Coxsackie Virus Valvulitis and Myocarditis Observed at Routine Autopsy

Recent evidence suggests that chronic valvulitis is associated with coxsackie B_4 myocarditis in experimentally infected animals[1-3]. Immunofluorescent techniques demonstrated the presence of specific coxsackie viral antigen within the affected valves, mural endocardium as well as the myocardium in the experimentally infected mice and monkeys[2-4]. It was postulated that some instances of chronic valvular heart disease in man may be of coxsackie viral origin[5]. This was thought to be especially likely in those cases where autopsy showed significant chronic valvular disease but where the clinical history lacked evidence of previous rheumatic fever. The widespread prevalence of mild viral respiratory tract infections with potentially cardiotrophic viruses, e.g. coxsackie, in children and young adults prompted a search for the presence of coxsackie viral antigen in the valves and myocardium of young patients dying of a variety of causes and coming to routine autopsy at the Charity Hospital of Louisiana at New Orleans.

Samples of heart tissue from the upper lateral aspect of the left ventricular wall and the mitral valve were obtained from 55 routine autopsies. A special effort was made to obtain tissue from individuals less than 30 years of age, and from those showing significant gross fibrotic thickening of the mitral valves regardless of age. Forty patients less than 30 years of age, and 15 patients of middle age with fibrotic lesions of the mitral valve were studied.

Both direct and indirect immunofluorescent techniques were employed[6]. Antiserum to coxsackie virus B_1, B_2, B_3, B_4, B_5 was prepared in different rabbits by 2 i.v. injections of the respective types of living virus suspension grown in monkey kidney cell medium. The inhibition titers of the different lots of the pooled sera varied from 1:64 to 1:723. For direct immunofluorescent technique the antiserum for each type of virus was fractionated and conjugated with fluorescein isothiocyanate[7]. Antiserum to the proteins of monkey kidney cell medium was similarly prepared in separate rabbits by 2 i.v. injections of virus-free monkey kidney suspensions and was labeled with fluorescein for use as a control of staining specificity. Aliquots of non-conjugated antisera from the same preparations were saved for use in the indirect method with fluorescein labeled anti-rabbit globulin prepared in sheep (Difco). The antisera were used to stain 8 μ cryostat

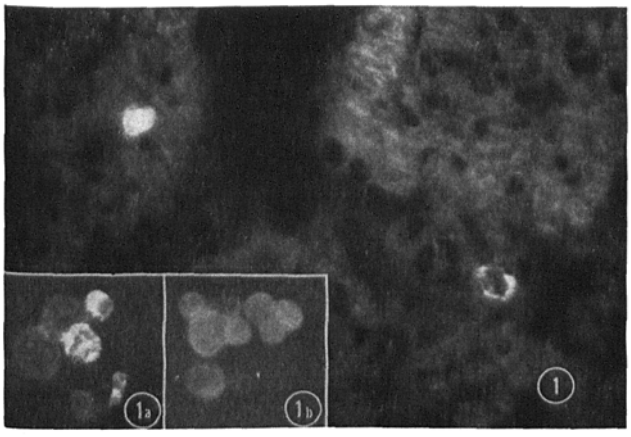

Fig. 1. Myocardium. Direct immunofluorescent stain with coxsackie virus B_2 antiserum. Note the fluorescent antigen within the cytoplasm of myocardial fibers. Three-day-old male infant dying of acute bronchopneumonia. 1a and 1b. Monkey kidney cell culture inoculated with coxsackie B_4 virus and stained with the conjugated coxsackie B_4 rabbit antiserum. Note the bright cytoplasmic fluorescence in the infected cells (1a). The monkey kidney cells stained with the control rabbit antiserum shows only a faint non-specific tissue fluorescence (1b). × 480.

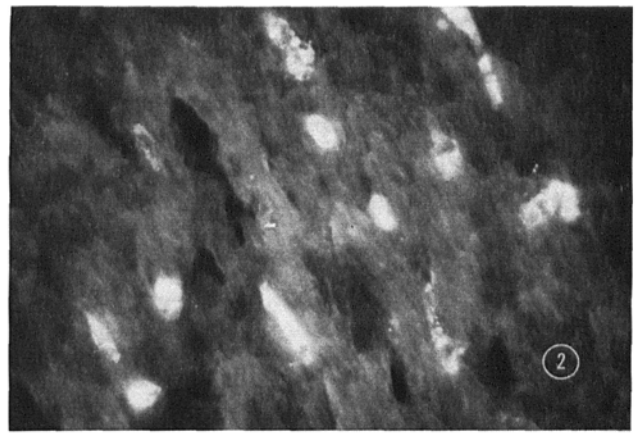

Fig. 2. Myocardium. Direct immunofluorescence stain by using coxsackie B_2 antiserum. Note discrete fluorescent antigen in the cytoplasm of myocardial fibers. Stillborn, premature infant. × 480.

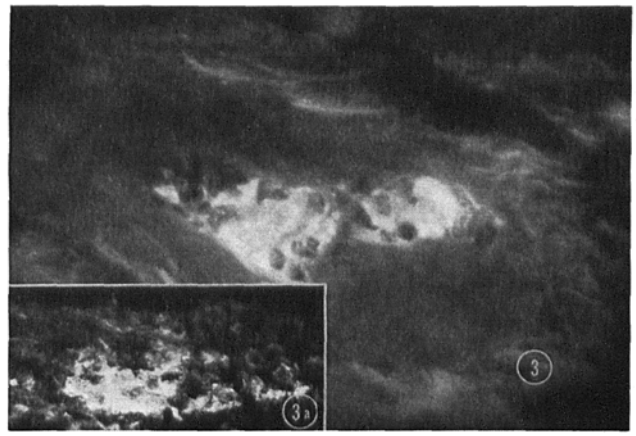

Fig. 3. Mitral valve. Indirect immunofluorescence stain by using coxsackie B_5 antiserum. Note fluorescent viral antigen within the stromal cells of the valve. 55-year-old male dying of acute bronchopneumonia and pancreatitis. 3a. Tricuspid valve from coxsackie B_4 virus infected mouse stained with conjugated B_4 rabbit antiserum. Note the similar cytoplasmic immunofluorescence in the stromal cells of the valve. × 480.

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sections of the valves and myocardium. Staining specificity of the different types of coxsackie B antisera was confirmed by: (a) positive fluorescence when the respective types of antisera were reacted with monkey kidney cell culture infected with known types of coxsackie virus (Figure 1a, 1b), (b) a positive staining reaction in both valves and myocardial tissue of mice infected with a B_4 strain of coxsackie virus when reacted with type specific (B_4) antiserum, (c) a negative staining reaction when antiserum to the proteins of monkey kidney cell medium was used in the positive human valvular and myocardial tissue sections, and (d) a strictly cytoplasmic localization of the fluorescence.

Seventeen hearts were positive for coxsackie viral antigens within the myocardium. Fourteen cases showed more than one type of coxsackie B antigen. The most frequent types were B_2 and B_5. Antigen displayed a bright intracellular cytoplasmic fluorescence scattered focally throughout the myocardium (Figures 1, 2). Of the 40 patients less than 30 years of age 15 showed varying degrees of antigen present within the myofibers. Focal chronic interstitial myocarditis was observed in the 17 patients in hematoxylin-eosin stained sections.

There were 3 cases of viral valvulitis. They were positive for types B_1, B_4 and B_5, respectively. Fluorescent viral antigen was located discretely within the stromal tissue elements of the mitral valves in all instances (Figure 3). Hematoxylin-eosin stained sections showed mononuclear cellular infiltration and fibrosis. One patient was 3 days old, another 5 years, and a third 55 years. The latter had gross valvular thickening.

This study demonstrates that coxsackie viral antigen can be identified in both valves and myocardium by immunofluorescent techniques in a significant number of patients coming to routine autopsy. It seems that the incidence of valvulitis and myocarditis may well be significantly higher than the current literature suggests. The demonstration that this antigen can invade and cause an inflammatory reaction within the valvular tissue seems important. It seems quite likely that a certain proportion of chronic valvular disease now thought to be of rheumatic or unknown origin may well be the result of viral infection. Since the incidence of these infections is much higher in the infants and the young adults it is likely that viral invasion at this time may produce a subclinical slowly progressive valvular damage. These studies will be reported more extensively elsewhere[8]. The role of the immunofluorescent methods in the diagnosis of viral disease of the heart and valves needs further exploration[9].

Zusammenfassung. Die Gegenwart von Coxsackie-Virus Antigen wurde mit Antikörper-Test in Klappen und Herzmuskel junger Patienten mit verschiedener Todesursache festgestellt. Von 55 Herzuntersuchungen waren 17 Herzmuskeln und 3 Mitralklappen für Coxsackie-Virus Antigen positiv. In den meisten Fällen konnte B-2 und B-5 beobachtet werden. In allen positiven Fällen zeigten die histologischen Untersuchungen interstitielle Herzmuskel- und chronische Klappenentzündung.

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Teratogene Wirkung von 1-Phenyl-3,3-dimethyl-triazen, Erzeugung von Gaumenspalten bei BD-Ratten

N-Äthyl-N-nitrosoharnstoff[1,2], 1,2-Diäthyl-hydrazin, Azo-, oder Azoxy-äthan (noch nicht veröffentlicht), als einmalige Dosis von 25% der LD 50 schwangeren Ratten des Stammes BD IX am 15. Tage post coitum gegeben, erzeugten bei fast allen Nachkommen Missbildungen der Pfoten, aber niemals Gaumenspalten. Demgegenüber fanden wir im Phenyl-dimethyl-triazen eine Substanz, die am gleichen Objekt und unter gleichen Bedingungen vorwiegend Gaumenspalten hervorruft. Da die Möglichkeit selektiver Wirkungen für die Teratogenese-Forschung wichtig erscheint, und da PEARN[3] kürzlich die spezifische Erzeugung gerade dieser Missbildung mit rohen Extrakten aus *Indigofera spicata* beschrieb, soll über unsere Ergebnisse kurz berichtet werden.

1-Phenyl-3,3-dimethyl-triazen (PDMT), C_6H_5-N=N-N-$(CH_3)_2$, ist ein ausgesprochen neurotropes Carcinogen[4]. Schwangere Ratten der Stämme BD VI (CPaH, schwarz) und BD IX (CPAH, wildfarben) erhielten am 15. Tage post coitum eine einmalige Dosis von 75 mg/kg Körpergewicht in Öl gelöst als s.c. Injektion. Die zeitgerecht spontan geborenen Nachkommen waren kurz nach der Geburt gestorben und z.T. von der Mutter aufgefressen. Daher konnten nur 7 Neugeborene untersucht werden. Sie hatten sämtlich Gaumenspalten (Figur a). Die Atemwege waren mit Schleim verstopft. Im Magen fand sich keine Milch. Das Ergebnis konnte in mehreren Wiederholungsversuchen bestätigt werden. Trotz zahlreicher Verluste beobachteten wir unter 23 Nachkommen 16 Fälle von Gaumenspalten, davon nur 5 gleichzeitig mit Missbildungen der Pfoten. Ein spontanes Auftreten von Gaumenspalten haben wir bei BD-Ratten niemals beobachtet.

Kleinere Dosen von PDMT, die zur Prüfung auf transplacentare carcinogene Wirkung gegeben wurden, waren nicht teratogen. Das Gleiche hatten wir bereits bei den oben genannten Substanzen beobachtet. In allen Fällen erwies sich die teratogene Wirkung – im Gegensatz zur carcinogenen – als Funktion der Konzentration des Giftes («Konzentrations-Wirkung»).

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