ADAPTATION OF GROUP B COXSACKIE VIRUS TO ADULT MOUSE PANCREAS

By GILBERT DALLDORF, M.D., AND REBECCA GIFFORD, D.V.M.

(*From the Division of Laboratories and Research, New York State Department of Health, Albany*)

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The present experiments were undertaken to resolve the reported discrepancies regarding the occurrence of pancreatic necrosis in mice infected with Group B strains of Coxsackie virus.

Pancreatic necrosis following Coxsackie virus infection was first reported by Pappenheimer, Daniels, Cheever, and Weller (1) in suckling mice infected with a strain of virus (Powers) isolated from a patient with atypical poliomyelitis. Necrosis of the acinar tissue was found in approximately half of the pancreases examined histologically.

Later Pappenheimer, Kunz, and Richardson (2) reported that they had produced illness and at times death in *adult* mice injected with two different generations of Conn. 5 virus. The most consistent and prominent lesions occurred in the pancreas, although necrosis of the visceral fat and of the liver was also noted. Successful serial passages in adult mice were made, using the pancreas as the source of virus. Unlike the suckling mice, older animals developed neither brain nor muscle lesions, regardless of the route of inoculation. The authors observed that pancreatitis was found only in mice inoculated when under 5 or over 15 days of age. Godman, Bunting, and Melnick (3) emphasized the selective susceptibility of very young animals to pancreatic necrosis.

These observations differed greatly from our own. Of more than 40 strains of Coxsackie virus studied histologically, representative of 14 serologic types from both groups and including most of the strains studied by Pappenheimer and his colleagues, only one (Powers) had produced pancreatic lesions in mice of any age (4). Of the several hundred mice examined histologically only 4 immature animals, inoculated with suspensions of leg muscle of the Powers virus, developed pancreatitis. Neither this nor any of the other strains tested affected adult mice. We learned that other workers had had similar experiences and undertook to explore the problem experimentally.

Comparative Susceptibility of Two Strains of Mice to Group B Coxsackie Virus.—It seemed possible that the strains of mice used in different laboratories might differ in susceptibility to the Group B Coxsackie viruses. This theory was promptly discarded as a result of the following tests.

Dr. Pappenheimer kindly sent to Albany a group of 7- to 8-week-old mice from his Harvard stock and a sample of the Conn. 5 virus that he had used in many of his tests. Similar mice of the Albany standard strain and a sample of the Albany P.O. virus were shipped to Boston. The animals were fed identical diets. The results in both laboratories were clear cut and similar (5).

Adults of both stocks developed loss of weight, illness, and pancreatic necrosis after inoculation of the Boston Conn. 5 strain, while none was affected by the P.O. virus. In the gross the Albany mice did show more extensive necrosis of the liver and of the visceral fat than the Boston animals and the P.O. virus proved to be more virulent for suckling mice than the Boston strain. Despite these differences, both viruses were completely neutralized by P.O. antiserum. The neutralization tests were made in both young and old mice.

This joint experiment emphasized that strains of virus of the same serologic type can behave differently. The differences were all the more puzzling because a Conn. 5 transfer supplied to our laboratories by Melnick in 1949 consistently failed to produce pancreatic lesions. Indeed there is no known justification for distinguishing between the Conn. 5 and the P.O. strains. Both are representative of a group of isolations made in the northeast during the summer of 1948 from patients with similar illnesses. They are serologically indistinguishable and are both capable of causing identical lesions.

In an effort to simplify the comparisons made in the present experiments, the three agents will be referred to as Boston (Pappenheimer's Conn. 5 strain), New Haven (Melnick's Conn. 5 strain sent to Albany in 1949), and Albany (the P.O. strain). The Boston strain was provided by Melnick in December, 1949, in the form of glycerinated *muscle-bone* (5). The material sent to Albany in January, 1952, by Pappenheimer consisted of a frozen muscle-bone suspension of the third passage of carcass suspensions made in his laboratory in June, 1951. The so called New Haven strain of Conn. 5 was originally sent to Albany by Melnick in May, 1949, in the form of suckling mouse *brains* in glycerol, marked Generation 6. Frequent passages of brain tissue in 3- to 5-day-old mice were subsequently made in Albany. The so called Albany strain since its isolation in 1948 had been propagated through at least 29 generations of brain tissue.

We suspected that the different methods of maintaining the agents might account for their different behavior. The Boston strain had been transferred largely in the form of carcass suspensions of newborn mice. The Albany and New Haven strains had been maintained in our laboratories by transfer of brain tissue, usually in mice from 3 to 5 days of age. This was done because the brain can be harvested with less risk of contamination by bacteria or other viruses. Only one tissue, that of the central nervous system, is involved in this means of transfer. Despite the exclusive use of brain tissue as seed, the muscles from suckling animals infected in this way remain at least as virulent as the brains. We used 3- to 5-day-old mice because they are more likely to survive injection than newborn animals and the signs of disease are more clear cut.

Albany Virus. Passage of Carcass Suspensions.—We first undertook to determine whether the Albany and New Haven strains could be adapted to adult mice by frequent passage of suspensions of the carcasses of infected immature animals.

Twelve passages of carcass suspension were made, beginning with the 29th brain generation of the Albany strain. The skinned, eviscerated, and beheaded carcasses were ground with

sand and broth-salt solution (physiologic salt solution containing 10 per cent infusion broth) in a mortar. Each suspension was tested in two litters of 2- or 3-day-old mice and a group of 5 or more adult animals, 8 to 9 weeks old. The injections were intraperitoneal, 0.05 ml. being used for the immature, and 0.1 ml. for the adult animals.

The results presented in Table I show that the young mice regularly became spastic or died on the 2nd day after inoculation. The old animals remained healthy and developed no lesions of their pancreases.

Five other transfers were made, again starting with the 29th brain generation, in which newborn mice were used whenever possible and the suspensions of

TABLE I

Occurrence of Pancreatic Lesions in Adult Mice Injected with 3 Strains of Group B, Type 1, Coxsackie Virus

Boston			New Haven			Albany		
No. of brain passages	No. of carcass passages	No. having lesions of pancreas	No. of brain passages	No. of carcass passages	No. having lesions of pancreas	No. of brain passages	No. of carcass passages	No. having lesions of pancreas
—	>3	20/20	25	1	1/5	29	1	0/8
				2	0/5		2	0/11
				3	1/5		3	0/10
				4	1/4		4	0/25
				5	1/5		5	0/15
				6	0/5		6	0/5
				7	0/5		7	0/5
							8	0/5
							9	0/5
							10	0/5
							11	0/5
							12	0/5

carcass prepared in a Waring blendor. All the adult mice remained well, and pancreatic lesions were found in none.

New Haven Virus. Passage of Carcass Suspensions.—

Finally seven passages of the New Haven virus were made, using suspensions of infected carcass and beginning with the 25th brain generation. The test mice were from 0 to 4 days and 8 to 9 weeks of age. The tissues were ground in a mortar. Preparation of the suspensions and inoculation of the animals were the same as before. Four of the adult mice, each from a separate generation (Generation 1, 3, 4, and 5), developed isolated small focal lesions of the pancreas. They had not appeared ill and no fat necrosis was observed. It was noteworthy that the lesions did not increase on further passage (Table I). The 0- to 4-day-old mice were markedly susceptible in each instance.

Since suspensions of carcass from the Boston strain were already pathogenic for adult mice on receipt, further passages were not made. Pappenheimer (5) reported that the brains of suckling mice injected with the third passage of carcass suspension had proved to be as virulent for adult mice as those injected

with the second passage. No further attempts to reduce the virulence by frequent brain passages in suckling animals were made.

Tropism for the adult pancreas therefore seemed to have been permanently lost in the case of both the Albany and the New Haven strains that had undergone repeated brain passages. We next undertook to learn whether passage of pancreatic suspensions might induce pancreatotropism.

Pancreatic Passages of the Three Strains.—

Groups of mice of four different ages—3 days, 7 days, 4 weeks, and 8 to 9 weeks—were inoculated intraperitoneally with suspensions of carcass of the Boston (third passage of

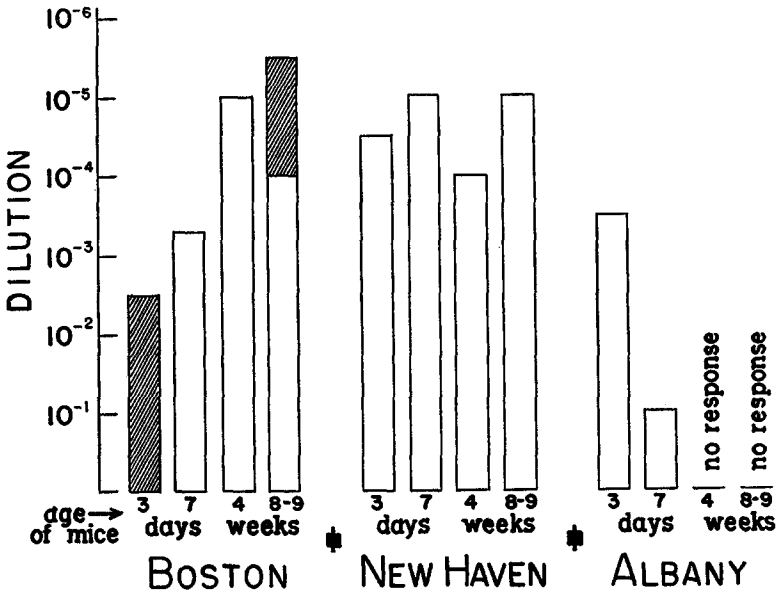

FIG. 1. Relative infectivity of pancreatic suspension from mice of various ages following infection with the three strains of virus. The hollow columns indicate titer as determined by death of the mice; the hatched columns indicate titer determined by signs of disease, none of the mice having died.

carcass suspension) and New Haven (25th passage of brain suspension, 6th passage of carcass suspension) strains of Conn. 5 and with a brain suspension (Generation 35) of the Albany P.O. strain. The inocula were 0.03 ml. of a 10 per cent suspension (1 per cent in the case of the Boston strain) for the two younger age groups and 0.1 ml. for the older animals. Mice from each group were sacrificed at intervals and their weighed pancreases titrated in 2- to 3-day-old mice. The results are shown in Figure 1.

The suspensions of the Albany strain prepared from infant mice were highly infectious, while those from both groups of weaned mice were not infectious at all. The Boston virus, on the contrary, was present in high titer only in the pancreases of the weaned animals. The New Haven virus gave intermediate results. Although the mice had shown no signs of illness, their pancreases

were moderately infectious. These results confirmed our suspicion that the Albany and New Haven strains had lost an affinity for the pancreas of adult mice through repeated brain passage. The question remained whether the Albany virus had originally been pancreatotropic or whether it had lacked that characteristic from the start. The agent was therefore reisolated from the patient's (P.O.) feces which had been stored at −70°C. since their collection in 1948. The fecal suspension was inoculated intraperitoneally in five litters of 0-day-old mice and in 5 adults. All the young animals died or became ill within 3 or 4 days. The adults remained healthy, although the pancreas of one contained a single focus of acinar necrosis. The pancreases, brains, and carcasses of the infant mice, harvested separately, were injected intraperitoneally in newborn and adult animals, to comprise Generation 2. The *pancreatic tissue* was once more markedly pathogenic for young mice. The adults were sick on the 4th day, one died and was eaten on the 5th day. Each of the 4 survivors had gross and microscopic lesions of the pancreas and the body fat indistinguishable from the type originally described by Pappenheimer *et al.* (2). The *brain tissue* was less virulent for newborn mice, and induced no lesions in the mature ones. The *carcass suspension* had an intermediate effect. Although the adult mice looked thrifty through the 10th day, pancreatitis was found in several on histologic study. It appeared that passage of pancreatic tissue of freshly isolated P.O. virus was an effective way of adapting the agent to adult mice. Passages were continued, using homologous tissues to perpetuate pancreas, brain, and carcass lines.

Generation 4 of each tissue line was injected intraperitoneally in 0- and 3-day-old, and adult mice. The pancreas and carcass suspensions were again more virulent than the brain. Adult mice of the pancreas line uniformly developed pancreatic lesions by the 7th day, while those of the carcass line did so less frequently. Mature animals inoculated with the brain line remained normal. Pancreases and brains of the 0-day-old mice in both the pancreas and brain lines were harvested, weighed, and suspended in broth-salt solution. Each of the four suspensions was titrated in newborn and adult mice. The results are shown in Table II.

It is evident that pancreatic passage had modified the Albany virus in such a way that pancreatic suspensions from the pancreas line were much more infectious than pancreatic suspensions from the brain line. This was true for both immature and mature test animals. Brain tissue suspensions from both lines had, on the other hand, a negligible activity in newborn mice and were avirulent for adult animals.

In view of these observations, it seemed necessary to determine whether a comparable series of pancreas passages of the strain of P.O. virus that was in its 35th brain generation could be adapted to adult animals. Five such passages were made in 0-day-old mice, inoculating adults with each generation. The pancreases of the older animals were harvested on the 7th day following inoculation and were examined microscopically. Although the young animals were uniformly susceptible, the adults developed neither gross nor microscopic

changes. These results were similar to the earlier experience in which repeated passage of suspensions of carcass had been made with the same strain, and confirmed the opinion that a tropism for the pancreas was permanently lost.

TABLE II

Titrations of Albany P. O. Strain, Generation 4, in Newborn and Adult Mice

0-day-old mouse tissue suspension	Tissue line	Age of mice	Dilutions of virus				
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Pancreas	Pancreas	0–1 day	8/8	7/7	8/8	8/8	7/7
"	"	Adult	5/5	5/5	5/5	4/5	0/5
"	Brain	0–1 day	7/7	8/8*	3/6*	7/7‡	1/7
"	"	Adult	0/5	0/5	0/5	0/5	0/5
Brain	Pancreas	0–1 day	4/7	1/7	0/5	0/5	0/4
"	"	Adult	0/5	0/5	0/5	0/5	0/5
"	Brain	0–1 day	2/6	1/4	0/7	0/4	0/6
"	"	Adult	0/5	0/5	0/5	0/5	0/5

The results of the titration in the 0–1 day old mice are based upon death or signs of disease; those of the adult mice are dependent upon histologic findings.

* Signs of disease but no deaths.

‡ Died on 1st day.

DISCUSSION

The results may be of practical importance for the classification of the Coxsackie viruses, in which the histologic criteria play a large role. It is, for example, evident that anatomic comparisons should take into account the past history of the strains, and the methods used in their propagation. We previously learned that the incidence of certain lesions varies with the route of inoculation (4). In the present experiments a single route of inoculation, the intraperitoneal, has been used. The selection of tissue for the inocula has instead been the critical factor. We had in earlier experiments resorted to intramuscular injection of muscle suspensions of Group B strains in order to accentuate muscle lesions.

Alteration of virus tropisms by adaptation to other hosts or tissues is of course commonplace. The results reported here simply show that such changes also occur when the Coxsackie viruses are adapted to mice. Actually the adaptation to *adult* mice takes one of two courses depending upon the tissue used for transfer and upon its generation. Brain passage promptly suppresses, it would seem for good, the inherent but limited affinity of a Group B virus for the adult pancreas. Pancreatic inocula intensify the pancreatotropism. It is likely that passage of carcass suspensions preserves and exaggerates the same tropism, for those workers who have reported pancreatic necrosis have

relied on passage of carcass suspensions.[1] If our own experiments are representative, it would seem that pancreatic passage exaggerates this characteristic still further and provides a more intensely pancreatotropic strain than has so far been described.

Coxsackie viruses may undergo other changes during their adaptation to mice. Lépine, Sautter, and Reinié (7) describe hernia formation in suckling mice inoculated intraperitoneally with human fecal suspensions and note that such lesions occurred regularly only in the first passage. The proportion of affected animals was less in the second generation and the lesion did not occur at all in later passages. If the lesion is due to Coxsackie virus, this observation would also imply a regressive change during adaptation.

Some reference should be made to the atypical response of suckling mice infected with the Boston (pancreatotropic) strain of virus. As indicated in Fig. 1, many of the mice became spastic but did not die. This reaction was so characteristic of the strain as to suggest that it had been altered in terms of suckling as well as adult mice.

Finally it may be noted that, despite the great affinity of the adapted strain for the pancreas of adult mice, few of these animals died. The susceptibility of mature animals to Coxsackie virus remains qualified by their limited response to infection, so that the original definition of the Coxsackie family is still valid.

SUMMARY

An alteration of tissue tropism of a Coxsackie virus has been observed following different methods of propagation of the virus in animals.

Tropism for the adult mouse pancreas, as described by Pappenheimer, appeared to be irrevocably lost following prolonged brain-to-brain transfer.

It was present in the same strain on reisolation from human feces, was intensified following pancreas transfers, and suppressed by brain transfers.

Pancreatotropism may be correlated with the titer of virus in the pancreas.

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[1] Dr. R. J. Huebner reports results similar to ours in a letter (6). He succeeded in passing a Group B, Type 3, Coxsackie virus as well as the Conn. 5 and Powers strains in adult mice by means of pancreatic suspensions and also describes the occurrence of acinar necrosis in early generations of suckling mice.