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Coxsackie A9 Myocarditis in Adult Mice.* (27927)

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In a previous report on the replicating capacity of Coxsackie A9 virus, Strain 13, in mice varying in age from less than 24 hours to 8 months, it was found that virus production occurred at all ages, but the relative yields of virus from myocardium and skeletal muscle varied markedly(1). In striated muscle the highest titers of virus were found in suckling mice, whereas in the myocardium the highest titers were in the oldest mice. A diffuse myositis was observed in suckling mice, moderate lesions in 20- and 40-day-old mice, and only focal areas of infiltration were seen in striated muscle of the 8-month old animals. No lesions were noted in the hearts of the sucklings, or of 20- or 40-day old mice, but frank myocarditis was seen in 8-month old animals.

Since multiplication of the Coxsackie A group of viruses with concomitant pathology in cardiac muscle of mice had not been reported previously and the number of adult mice included in the initial study was small, further experiments were done to determine the incidence of these myocardial infections and concomitant lesions. Further data on strain specificity were also obtained.

Materials and methods. Swiss mice from the Hauschka and Marand Institute of Cancer Research‡ or Webster§ strains were used. Three strains of Coxsackie A9 virus were employed, *viz.,* Strain 13, used as second rhesus kidney (MKTC) passage, NIH-1, and Wiederhold. The sources and passage histories of the first 2 strains have been described(1); the Wiederhold strain was obtained from Dr. Sidney Kibrick; it was isolated originally by Dr. Frederick C. Robbins(2) in suspended cell culture of mature human kidney inoculated with feces of a patient diagnosed as having nonparalytic poliomyelitis and was then passaged in the same tissue and in human embryonic skin muscle cultures and was received after many additional passages in

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rhesus kidney tissue culture. The methods used here were the same as in the earlier study(1).

Experimental infections. Mice approximately 1 year old were inoculated intraperitoneally (i.p.) with 0.05 ml of virus containing about 10^5 $TCID_{50}$‖ of 1 of the 3 strains of Coxsackie A9, 30 mice each with Strain 13 and Wiederhold and 24 with NIH-1; 12 control mice of the same age were inoculated with noninfected tissue debris and medium from the MKTC. On the 6th and 9th days after inoculation of Strain 13 and Wiederhold, 12 mice of each of these 2 groups were sacrificed; blood, one hind limb and a coronal section of heart of each were collected for isolation of virus and the other hind limb and the other half of the individual hearts were simultaneously collected for histologic examination. The mice inoculated with NIH-1 were handled in the same way except that only 5 were sacrificed on the 9th day. Half of the control mice were sacrificed on day 6, the rest on day 9, and were similarly handled. The remaining infected mice of each group were sacrificed 14 days after inoculation and pooled sera of each group were tested for neutralizing antibody (NA) to Strain 13.

Pathology. Tissue blocks were prepared from heart and skeletal muscle of the proximal portion of a hind limb. These were fixed in 10% formalin; sections 6 μ thick were cut and stained with hematoxylin and eosin, 3 sections about 36 μ apart from each block of tissue.

Results. Infections of one-year-old mice with Coxsackie A9, Strain 13. None of these mice showed any signs of illness. Of the 12 inoculated with approximately 10^5 $TCID_{50}$, 7 were infected, as evidenced by recovery of virus in MKTC from 20% suspensions of heart obtained 6 days after i.p. inoculation of virus (Table I). Coxsackie A9 virus was isolated from only 2 of the hind limbs of these infected animals. Significant pathologic lesions (graded ++ to ++++) were seen in the hearts of 2 of the 12 inoculated mice, and in none of the sections of striated muscle. Among mice sacrificed on day 9 after inoculation, Coxsackie A9 virus was recovered from 2 additional hearts, but none of the hind limbs contained detectable virus; 3 additional mice showed myocarditis at this time. The 6 remaining mice were sacrificed on day 14; the NA titer of the pooled serum from these mice against Strain 13 was 1:40. None of the 12 control mice examined on days 6 and 9 yielded virus or showed pathologic lesions in the heart or skeletal muscle.

Thus, the hearts of 7 of the 12 inoculated animals yielded virus when tested at an optimum time, namely, 6 days. It is likely that a similar number of infections occurred among the 12 mice inoculated simultaneously and sacrificed on day 9, when recovery of virus was poorer, but the pathologic changes in cardiac muscle were more striking. The cardiac lesions in the mice sacrificed on days 6 or 9 were considered separately. Infection of the mice and myocarditis were related to the i.p. introduction of Coxsackie A9, Strain 13 ($P = 0.05$)(3). In the one-year old mice, in contrast to the younger animals studied, the lesion produced was an isolated focal myocarditis, without significant pathologic lesions in any of the limbs examined.

Inoculation of one-year old mice with other strains of Coxsackie A9 virus. Virus could not be isolated from any of the mice inoculated with the NIH-1 or Wiederhold strains of Coxsackie A9, nor were any pathologic lesions found in the hearts or limbs of these animals. Pooled sera from both groups collected 14 days after inoculation showed no neutralizing antibody. Thus, these 2 strains of Coxsackie A9 virus did not infect the one-year old mice.

Pathologic findings. Significant histopathologic changes were noted only in the animals infected with Strain 13; these consisted of foci of lymphoid cells and an occasional polymorphonuclear leukocyte within the myocardium (Fig. 1). The inflammatory cells frequently surrounded an eosinophilic, necrotic myocardial fiber. In one animal (No. 8, day 6, Table I) there was a marked inflammatory infiltrate around and involving a large coronary artery; this consisted of lymphocytes, large mononuclear cells, and

‖ Dose which infects 50% of tissue culture tubes.

TABLE I. Demonstration of Virus and Lesions in One-Year-Old Mice After Intraperitoneal Inoculation of Coxsackie A9, Strain 13.

Days after inoculation	Mouse No.	Heart		Hind limb	
		Virus	Lesion*	Virus	Lesion*
6	1	+	0	0	0
	2	+	0	0	0
	3	0	0	0	0
	4	+	+(F)	+	0
	5	0	0	0	0
	6	+	0	+	0
	7	0	0	0	0
	8	+	++++(PV, N)	0	+(PV)
	9	0	0	0	0
	10	0	0	0	0
	11	+	++(F)	0	0
	12	+	0	0	0
Total	12	7	2	2	0
Controls†	1–6	0	0	0	0
9	1	0	+++(F)	0	0
	2	0	0	0	0
	3	+	0	0	0
	4	+	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	++(F)	0	0
	9	0	0	0	0
	10	0	++(PV)	0	0
	11	0	0	0	0
Total	12	2	3	0	0
Controls†	1–6	0	0	0	0

* Graded by number of inflammatory cells: +, only a few, probably nonspecific and not included in totals; F, foci of mononuclear cells; PV, perivascular infiltration with mononuclear cells; N, polynuclear neutrophile infiltration.

† Inoculated with MKTC debris and medium without virus.

polymorphonuclear leukocytes, with nuclear fragments from necrotic cells scattered among the inflammatory cells. The adventitia of the artery was obscured by the dense inflammatory infiltrate, and a few inflammatory cells extended into the media. The relation of this lesion to the infection of that mouse is not clear. No endocardial or pericardial lesions were seen.

No necrosis of muscle fibers nor significant inflammation was seen in the skeletal muscle.

Discussion. Changes in susceptibility to infection involve cellular and humoral factors. The cellular factors are well demonstrated in the changing capacity of the heart and striated muscle of the mouse to replicate Strain 13 of Coxsackie A9 virus. In the present experiments limbs of newborn mice produced about 10,000 times more virus than the heart of the same animal (Table II). When compared with the results previously obtained in mice of different ages up to 7 months(1), it is seen that this ratio changed with the age of the animals so that, by the 7th month, the cardiac muscle produced about 2000 times as much infectious virus as did skeletal muscle. This enhanced capacity of the heart to replicate this virus was even more marked in 1-yr old mice.

TABLE II. Production of Coxsackie A9 Virus, Strain 13 in Skeletal Muscle and Myocardium of Mice.

Age of Mice	Titer of virus, $TCID_{05}$		
	Hind limb (A)	Heart (B)	A/B
Newborn*	10^5	10	10,000
1 mo*	10^4	$10^{1.6}$	143
7 " *	5	10^4	0.005
12 "	0–10†	10^4	$\leq$0.0001

* Data previously reported.

† Virus demonstrated in only 2 of 12 mice tested.

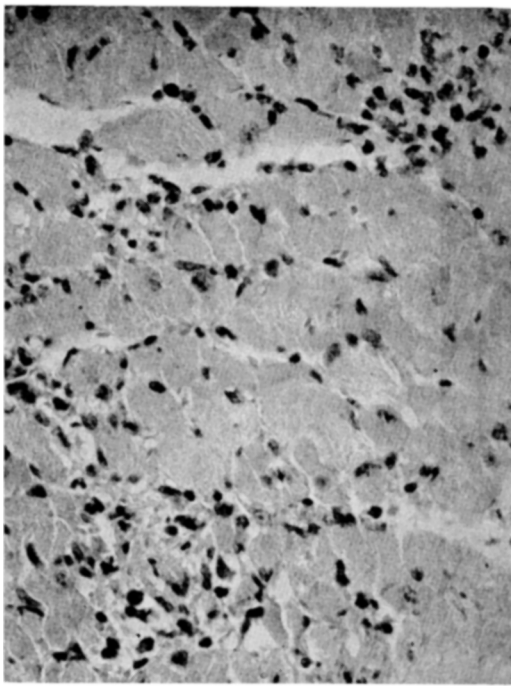

FIG. 1. Myocardium of 1-yr-old mouse 9 days after inoculation with Coxsackie A9 virus, Strain 13, showing foci of lymphoid cells (×400).

Pathologically, the newborn mice showed only myositis; those 1-yr old, only myocarditis, and mice of intermediate ages usually showed both lesions.

Coxsackie B virus infections in mice have been found to vary with the age of the experimental host. Encephalitis, hepatitis, and myocarditis are usually most marked in suckling mice(4), while pancreatitis(5,6) or myocarditis(7) may be the only findings in older mice. These changes in the host cell may involve changes in numbers of specific cellular receptors at the membrane(8,9), changes in the ability of these cells to produce ribonucleic acid or protein of the viruses in question, or both. Similar alterations in the patterns of disease with age have been noted in man. For example, myocarditis and rashes have been noted most frequently in infants, aseptic meningitis in children, and pleurodynia in adults with Coxsackie B5 virus infections (10). The nature of these changes deserves further study, particularly at the molecular level.

Spontaneous cardiac lesions have been noted previously in adult mice(11,12,13); however, statistical analysis of the data presented here attests to the association of the cardiac infiltrates with infection by Coxsackie A9, Strain 13, infections. Myocarditis was not seen and virus was not isolated from control mice.

The NIH-1 and Wiederhold strains of Coxsackie A9 virus which had become well adapted to passage in MKTC did not infect one-year old mice. The differences in capacity of Coxsackie viruses of the same type and strain to infect mice or tissue cultures may reflect genetic differences in the capsomeres necessary for cell-receptor union. These may involve variations in the amino acid sequence of the protein subunits and may be subject to selective forces during passage in mice or in cultures. From the data presented, the capacity of hearts and limbs of mice to replicate Coxsackie A9, Strain 13 probably does not involve alterations in the virus, because the same stock of virus was used to infect all of the mice.

Summary. Coxsackie A9 virus, Strain 13, replicated in the hearts of 1-yr old mice and produced an isolated myocarditis in some of them without concomitant lesions of striated muscle. The differential susceptibility of striated muscle and heart was shown to vary with age; in suckling mice there was only myositis with 10,000 times more virus per gram produced in skeletal muscle than in myocardium, whereas in 1-yr old mice, myocardium contained 10,000 times as much virus as the limb.

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Effect of Highly Purified Vitamin B_{12} Binders from Human Gastric Juice on B_{12} Absorption in Rats. (27928)

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There is considerable evidence to suggest that species specificity exists among the intrinsic factors (IF's) of various animals(1,2, 3). Inasmuch as chemically pure IF has not been available, and contamination of some of the preparations by an inhibitory impurity has been indicated(4,5), species specificity has yet to be established with exclusion of impurities. Such studies would contribute to the understanding of the chemical nature of IF. Although some investigators(3,6,7) failed to demonstrate IF activity in human gastric juice when applied on rat intestine, the possibility still remains that purified human IF might be active in the rat, as suggested by Taylor *et al*(8).

Gräsbeck and his coworkers have recently been successful in purification of IF from human gastric juice to the extent that doses of the order of 50 μg of their preparations are active in pernicious anemia patients(9, 10). They further demonstrated the presence of at least 2 B_{12} binding components in gastric juice, one migrating slowly, the other rapidly in immunoelectrophoresis(11). The slow binder possesses IF activity(10) whereas some preparations of the rapid binder have no IF activity. The purpose of the present investigation was to test in rats these purified fractions of human gastric juice which do not contain impurity to any physiologically significant extent.

Materials and methods. Young adult rats of the Wistar strain were used. Cobalt-60* labeled cyanocobalamin (Co^{60}-B_{12}) with the original specific activity of 1 μc/μg was employed either diluted isotopically 5-fold for fractionation of IF from human gastric juice, or undiluted for absorption in the rat loop.

Purification of human IF. Purification was carried out by a series of column chromatographic procedures on diethylaminoethyl (DEAE) and carboxymethyl cellulose and dextrans (Sephadex®)(9,10). The purified binders were homogeneous in immunoelectrophoresis in which rabbit antigastric juice antiserum was allowed to diffuse against the purified fraction that had been subjected to agar gel electrophoresis. They are named S and R (from "slow" and "rapid" electrophoretic mobility). Both are in the form of their B_{12} complexes, being saturated but not oversaturated with Co^{60}-B_{12}. In the present preparations, the ratio of protein to B_{12} was about 64 μg/μg B_{12} in the former and 210-240 μg/μg B_{12} in the latter. They were prepared and stored frozen in 5 mM phosphate buffer of pH 5.5, diluted with a physiological saline and used in the dose of 30 mμg with respect to the bound Co^{60}-B_{12}, because of its low specific activity. This was the smallest dose possible.

Hog and rat IF. Two hog stomach preparations of WES #727 and #942† which are active at a dose of a few mg in pernicious anemia patients were used. Rat IF concentrate was prepared from rat stomach mucosa

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