantly in the left ventricle. It occurred during 29–78 weeks after withdrawal of the carcinogen in 5% and 79–108 weeks after withdrawal in 20% of the experimental animals, but was never observed in controls of the same age. We suggest that endomyocardial fibrosis was induced by a direct effect of the carcinogen on the fibroblasts of the endomyocardium.

Key words: Endocardium – Myocardium – Fibrosis – Toxic response

Introduction

Endomyocardial fibrosis (EF) is a rare human disease in most areas of the world, but in certain regions of Africa, e.g. in Uganda, it is the commonest cause of heart failure (Davies and Ball 1955). The pathogenesis of EF is largely obscure. A variety of possible aetiologic factors such as infections, especially viral infections during early childhood, toxins or metabolic disorders (carcinoid-syndrome, glycogenosis) have been discussed (Doerr 1970 and 1974). In Burmese cats a primary endomyocardial fibroelastosis has been described as a naturally occurring familial cardiac disorder (Zook et al. 1981).

In the rat EF has been reported in different strains as a "naturally occurring disease" the incidence of which varied from 1 to 7% and had a tendency to increase with age (Boorman et al. 1973). In our own laboratory we have never seen this change in untreated control animals of the rat strain used (Sprague Dawley). However, a number of rats treated with N-nitrosomorpholine (NNM), a carcinogen which produces predominantly liver and kidney tumors in the rat (Druckrey et al. 1967; IARC Monograph 1978; Bannasch et al. 1981a), developed this disease after long lag periods.

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Table 1. Endomyocardial fibrosis in rats after treatment with NNM for 1–14 weeks in concentrations of 120–500 mg/l in drinking water (Total dose at the end of treatment: 24–200 mg/animal)

Time after cessation of NNM (weeks)	Number of animals		Animals with endomyocardial fibrosis	
	Controls	Treated	Controls	Treated
2–28	58	84	0	0 (0%)
29–78	75	121	0	6 (5%)
79–108	24	25	0	5 (20%)

Our observations point to a toxic aetiology for EF in the NNM-treated rats and might also be of interest for a better understanding of this disease in man. It appears noteworthy in this context that an industrial human exposure (rubber manufacture, hydraulic fluids) to NNM has been detected recently (Preussmann et al. 1979).

Material and methods

Male Sprague-Dawley rats, each weighing approximately 200 g at the beginning of the experiment, were kept in groups of two to three animals in Makrolon cages under standardized conditions. They were fed a semisynthetic basal diet (Altromin®, Lage, Lippe, Germany) ad libitum. In addition, ordinary tap water was given. In the experimental series the NNM was dissolved in the tap water for the period of treatment.

A total of 255 rats received NNM in concentrations of 120–500 mg/l drinking water for 1–14 weeks. The dose of NNM taken up by each set of animals was determined by measuring the difference between the drinking water supplied and the remaining volume. Individual doses were calculated by dividing this value by the number of animals housed in the respective cages. The total dose of NNM at the end of treatment ranged between 24 and 200 mg/animal.

The 25 experimental animals which were sacrificed at the end of treatment did not show any remarkable histological changes in their hearts. The 230 animals which survived the period of treatment are listed in Table 1. These animals were maintained for 2–108 weeks after cessation of treatment. Groups of three animals each were killed in intervals of 4–8 weeks. The remaining animals were kept until they became moribund or died spontaneously. Hundred and fifty-seven animals served as controls. They were killed together with the experimental groups or with moribund animals (see Table 1 for details).

The hearts were removed under ether anaesthesia, divided sagitally with a razor blade through the upper half of the ventricles and fixed in either a 4% formaldehyde solution or in Carnoy's mixture. After being further automatically processed, the specimens were embedded in Paraplast and cut in steps. The sections were stained with haematoxylin-eosin, Masson-Goldner, alcian blue and the periodic acid-Schiff reaction. Elastic fibers were demonstrated according to Weigert.

Results and discussion

None of the 157 untreated control rats showed EF, but one control animal had small inflammatory infiltrations composed of lymphocytes, so-called Anitschkow-cells and fibroblasts in the myocardium. In the treated animals EF was not observed up to 28 weeks after withdrawal of NNM (Table 1). However, during the period of 29–78 weeks after cessation of treatment 5% of the animals exhibited endomyocardial fibrosis. Later on (79–108 weeks) as many as 20% of the treated rats developed this disease.

Sometimes the hearts with EF showed hypertrophy (Fig. 1), but in most cases the endocardial changes did not cause obvious macroscopic alterations and were detected by histological examination only (Figs. 2–4). In all cases the left ventricle was affected. In three cases, however, fibrosis of the endocardium was also present in the right ventricle, although to a much lesser extent. In this respect the NNM-induced EF of the rat appears to be very similar to the "spontaneous" lesions described in the rat by Boorman et al. (1973) and to the human disease as observed in Africa (Davies and Ball 1955; Weber 1962).

The leading histological finding was a thickening of the endocardium (Figs. 2–4) in large areas of, or even throughout, the left ventricle. The thickened endocardium was composed of fibroblasts and collagenic fibers which were arranged in a highly ordered fashion and were always associated with glycosaminoglycans as demonstrated histochemically by alcian blue. The increase in fibrotic tissue was, as a rule, uniformly distributed over large areas of the endocardium. Sometimes, however, polyp-like formations protruded into the ventricular lumen (Fig. 3). Unlike the well known changes in patients with carcinoid-syndrome (Hedinger 1955 and 1957; Smith and Campbell 1956; Eder and Schauer 1959), which predominate in the right ventricle, the valves were not involved in the fibrotic process. Moreover, the additional increase in elastic fibers which is characteristic for cardiac fibroelastosis in man and animals (Zook et al. 1981) was not seen.

Frequently the superficial layer of the inner myocardium of the NNM-treated rats was involved in the disease (Fig. 4). In these cases the myocardium was interwoven with thin bundles of collagen fibers or split by broader fibrotic septae which were at places connected with the thickened endocardium. It is noteworthy that the inflammatory infiltrations of the myocardium as mentioned in one control rat were also seen in one treated animal, but they were not combined with EF. About half of the animals with EF exhibited a considerable myocardial storage of glycogen as shown by the periodic acid-Schiff reaction. However, a clearcut glycogenosis of the myocardium which may be associated with EF in humans (Doerr 1974) could not be proven although the majority of the experimental animals developed focal hepatic and renal thesaurismoses (glycogenosis, mucopolysaccharidosis or lipidosis). These storage phenomena were characteristic of preneoplastic or neoplastic cell populations and have been described in detail earlier (Bannasch et al. 1980 and 1981 a).

In addition to the focal thesaurismoses a number of pathological changes were found in other organs of the animals with EF. Most interesting in the context of this communication were the following liver lesions which were observed in one animal each: fibrosis, cholangiofibrosis, cholangiofibroma, fibrosarcoma and angiosarcoma (see Bannasch et al. 1981 a; Bannasch et al. 1981 c). The liver fibrosis was combined with a fibrosis of the spleen. Eight animals developed hepatocellular carcinomas and/or neoplastic hepatic nodules (see Bannasch et al. 1980), 7 cystic cholangiomas (see Bannasch et al. 1981 a) and 6 hepatic spongiosis (see Bannasch et al. 1981 b). Three animals showed renal adenomas as previously reported (see Bannasch et al. 1981 a). One animal had a neurinoma of the oculomotor nerve.

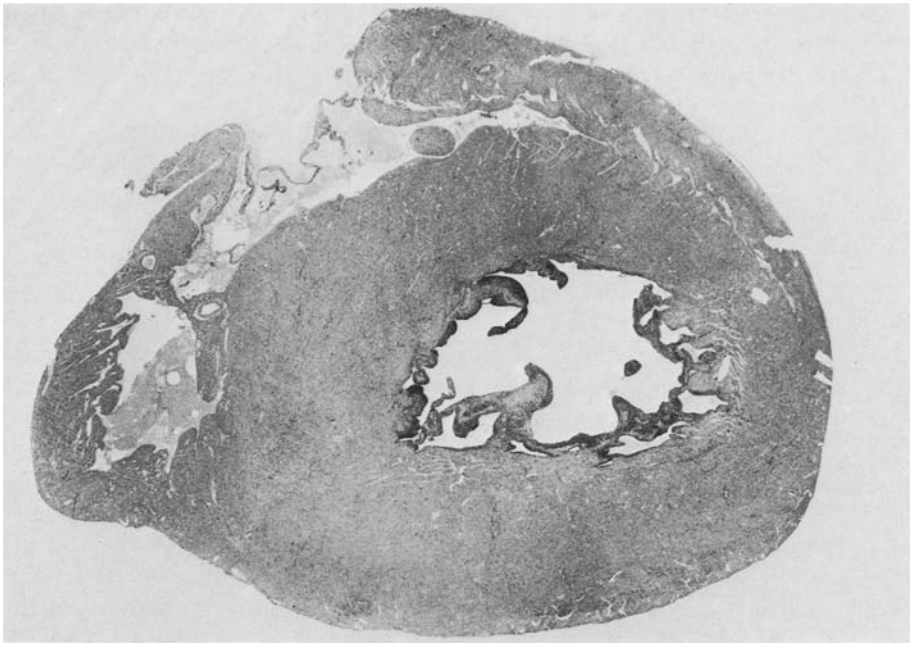

Fig. 1. Rat heart showing NNM-induced endocardial fibrosis in the left ventricle. Note additional hypertrophy of the myocardium. Haematoxylin-Eosin, × 8

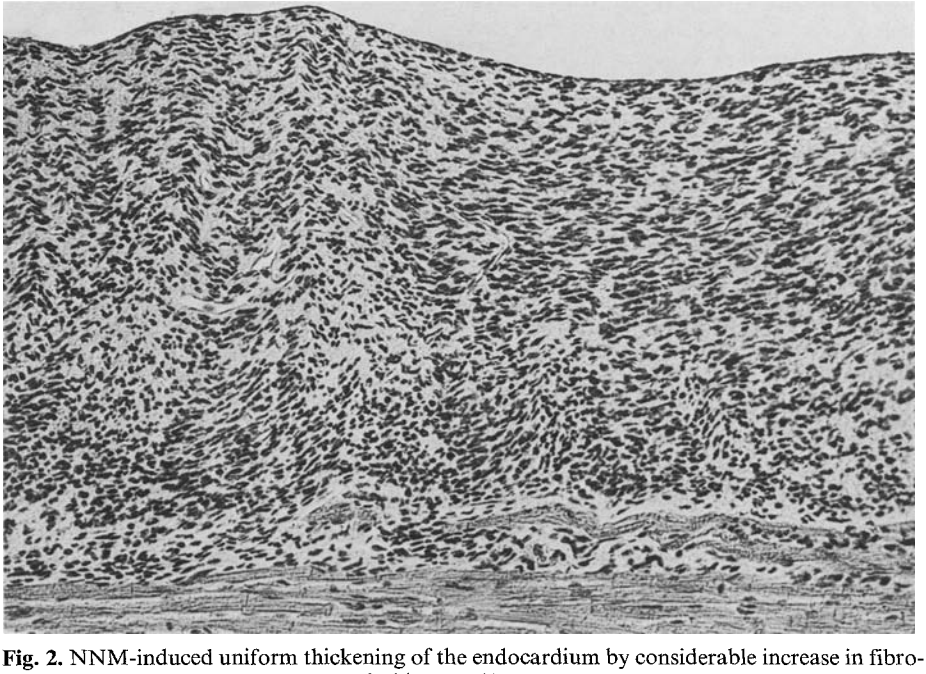

Fig. 2. NNM-induced uniform thickening of the endocardium by considerable increase in fibroblasts and collagenic fibers. Masson-Goldner, × 190

Fig. 3. NNM-induced polyp-like formation of endocardial fibrotic tissue. Masson-Goldner, × 90

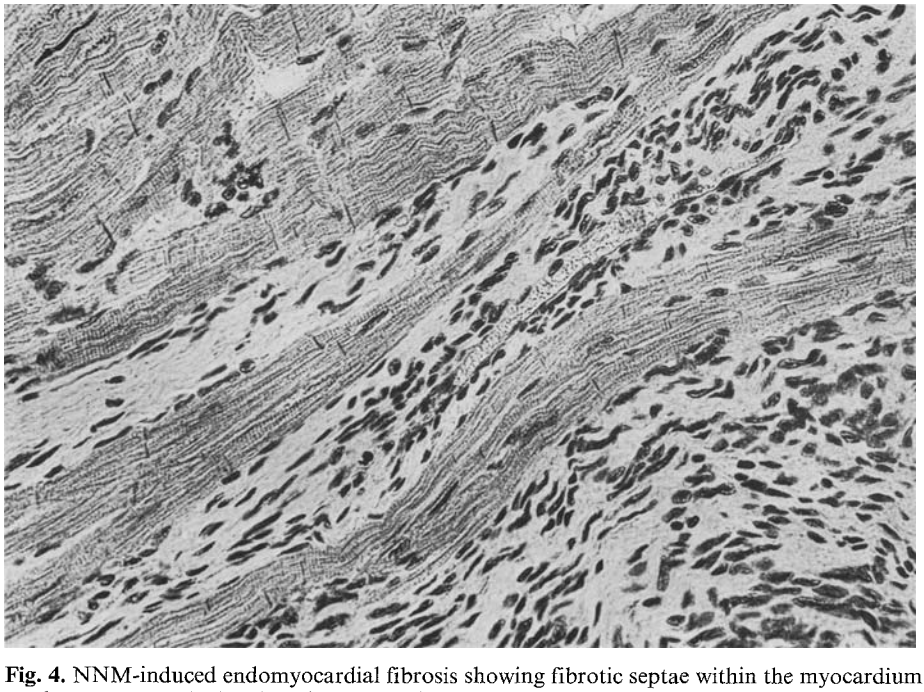

Fig. 4. NNM-induced endomyocardial fibrosis showing fibrotic septae within the myocardium, at places connected with the thickened endocardium. Masson-Goldner, × 450

The natural history of the EF appearing in the NNM-treated rats could not be fully elucidated. The experimental production of EF in rats by a brief period of treatment with certain corticoids plus Na salts has been reported by Selye (1958). In this case myocordial necrosis which was predominantly localized in the subendocardial layers appeared to be responsible for the development of the disease. In our material, however, comparable myocardial necroses were not observed in any stage of the experiment. Similarly thrombotic changes, which have been discussed as a possible aetiologic factor in EF in man were never seen. The secretion of a fibrogenic factor from neoplastic cells has been postulated for the development of EF in the human carcinoid syndrome. However, although hepatic or renal neoplasms were present in many of the NNM-treated animals a production of a fibrogenic factor from these tumors is unlikely since most of the tumor-bearing animals of the whole series did not show EF.

The natural history of the "spontaneous" EF described in rats by Boorman and colleagues (1973) is also unclear. In contrast to these authors we did not find EF in untreated rats. We suggest, therefore, that in our animals EF was induced by a direct effect of NNM on the endocardial fibroblasts which might be irreversibly altered by the carcinogen and produce fibrosis after long lag periods. This suggestion is supported by the observation of neoplastic or non-neoplastic lesions originating from fibroblasts in other organs, especially in the liver, of the same animals. The combination of EF with fibrotic or cirrhotic alterations of the liver has also been reported in man (Davies and Ball 1975).

From the finding of polyp-like fibrotic changes in the hearts of some animals treated with NNM it appears that EF has the potential to progress to tumorous growth in the rat. To the best of our knowledge such a progression has not been described in the human disease. But Boorman et al. (1973) reported that in the ventricles or atria of six rats they did indeed observe large tumor-like masses some of which showed considerable nuclear atypia and mitoses. Sarcomas of the heart, apparently originating from the endocardium, have been induced by urethane in rats and by ethylnitrosourea in mice (Vesselinovitch et al. 1968, 1974). In both studies a high incidence of liver tumors has also been observed.

It appears worth mentioning that we also found EF in another experiment in one rat 60 weeks after oral application of 2 mg/kg bw of the naturally occurring carcinogen aflatoxin B_1. This observation might be particularly interesting with respect to the high incidence of EF in certain regions of Africa since it is well known that the food is frequently contaminated with aflatoxin in these areas (Martin et al. 1971). Moreover, a contamination of various animal diets with small amounts of carcinogens, such as aflatoxin or dimethylnitrosamine, has been detected in several laboratories (Newberne 1976; Kann et al. 1977; Walker et al. 1979). Thus, it is tempting to speculate that the spontaneous EF as described by Boorman et al. (1973) might be due to chronic intoxications with carcinogens, too.

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