Experimental aspects of cobalt cardiomyopathy

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All chambers of the heart are affected in experimental cobalt cardiomyopathy, with atrial pre-
dilection. The primary morphological alteration is mitochondrial damage that possibly reflects
an enzymatic block of oxidative decarboxylation at pyruvate and ketogluterate levels. In acute
cobalt toxicity chelation of calcium may also be a contributory factor, resulting in deficient
utilization of high-energy phosphates. Experimental cobalt cardiomyopathy requires precon-
ditioning factors: protein deficiency appears to be one of them. Vegetative polypoid endocarditis
is an important accompaniment in the model used, suggesting that in rats on a protein deficient
*diet cobalt produced endothelial damage in addition to a cardiocyte injury.*

In 1965 investigators in Quebec (Morin *et al.*, 1967) and Omaha (McDermott *et al.*, 1966) observed an endemic cardiomyopathy in heavy beer drinkers. Though epidemiological studies suggested a multicausal origin, cobalt, a beer additive, appeared to play a major part (Kesteloot *et al.*, 1968). Since the Quebec outbreak, studies on experimental animals have been carried out in collaboration with Dr. C. I. Chappel to reproduce the characteristic vacuolar and dystrophic 'myocardosis' of humans (Bonenfant, Miller, and Roy, 1967; Rona, 1968) and to gain an insight into the pathogenesis of this condition, using cobalt administration alone or in combination with hormonal and nutritional factors.

Methods and results

Male Sprague-Dawley rats weighing 200–250 g. were used. Cobalt sulphate alone, administered orally in high dosage (100 mg./kg.), was cardiotoxic. However, the incidence of myocardial changes was low as the rats died prematurely in systemic circulatory failure. The lesion consisted mainly of fatty change, contraction bands, and hyaline myofibre necrosis (Fig. 1). This acute cardiotoxicity of cobalt was probably related to a competitive inhibition of Ca (Kaufmann and Fleckenstein, 1965) that resulted in an inability of cardiac muscle cells to utilize high energy phosphate and to sustain mechanical tension in the state of excitation.

As cobalt inhibits the biosynthesis of thyroxin and cobalt administration results in thyroid alterations (Bonenfant *et al.*, 1967),

FIG. 1 *'Hyalin necrosis' of cardiac muscle cell with contraction bands* (bottom right). *Rat treated daily with 100 mg./kg. cobalt sulphate.* (*Electron micrograph: uranyl acetate and lead citrate staining.* × *6,800.*)
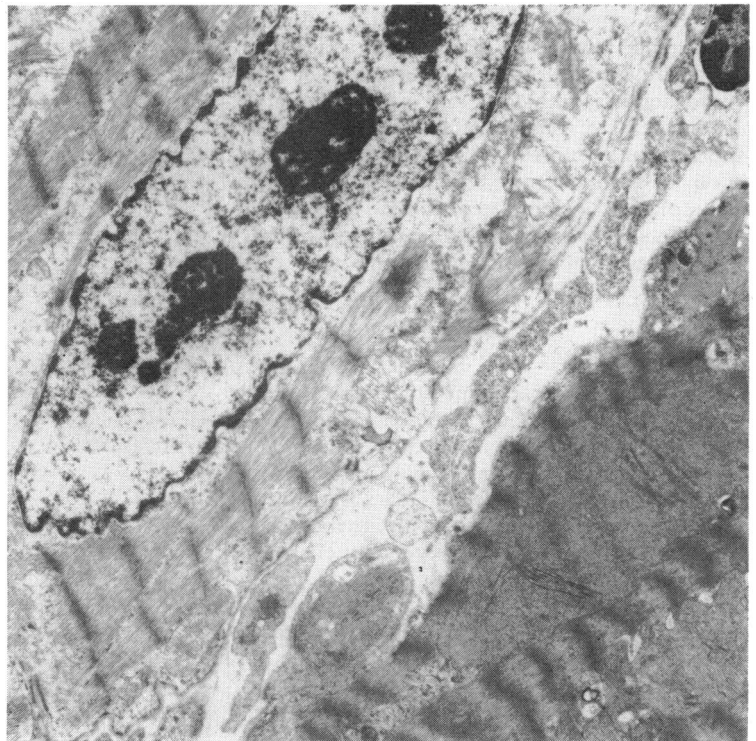
the predisposing role of hypothyroidism was considered. Though both prior treatment with propylthiouracil and thyroidectomy aggravated the myocardial changes of cobalt-treated rats, no distinct dystrophic-vacuolar cardiomyopathy was produced. The same results were obtained after the simultaneous administration of a sympathomimetic drug (isoprenaline) and cobalt, or by a combination of thiamine deficiency and cobalt.

A combination of protein-deficient diet and cobalt administration was also studied. In these experiments cobalt sulphate, containing from 4·0 to 12·5 mg./kg. cobalt ion, was given daily in 1·0 ml. distilled water by gavage to 280 rats for two weeks. Before this procedure the rats were preconditioned for 10 days by protein restriction. Severe cardiomyopathy occurred in rats receiving the low protein diet consisting of 4 per cent casein and treated with 12·5 mg./kg. cobalt ion. All chambers of the heart were involved; apparently the atrial myocardium underwent more extensive damage than that of the ventricles. At the light microscopical level the swollen cardiac muscle cells contained coarse irregular myofibrils. Hydropic vacuoles alternated with stippled basophilic areas. The latter change was particularly prominent in 1 μm. thick sections of Epon embedded material stained with toluidine blue (Fig. 2). In advanced cases there was partial or complete lysis of the muscle cells leaving empty sarcolemmal sheaths behind. The most ubiquitous fine structural feature of the myocardial lesions was swelling of the mitochondria, with decreased matrical density and, finally, disruption of the cristae and the outer membrane (Fig. 3). In addition, dilatation of sarcoplasmic reticulum and intra-sarcoplasmic oedema were noted. The myofilaments were pushed to the periphery of the cell or occupied a perinuclear position.

Discussion

These histological and fine structural alterations correlate well with the metabolic derangement of protein deficiency (Svoboda, Grady, and Higginson, 1966) and cobalt toxicity (Grice et al., 1969; Hall and Smith, 1968). Cobalt interferes with myocardial energy metabolism, as it irreversibly chelates with the dithiol form of lipoic acid (Dingle et al., 1962; Webb, 1962), inactivating the coenzyme required for the oxidative decarboxylation of pyruvate to acetyl coenzyme A and alpha ketoglutarate to succinate (Alexander, 1969). Pyruvate and lactate accumulation in the mitochondria increases osmotic pressure
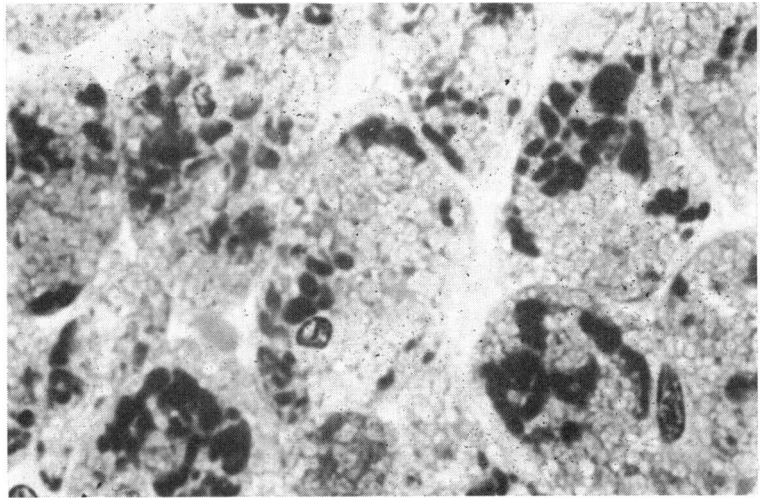

FIG. 2   Pale perinuclear areas of cardiocytes represent swollen mitochondria. (Epon embedded 1 μm. thick section, stained with toluidine blue. × 1280.)

FIG. 3   Electron microscopic presentation of swollen mitochondria, dilated sarcoplasmic reticulum, and intrasarcoplasmic oedema. (× 20,800.)

FIG. 4 *Electron dense intramitochondrial particles represent cobalt-protein complexes.* (× 16,300.)

and results in water binding, oedema, and finally structural disruption. Aminoacids combine readily with cobalt and prevent its chelation with sulphydryl groups of the myocardial tissue (Alexander, 1969; Wiberg *et al.*, 1969). Protein deficiency blocks this protective mechanism. Whereas the fine structural changes the mitochondria presented are nonspecific, a pathognomonic feature of cobalt intoxication was the appearance of dense osmophilic intramitochondrial particles, measuring 0·3–0·4 μm. in diameter (Fig. 4), considered to represent cobalt-protein complexes (Knieriem and Herbertz, 1969; Kasperek, Siller, and Knieriem, 1969). These differed from the fine granular intramitochondrial calcium deposit described in a variety of myocardial injuries.

In addition to the myocardial lesion, polypoid vegetative endocarditis developed in 10–35 per cent of rats receiving the low-protein diet and treated with cobalt. The mitral valve was affected predominantly (Fig. 5), but vegetations also occurred occasionally on the aortic and tricuspid valves and on the parietal endocardium. A non-necrotizing granulomatous inflammatory process developed around the vegetations (Fig. 6). Blood cultures from these rats were negative. Electron microscopical findings consisted of endothelial cell alteration leading to endothelial discontinuity that allowed platelets to be carried into the subendothelial stroma. In the depth of the valve there was polymerized fibrin among proliferating fibroblasts (Fig. 7). Though it is conceivable that endothelial damage may be the primary factor in the genesis of endocarditis, platelet conglutination may also be important, since oxidation of the free SH groups on the platelet surface, a step in platelet conglutination (Skålhegg, Hellem, and Ödegaard, 1964), may be affected by cobalt and protein deficiency.
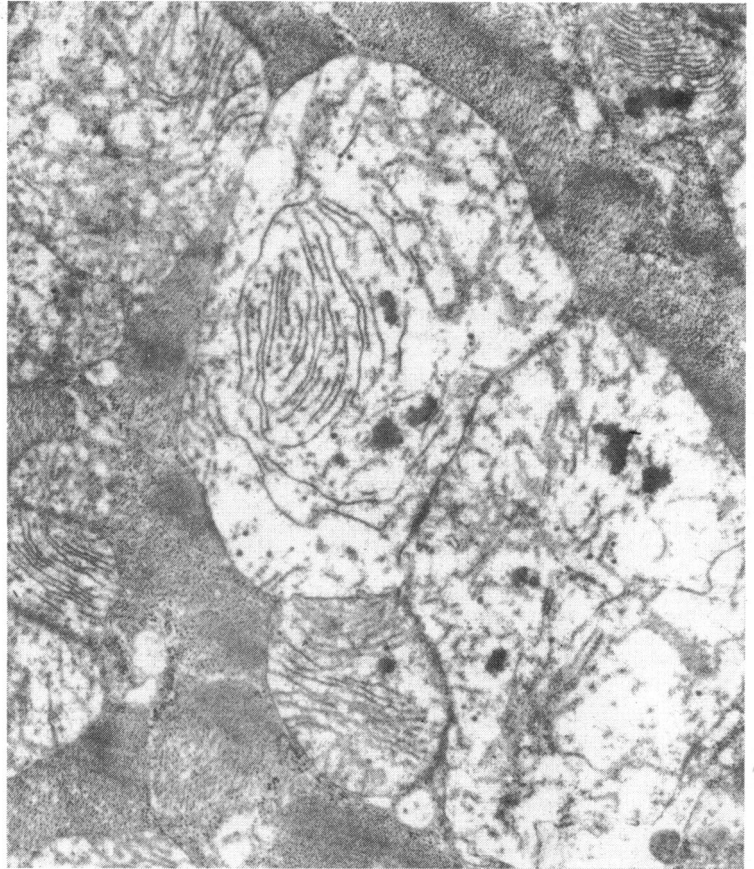

FIG. 5 *Gross presentation of polypoid endocarditis in the mitral valve of a rat on protein-deficient diet treated with 12·5 mg./kg. cobalt ion daily.*

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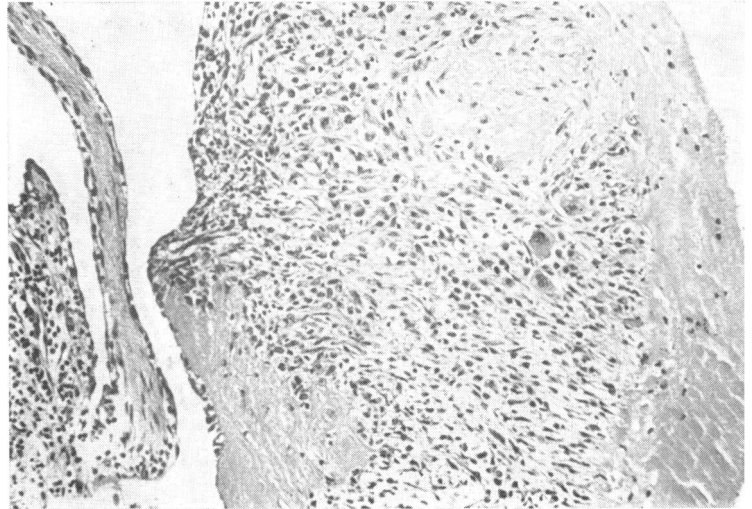

FIG. 6 *Non-necrotizing granulomatous reaction around the valvular vegetations. (Haematoxylin and eosin. ×150.)*

FIG. 7 *Electron dense fibrin bundles among the proliferating cells of the valvular vegetations. (×4,300.)*