Pathologic Changes of Aorta and Coronary Arteries of Mice Infected with Coxsackie B_4 Virus[1] (35641)

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Vascular lesions associated with possible viral cytoplasmic inclusions in endothelial cells of capillaries in man (1–4) and viral crystals in capillary endothelial cells in animals (2, 5–7) have been reported. A high percentage of intracytoplasmic inclusions or virus-like crystals in capillary endothelial cells of glomeruli in patients with lupus nephritis or systemic lupus erythematosus (2–4, 8) suggests a possible viral etiology for some of the clinical arterites of unknown etiology, such as giant cell arteritis and polyarteritis nodosa. That viruses can cause arteritis, or at least invade capillaries, in experimental animals has been well established. The finding of viral crystals in the endothelial cells of blood vessels is strong evidence of viral infection (6, 7) Coxsackie virus B_4, a common cardiotropic virus infectious to man, can cause coronary arteritis in monkeys (9, 10). Capillary damage associated with virus-like particles in the endothelial cell in the myocardium of mice infected with Coxsackie virus B_4 has been reported from this laboratory (11), showing further that this virus can infect blood vessels of experimental animals.

This report is concerned with the production of changes in both the aorta and coronary arteries of mice by Coxsackie virus B_4. The possible relationship of such lesions to chronic arterial disease and arteriosclerosis deserves consideration.

Materials and Methods. Virus stock. The Coxsackie virus B_4 used in these experiments was originally recovered by Kibrick and Benirschke (12) from a 10-day-old infant who died of encephalohepatomyocarditis. The virus was obtained as monkey kidney culture passage strain. For these experiments the virus was prepared in rhesus monkey kidney cultures according to techniques previously described (13). Control fluid from monkey kidney cell cultures free of virus was also obtained. The virus and control fluid were stored at −65°.

Mice. A random breed strain of HaM/ICR mice was used in the experiments.

Inoculation of virus and collection of tissues. Twenty 2-day-old and twenty-one 12-day-old suckling mice were inoculated intraperitoneally with 0.025–0.05 ml and 0.1 ml, respectively, of monkey kidney cell culture fluid containing 10^5 $TCID_{50}$/ml Coxsackie virus B_4. The 2-day-old mice were killed daily up to the third day after inoculation and the 12-day-old mice were killed daily up to the fifth day after inoculation.

Control experiment. Fifteen 2- to 12-day-old suckling mice were inoculated with 0.025 to 0.1 ml of virus-free tissue culture fluid and killed in the same manner as the infected animals.

Histopathologic studies. The aortas and hearts were collected and fixed in 10% neutral formalin. Fixed tissues were processed, embedded in paraffin, and cut serially in sections of 6-μ thickness. Lungs and some mesenteric tissue were also collected and processed. The sections were stained with hematoxylin and eosin and examined with a light microscope.

Results. Almost all of the 2-day-old infected mice killed within 2 days after inoculation showed vascular changes. The 12-day-old infected mice had vascular changes similar to those of the younger mice, but the changes were not as numerous nor as extensive as in

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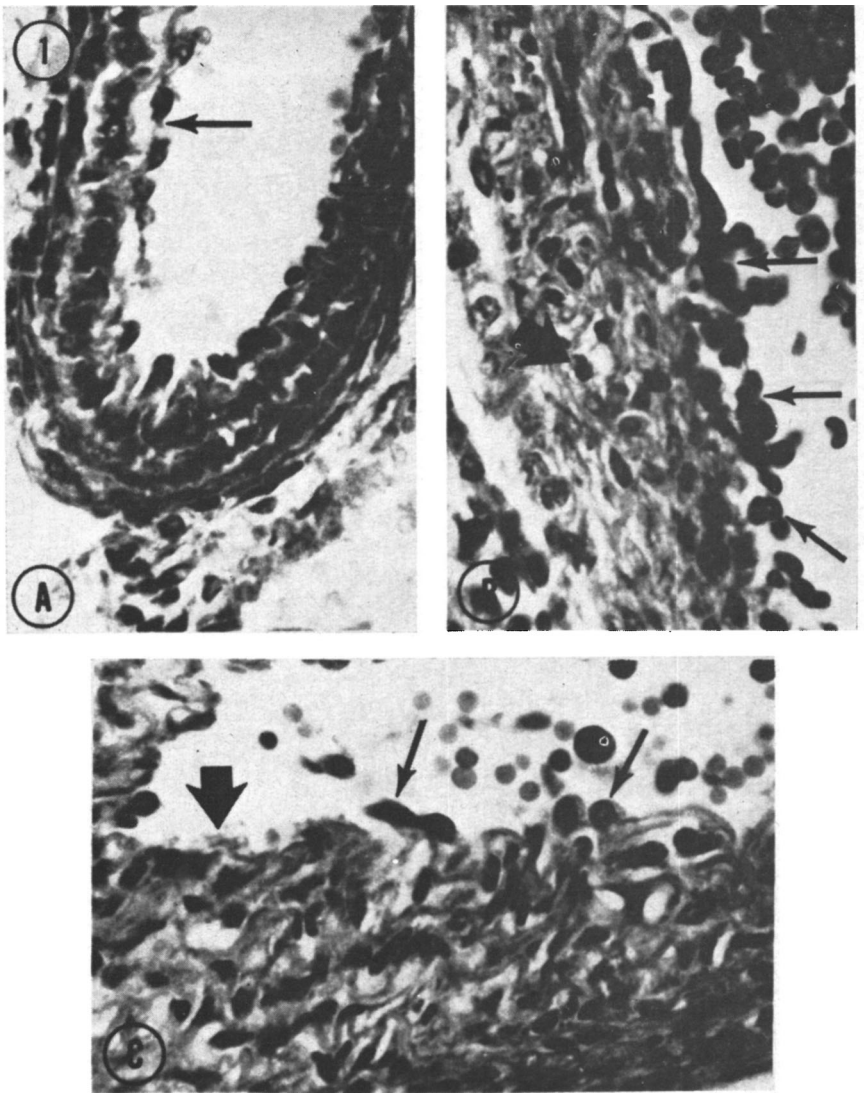

FIG. 1. Sections of aorta of mice infected with Coxsackie virus B_4: (A) Aorta of a 4-day-old mouse 48 hr after inoculation, showing focal edema of aortic wall and subendothelial area with overlying swollen cells (arrow) and denuded intimal surface in the opposite area. (B) Aorta in region of sinus of valsalva of a 3-day-old mouse killed 24 hr after inoculation. Note swollen lining endothelial cells with edema and a few inflammatory cell infiltrates in the subendothelial region (small arrows) and a few cells in the media with pyknotic nuclei (large arrow). (C) Aorta of a 3-day-old mouse killed 24 hr after inoculation, showing swollen endothelial cells (small arrows) and denuded intimal surface of the aorta (large arrow). H & E ×450.

the younger mice.

The histopathologic changes in the aorta consisted of small focal degenerative and necrotic lesions of the endothelial cells with edema of the subendothelial and medial layers of the aortic wall. There was occasional mild inflammatory cell infiltration. Endothelial cells were desquamated in some areas, leaving a denuded intimal surface (Fig. 1). The cells in the media in some areas displayed necrosis of individual cells evidenced by pyknotic nuclei.

The coronary arteries showed essentially the same changes as the aorta. The intima

had degeneration of endothelial cells with necrosis and denudation. Edema of the vascular wall was usually present (Fig. 2). Thrombus formation was not found in any of the animals.

The changes usually developed within 24 hr after inoculation. Mitotic figures of endothelial cells were occasionally seen 48 hr after inoculation. Lesions became less impressive by the third day after inoculation. The same vascular changes were found in four 2-day-old mice that died spontaneously 48 hr after infection.

Similar vascular changes were present in the pulmonary veins and in the mesenteric arteries and veins. They were probably also present in arteries, veins, and vessels elsewhere although a thorough search was not made.

Focal myocardial necrosis usually seen in Coxsackie virus B_4 infected mice was also present (Fig. 2). There were no vascular or myocardial lesions noted in the control animals.

Discussion. Vascular lesions in man due to viral infections have received very little attention. The recent reports of cytoplasmic inclusions or virus-like crystals in the lining endothelial cells of the capillaries in the patients with lupus nephritis or systemic lupus erythematosus suggest strongly that some types of clinical vasculitis of unknown etiology may be produced by direct invasion of blood vessels by viruses. Viral infections also might initiate autoimmune-type reactions. Several viruses have been shown to cause vasculitis by direct invasion of the capillaries in animals, as supported by the finding of viral crystals in endothelial cells (6, 7). The Coxsackie virus B_4, a common viral infective agent in man, can cause vascular lesions in the heart of mice (11). In the present experiments, mice inoculated with Coxsackie virus B_4 developed lesions of the great blood vessels. These mice showed remarkable endothelial damage with denuded areas of the intima by desquamation of endothelial cells in both aorta and coronary arteries.

It has been reported that one important requirement necessary for the development of venous thrombosis is damage to endothelium of the veins (14). The destruction of endothelium initiates the adherence of platelets to the exposed subendothelial tissue rather than to the endothelium itself, as shown in recent animal experiments (15, 16). It is interesting that McKay and Margaretten (17) suggested that most intravascular thrombosis in viral infection in man is caused by viral invasion with damage to endothelial cells.

There were no thrombi seen in the animals used in the studies reported here. It is probable that the small damaged foci or denuded areas were repaired rapidly by endothelial cell regeneration or re-endothelialization. The failure of thrombosis to develop may be characteristic of mice, an animal that is not prone to atherosclerosis or similar types of vascular lesions. However, the response may be quite different in man and other animals.

Little is known of the damage to blood vessels by Coxsackie virus in man. Because Coxsackie viral infections are common in people, these vascular lesions need careful study. Since Coxsackie virus B_4 damages the great blood vessels of mice, it is likely that it also damages the vessels of man. The conditioning factors which determine the extent and nature of the response of the host need investigation. Young human beings (sucking babies) are especially prone to Coxsackie and other viral infections. Endothelial lesions could be followed by fibrin deposition and thrombus formation with subsequent development of atheroma. Viruses other than Coxsackie virus B_4 most probably can also damage blood vessels and may even induce thrombus formation. Such concepts need investigation, especially in man. Nevertheless, it is important to realize that with certain viral infections and the associated viremia, all blood vessels of the animal, including those of man, can become infected.

Summary. Coxsackie virus B_4 has been shown to produce significant lesions in the large and small blood vessels of 2-day-old and 12-day-old mice. The Coxsackie virus B_4-infected mice developed focal endothelial cell degeneration and necrosis with subsequent desquamation and denudation of in-

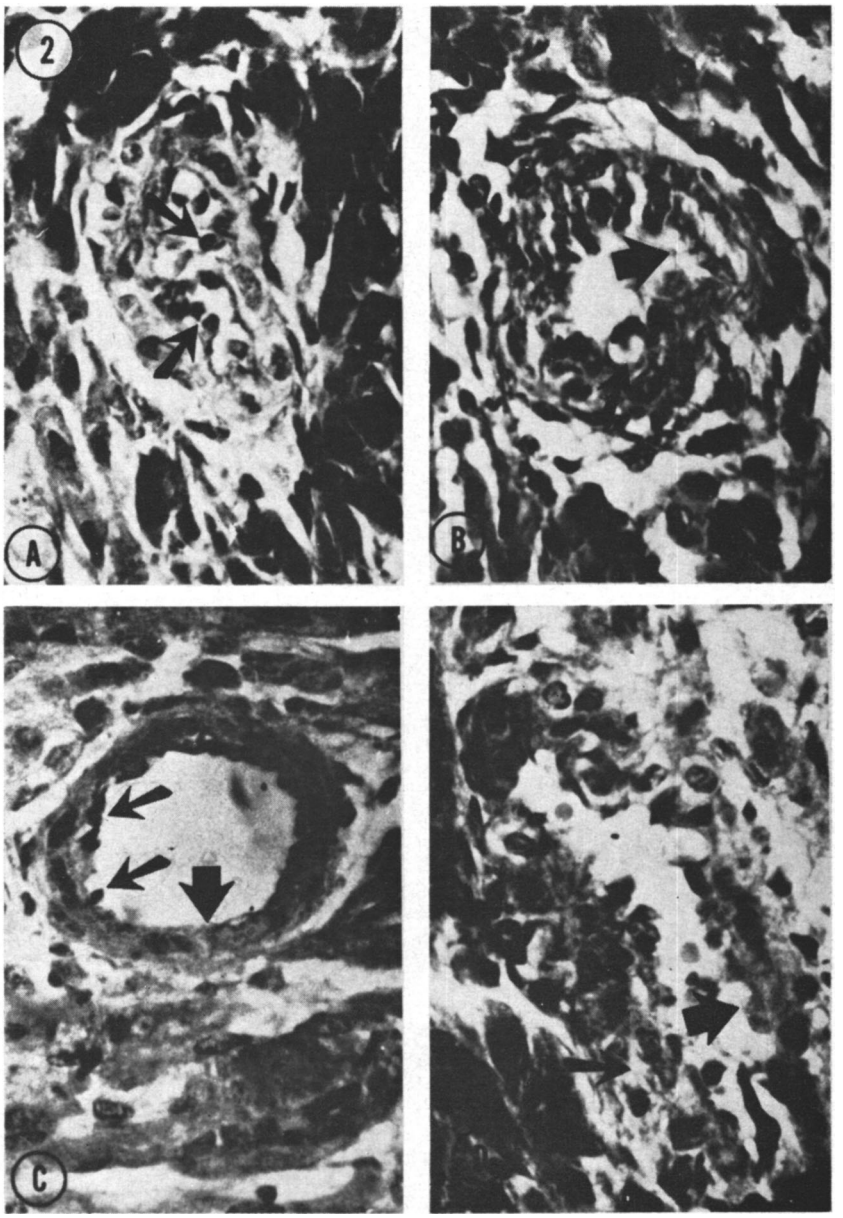

FIG. 2. Sections of coronary arteries of mice infected with Coxsackie virus B_4: (A) Branch of coronary artery of a 4-day-old mouse killed 2 days after inoculation, showing swollen endothelial cells (arrows), three with pyknotic nuclei. (B) Branch of coronary artery of a 3-day-old mouse killed 1 day after inoculation, showing vacuolated endothelial cells (small arrow) and denuded intimal surface (large arrows). (C) Branch of coronary artery of 14-day-old mouse killed 2 days after inoculation, showing degenerative endothelial lining cells (small arrows) and denuded intimal surface (large arrow). Myocardial necrosis can be seen in the right lower portion of the illustration. D) Branch of coronary artery of a 4-day-old mouse killed 2 days after inoculation, showing degenerative and sloughing endothelial cells, denuded intimal surface (large arrow) and edematous media (small arrow). Myocardial necrosis is also noted in the right upper portion of illustration. H & E ×450.

tima of the aorta, coronary arteries, large veins, and other blood vessels.

Viral infections in man are common, but infection of the great blood vessels remains little studied. Viral lesions are suggested as an initiating factor in the production of vascular diseases in man, possibly including atherosclerosis.

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