Cardiomyopathy in Crowded Rabbits

A PRELIMINARY REPORT

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SUMMARY

Rabbits were crowded 4 to a cage for 2 weeks, then released for 1 week, crowded again for 1 week, and so on. Of 44 rabbits subjected to intermittent crowding only 9 survived longer than 10 months, 20 died during the 1st month and 15 died between the 2nd and 9th month of the experiment. Autopsy findings were indicative of heart failure. Light-microscopical sections of the myocardium showed myocytolysis, interstitial oedema, and an increased amount of acid mucopolysaccharides in rabbits surviving for 2 weeks and more. Some rabbits surviving longer than 2 weeks had in addition coagulative myocardial necrosis. The accumulations of acid mucopolysaccharides apparently were unrelated to necrotic foci. Longtime survivors frequently showed myocardial fibrosis and endocardial fibro-elastosis, as well as basophilic degeneration of myocardial fibres. The lesions observed were similar to those described in idiopathic endomyocardopathy in Southern Africa. Therefore, the rabbit may be of some value for research in cardiomyopathies.


Research into idiopathic endomyocardopathy (IECP) which is common in Southern Africa and may, possibly, be identical with congestive cardiomyopathy is hampered by the lack of a suitable animal model. Cardiomyopathies in many different species have been studied, but all differ from the human condition. The golden hamster of the BJO 14,6 strain with its hereditary myopathy is extensively used as a research model in cardiomyopathies. However, Van der Walt demonstrated that the hamster cardiomyopathy differs from the human disease in various important aspects. The morphological heart lesion is unlike that found in man and is an inborn metabolic aberration, whereas the human disease is acquired. Various drugs and hormones have been used to produce cardiomyopathies, but again, the lesions in the experimental animals differ from those in man. Weber et al. found a spontaneous cardiomyopathy in Chacma baboons (Papio ursinus), with acute necrotizing lesions possibly caused by stress. However, the human IECP is characterized by chronic fibrotic heart lesions.

It would appear that among the common laboratory animals the rabbit is the least used in studies on cardiomyopathies. We decided therefore to see whether or not under suitable conditions this species could develop cardiac lesions similar to IECP. In order to be comparable, the lesions are required to be chronic and to affect both the endocardium and myocardium. As IECP is observed at times in well-fed middle-class White patients, it did not seem logical to produce a cardiomyopathy by a dietary or electrolyte imbalance. Medication was likewise rejected. However, as it is well known from Bajusz’s work that stress affects the heart, it was decided to apply a chronic, easily controllable stress to the animals. This stress had to be of such a nature that it could not, in itself, affect the myocardium. Prolonged anaemia, hypertension, strenuous exercise, etc. could not be applied, as they would affect the heart directly. Crowding of rabbits seemed to be easily produced and controlled by daily weighing. It soon became apparent that continued crowding led to a high mortality rate within a short time, the animals dying with dilated hearts and acutely congested internal organs. Continued crowding over prolonged periods had to be abandoned. A regimen of intermittent crowding was introduced; this reduced the mortality and has led to the survival of some animals for more than 10 months at the time of writing.

MATERIALS AND METHODS

The size of the wire cages used in the experiments was 60 × 45 × 30 cm. Tap water and rabbit food pellets supplied by Victory Mills, Worcester, CP, were provided ad libitum. The room temperature varied between 15° and 24°, according to the season.

The animals used were New Zealand White rabbits. Their initial weight ranged from 2 200 to 3 700 g. The experimental rabbits were weighed daily. The sexes were kept apart in order to prevent litters. Six rabbits housed singly in cages of the size recommended by the US Institute of Laboratory Animal Resources during a 2 months’ immunization project, were used as controls. Four rabbits were crowded in a cage for 2 weeks and then housed singly for 1 week, whereupon they were crowded again, released, and so on. Rabbits succumbing to the regimen were replaced by new ones. Forty-eight rabbits were used in the experiment. Four animals that died from causes other than cardiac failure are not included in this report.

Six additional rabbits were placed singly into small compartments, the size of which allowed the rabbit to stretch and turn around, but not to move freely. One crowded animal in extremis was sacrificed in order to get tissues for electron microscopy. All the other experiments lasted until the spontaneous death of the animal. An autopsy of the thoracic and abdominal organs

*Date received: 8 March 1973.
was carried out. Tissue from heart, lungs, liver, spleen, adrenals and kidneys was fixed in formal saline and embedded in paraffin wax. Sections were stained with haematoxylin and eosin, toluidine blue, Verhoeff-van Gieson, Alcian blue - periodic-acid-Schiff reagent (PAS), Hale-PAS, and Mallory's phosphotungstic acid haematoxylin (PTAH).

RESULTS

Once crowded, the rabbits became aggressive, fighting and biting one another. They had wounds on their backs and ears. After a short while some had holes gnawed in their external ears. The fighting subsided after a few days, once a social order had been established, apparently, but it flared up occasionally. The wounds, however, never endangered the life of the animals.

At the time of writing 35 experimental rabbits had died and 9 had survived for periods up to 10 months. Twenty rabbits died during the first month, 10 of these during the first 2 weeks (Table I). The daily weight controls showed that all except 4 rabbits succumbing to the experiment, lost an average of 425 g of weight. The weight loss occurred during the last week of life in the majority of cases. Loss of weight was observed to a much lesser degree in rabbits who had survived for 2 months and more at the time of writing. There were 9 such rabbits, of which only 3 showed an average weight loss of 300 g, whereas 6 gained an average weight of 550 g, the gains ranging from 100 - 1000 g. The loss of weight was not caused by starvation, for at autopsy the stomachs were always normally filled and the intestinal contents also appeared to be normal.

TABLE I. TIME OF SURVIVAL OF CROWDED RABBITS

<table>
<thead>
<tr>
<th>Survival time</th>
<th>Weeks</th>
<th>Months</th>
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<tbody>
<tr>
<td></td>
<td>2</td>
<td>1 2 3 4 5 7 9</td>
</tr>
<tr>
<td>Number of rabbits</td>
<td>10</td>
<td>10 2 5 4 1 1 2</td>
</tr>
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Animals that died were examined as soon as possible. At autopsy the hearts were usually dilated. There was pulmonary oedema and congestion of the internal organs. The autopsied hearts were cleared of blood clots, weighed, and 15 were found to be hypertrophic. A heart weight of 0,19%-0,36% of the body weight was considered as normal. One heart was 9 g heavier than normal. This animal died 3 months after the beginning of the experiment. The increase in cardiac weight above the normal range averaged 1,8 g.

Control Animals

Histological sections of the control hearts showed that besides the periartrial tissue, the rabbit myocardium contained very little fibrous tissue. Three hearts had a very mild basophilic degeneration of myocardial fibres. Only 2 - 3 fibres with basophilic degeneration could be detected per section. One heart had a minute focus of recent subendocardial coagulative necrosis next to the left apex. Except for 1 heart, the endocardium was thin throughout and did not contain elastic fibres in the intertrabecular and apical portions. The other exceptional heart referred to above showed a mild fibrous thickening of the right ventricular endocardium near the apex. No elastic fibres were visible.

Experimental Animals

The experimental animals could be divided into a group surviving for less than 2 weeks and a group of longer survivors. In the former group there was no myocardial or endocardial fibrosis whereas cardiac fibrosis was present in the latter group.

Short-term survivors: The hearts of the short-time survivors frequently showed 2 kinds of myocardial lesions, viz. focal myocytolysis and interstitial oedema. Lytic necrosis was even found in 2 rabbits that died within 24 hours of the beginning of the experiment. Myocytolysis (Fig. 1), which was found more often in the inner third of the ventricles than in other portions of the heart, elicited no, or only a very minor cellular reaction, but it was accompanied by interstitial oedema. The oedema-
Longer-term survivors: The hearts of rabbits from this group were somewhat different. In addition to the interstitial oedema, the accumulation of acid mucopolysaccharides and the foci of myocytolysis, there were also areas with coagulative necrosis of groups of myofibres. However, myocytolysis was more common than coagulative necrosis. Foci of recent necrosis could also be found in long-term survivors. The lytic myofibres had collapsed, producing linear or stellate areas of delicate fibrosis with thin collagen fibres (Figs 3 and 4). The organization of coagulative necrosis had progressed to fusiform foci of myocardial fibrosis with collagen fibres of the usual thickness. Either of these kinds of fibrosis was found regularly in animals that died after the 17th day. Occasionally larger areas of fibrosis were found which were irregular in shape and contained a few intact myofibres separated from one another by thick collagen fibres (Fig. 5). One rabbit which died 2½ months after the beginning of the experiment had a diffuse dense fibrosis of the wall of the right ventricle, the myofibres being separated by increased amounts of ground substance and broad strands of connective tissue. Within or near areas of myocardial fibrosis, hypertrophy of the remaining and adjacent myofibres was a common finding. Basophilic myocardial degeneration became widespread and obvious in animals surviving for more than 2 months.

There were also changes in the endocardium of animals surviving for more than 1 month. One could find patchy thickening with focal proliferation of endocardial cells, mucoid oedema, or focal fibrous thickening of the endocardium, particularly near the apices. Some animals had small round cell infiltrations in the endocardium. Hearts from animals which survived 2½ months and more also showed the presence of elastic fibres in the thickened endocardium (Fig. 6). Only 1 animal showed a diffuse fibrous endocardial thickening. Mural thrombi were not found. There were, however, 2 animals which had some
fibrin incorporated in the thickened endocardium and 2 more with several small fibrin films covering the surface of the thickened endocardium (Fig. 7).

The degree of histopathological cardiac changes did not always correspond with the duration of survival. It was apparently influenced by the sensitivity of the individual animal during the terminal period of the experiment.

The most important histological findings in the other organs were acute or sometimes chronic systemic congestion and pulmonary oedema. The hepatic congestion occasionally caused centrilobular necrosis. In a few rabbits cytolytic necrosis of groups of cells in the adrenal cortex was found. Slight interstitial nephritis and mild pyelonephritis were other incidental findings.

**DISCUSSION**

The results show clearly that New Zealand White rabbits are well suited for research in cardiomyopathies. Under the experimental conditions they developed a severe cardiomyopathy which caused the spontaneous death from cardiac failure in 34 out of 44 experimental animals. One animal was sacrificed in the agonal phase and at the time of writing, 9 animals were still surviving.

The cardiomyopathy of the crowded rabbits was characterized by myocardial necrosis and interstitial oedema which appeared soon after the beginning of the experiment. At later stages there were myocardial fibrosis and patchy endocardial thickenings caused by cellular endocardial proliferation as well as fibrous thickening and fibro-elastosis of the endocardium. Fibrin deposits within or on the endocardium were found in 4 animals. The myocardium showed areas of hypertrophy and a pronounced basophilic degeneration.

The necrotic myocardial lesions were lytic or coagulative, the latter being less frequent than the former. Animals surviving longer than 1 month were more affected by coagulative necrosis than were animals dying during the 1st month. It was confined to the myofibres only, the adjacent connective tissue being intact. This necrosis is, therefore, similar to the lesion caused by isoproterenol injections in rats and may possibly be caused in the experimental animals by elevated blood levels of adrenomedullary hormones. The pathogenesis of the myocytolysis in the experimental animals remains obscure for the time being, but is well known to occur in many different conditions.

The necrotic lesions were accompanied by oedema which, however, was not confined to the vicinity of the necrotic lesions only. There were many patchy accumulations of acid mucopolysaccharides in the subendocardium and myocardium. In some instances the mucoid oedema was certainly secondary to the necrosis, but in many areas it appeared to have arisen independently of the necrosis. A lesser degree of a similar accumulation of acid mucopolysaccharides which led to myocardial fibrosis was observed in hypertensive rats by Hauss et al. Rössle observed, in what he called 'serous inflammation', a diffuse pericellular fibrosis subsequent to the acute phase of the serous inflammation which is similar in appearance to the above-mentioned oedema. As it is known that fibroblasts form acid mucopolysaccharides and tropocollagen simultaneously, it needs to be considered whether the interstitial mucoid oedema is a forerunner of some of the myocardial fibrosis. Mucoid oedema is a common finding in cardiomyopathies, e.g. it was found in isoproterenol rat heart and it is a common finding in the human IECF. However, the myocardial fibrosis of the experimental rabbits is also caused, in part, by the collapse of lytic myofibres and the organization of coagulative necrosis.
Endocardial thickening was caused by various processes. There were areas of subendocardial oedema containing acid mucopolysaccharides. There were also patches of endocardial cell proliferation which may have caused thickening, and lastly there were occasional subendocardial necroses, the organization of which could have caused the endocardial change. Whereas the apical endocardium of a normal rabbit contains no elastic fibres, endocardial fibro-elastic tissue of varying degree was observed near the apices in rabbits surviving the experiment for 2½ months or more. However, not all the long-term survivors showed this change, the cause of which remains obscure. Prolonged dilatation of the ventricles may be a cause. In 4 rabbits small amounts of fibrin were incorporated in, or deposited on, the endocardium. This affected only areas of altered thickened endocardium and it is therefore likely that the endocardial lesion is the cause of the fibrinous change. Whether or not this is a forerunner of mural thrombosis is still unknown.

In rabbits with long-standing cardiomyopathy 2 additional kinds of myocardial lesions were observed. The rabbits developed myocardial hypertrophy in groups of myofibres adjacent to fibrotic areas which showed a pronounced basophilic myocardial degeneration. It is easy to see the cause of the hypertrophy, but the significance of the basophilic degeneration is unknown. However, it is noteworthy that basophilic degeneration which increases with age is a common finding in IEC.

There are obviously 2 main lesions, but it is difficult to determine which is the primary lesion, the necrosis or the oedema. There are arguments in favour of each. However, one has to bear in mind that the accumulation of acid mucopolysaccharides in the myocardial interstitium was frequently found far away from, and apparently unrelated to, necrotic foci. Furthermore, it is known that interstitial oedema such as is found in serous inflammation, can cause scarring and hyaline lesions.

In oedematous tissue the transitional zone between capillaries and myofibres is enlarged. Thus diffusion from capillaries to myofibres and back is rendered more difficult. The same factors are operative when an excess of acid mucopolysaccharides, possibly locally formed rather than derived from the blood, is present.

From the light-microscopical findings, one cannot draw conclusions whether or not the lesions should be interpreted as inflammatory changes. It may well be that the mucoid oedema of IEC and the accumulation of acid mucopolysaccharides in the hearts described, are not reactive lesions but manifestations of a non-inflammatory metabolic aberration.

If one compares the lesions in the rabbit hearts with those in IEC one finds certain similarities. In both conditions there is myocardial fibrosis, interstitial myocardial oedema, basophilic degeneration of myofibres, involvement of the inner third of the myocardium, patchy fibrous or elastic thickening of the endocardium and fibrin deposits on or in the endocardium. However, mural thrombi, which are very common in IEC, were not observed in the rabbits, and necrotic myocardial lesions, which are uncommon in IEC, were frequently found in rabbits. These dissimilarities may be essential, but it may also be that the mural thrombosis occurs only late in the course of the cardiomyopathy of rabbits and that the necrosis can no longer be recognized in the burnt-out lesions of patients with prolonged treatment for IEC.

The results show that the rabbit is an experimental animal in which one can easily produce a severe chronic cardiomyopathy terminating in cardiac failure. This can be obtained without upsetting the dietary requirements or causing a hormonal imbalance through medication.

The rabbit cardiomyopathy has some similarities with the human IEC. However, these results have not provoked us to assert that the IEC may be the result of a stress reaction in man. For the time being, such an assertion would seem unfounded.

REFERENCES