SUSCEPTIBILITY OF GRAVID MICE TO COXSACKIE VIRUS INFECTION

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The natural resistance of mature mice to Coxsackie virus infection can be overcome, in the case of Group B strains, by administering cortisone (1) or by intensifying the native tropism of these strains for the pancreas through laboratory manipulation (2). Resistance also weakens during pregnancy as the present report indicates.

Materials and Methods

Coxsackie viruses A-8 (No. 5010) and B-1 (No. 49683) were selected to represent Groups A and B. Of the second, 2 substrains were used which have been characterized in another report (2) as the *brain line* and the *pancreatic line*. The brain line seems to have completely and irreversibly lost the affinity for the pancreas which the B-1 strain showed upon its isolation. The pancreatic line, on the other hand, has acquired a greater affinity for the pancreas, following passage of pancreatic suspensions. The mice were of the Albany standard strain, of which only the suckling young are highly susceptible to the Coxsackie viruses of both groups. The pancreatic line of B-1, however, multiplies actively in adult mice and rather regularly induces pancreatic necrosis without necessarily causing sickness or death.

A number of experiments were performed with an early pancreatic passage of the B-1 strain. In order to obtain enough material for the large inocula desired, a 10 per cent suspension of both infected pancreatic and carcass tissue from suckling mice was prepared in broth-salt solution (physiologic salt solution containing 10 per cent infusion broth). In all but one experiment, in which the subcutaneous route was used, 0.1 ml. of the suspension was inoculated intraperitoneally. The brain line was represented by a similar preparation of infected suckling mouse brains. A suspension of brain or pancreatic tissue of uninfected mice was injected into the controls. The Group A-8 strain consisted of a 10 per cent suspension of the tissue of infected suckling mouse legs.

Many of the animals were sacrificed and autopsied. As a rule the pancreases of mice infected with the pancreatic line were examined histologically, except when the presence of widespread necrosis of visceral fat provided gross proof

of pancreatic disease and made further examination unnecessary. In other animals that it was important to keep alive, evidence of pancreatitis was obtained by testing 10 per cent fecal suspensions for trypsin by means of a simple procedure involving the use of strips of x-ray film (3).

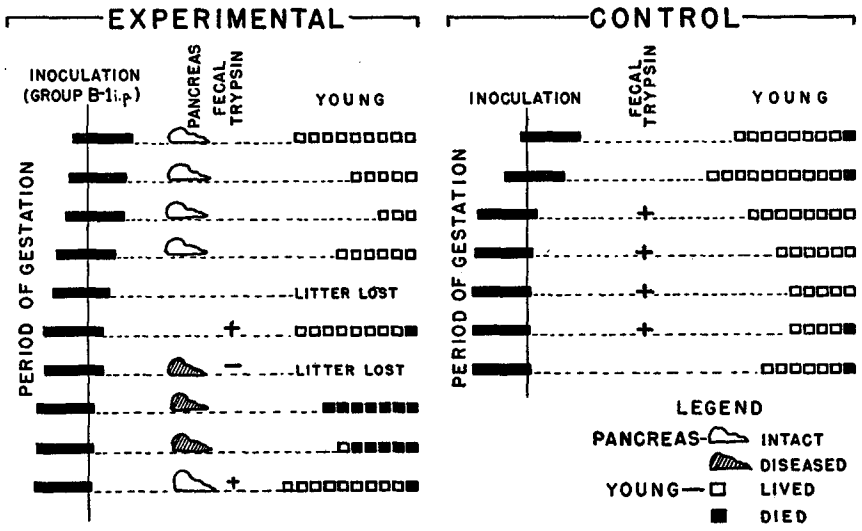

FIG. 1. The outcome of a representative experiment in which gravid mice were infected with a pancreatotropic strain of Group B-1 virus.

TABLE I

Death of the Young of Mothers Infected with Group B-1 Coxsackie Virus

Week of pregnancy when inoculated	No. of litters	Normal litters		Abnormal or lost litters	
		No.	*per cent*	*No.*	*per cent*
1st	15	12	80	3	20
2nd	14	8	57	6	43
3rd	52	12	23	40	77
Controls	33	28	85	5	15

RESULTS

The Consequences of Coxsackie B-1 Virus Infection in Gravid Mice

The results in a representative experiment are shown graphically in Fig. 1. They are typical of the experiments with the *pancreatic line* of B-1, in that signs of disease and loss of litters were more frequent in mice inoculated during the *3rd* week of gestation. Table I summarizes the outcome of several experiments, in which 80 per cent of the mice inoculated in the *1st* week of gestation gave

birth to an average number of healthy viable young. Only 57 per cent of those infected during the *2nd* week had normal litters, while only 23 per cent of the mice inoculated during the *3rd* week of pregnancy produced normal young. In the last group, the other young were either born dead or had disappeared before we had seen them. 85 per cent of the control mice had healthy litters comparable in numbers (6 to 8) to those in our breeding colonies and to the experimental animals infected during the 1st week of their pregnancy.

One of the mice shown in Fig. 1 delivered a healthy viable litter the day after it had been inoculated. The feces of this animal, collected 24 days after injection, contained a normal amount of trypsin, and its pancreas in the gross and histologically was normal on the 24th day. Since this appeared to be inconsistent with the other results, the observation was verified and extended by inoculating an additional 14 females near term. Of the 6 that delivered within 48 hours, all produced living young, all but 1 of the 47 young survived, and the mothers showed but slight and brief signs of disease. Of the 8 that delivered subsequently, 5 of them on the 3rd day and 1 each on the 4th and 5th days, only 1 gave birth to a litter that survived, while but half of the young born to a second animal lived. The remaining 6 litters died or were destroyed at birth by their mothers. 6 of the 8 mothers in this group had markedly rough coats and were in poor condition for at least a week postpartum.

6 mice that had delivered 44 young among them were inoculated with the same material within 24 hours after they had delivered and all were placed in the same cage. Although these mothers showed only slight and transient signs of disease, 27 of their young were missing before the end of the 2 week period of observation. It is possible that over-crowding in the cage was responsible for the losses, or that one mother had cannibalistic tendencies. The living young appeared healthy before they were eaten, and their deaths were distributed over a period of 10 days. 6 other mice, that had delivered normal young 13 days before, were also inoculated. They did not become ill. Their young had been removed and destroyed before the injection was made.

In one experiment, in which the mice were observed for more than 3 weeks after delivery, no deaths occurred among 12 females infected during the 1st week of pregnancy and only 1 among 23 infected during the 2nd week. Of those inoculated during the 3rd week of gestation, 6 of 40 died, a mortality of 15 per cent. Some of the infected animals from the third group were sacrificed shortly after the death of their young, and were found to have severe gross and microscopic lesions of the pancreas.

The *brain line* of B-1 was injected in the same manner into 41 pregnant mice. Only 5 animals lost their litters, in contrast to the high mortality rate among the young of mice infected during the 3rd week with the pancreatic line. Of the 5, 3 had been inoculated during the 3rd week of pregnancy, and 1 each in the 1st and 2nd weeks. Pancreatic necrosis was not found in the 3 infected females

that were examined postmortem. Only one of 14 control mice, injected with normal brain tissue, lost its litter. Most of the young of this series were weighed when 21 days old. The average weight of the experimental groups did not vary by more than 0.5 gm. from that of the controls.

The Group A-8 strain of Coxsackie virus, which is highly virulent for suckling mice, induced no illness or death among 12 gravid animals inoculated near term. The young in the litters were average in number and in subsequent development. On the basis of these results, further experiments of this type with the Group A strains were not performed.

The Cause of Death of Newborn Mice and Their Mothers

Whether the death of the young was primary or followed maternal disease was investigated in several ways. It was plain that poor health in mothers and in their young was commonly associated. Therefore 4 litters born of mothers infected with the pancreatic line during the last week of pregnancy were given healthy, uninoculated mothers. The infected mothers were caged with normal young and suitable controls for each group were maintained. The animals were observed for 7½ weeks and were periodically weighed. At the end of the experiment the average weight of all litters (19 ± 0.4 gm.) did not deviate significantly from that of the controls. Earlier in the experiment, on the 21st day, the experimental groups looked distinctly larger than the controls. The heaviest young (11.3 gm.) were those born to infected mothers and nursed by healthy foster mothers. However, similar mice nursed by their own mothers were only 0.6 gm. lighter. The smallest mice were the controls (9.2 gm.). The irregular sizes at 21 days could be explained by the smaller number of suckling young in the experimental litters, roughly 4 rather than 6. Since all grew to normal size 3 weeks after weaning, it was concluded that the young of mothers infected with the pancreatic line of Coxsackie B-1 are not stigmatized in a way that stunts their development. Clearly all the young of infected mothers grew as well as those of normal mothers. None of them were recognized as abnormal in appearance. 63 born of mothers inoculated during the 1st week of gestation, and 36 born of mothers inoculated during the 2nd week were observed for more than 8 weeks and were examined in particular for congenital defects. No defects were recognized.

14 of the young in the experiment shown in Fig. 1 that survived despite the fact that their mothers were inoculated almost at term with the pancreatic line were tested for the presence of fecal trypsin when they were 3 weeks old. Since the feces of 2 of the 14 failed to liquefy gelatin, it was thought that the young of mice so infected with this strain might have pancreatic disease. Although histologic examination was not made of the 2, the pancreases of 27 healthy or unhealthy young of infected mothers were collected for microscopic study within a few hours or days after birth. Pancreatitis was never found. 14 of the mice did have pancreatic acini remarkable because of the intense

acidophilia of the secretory cells. However, death or increase of cells was never seen in this group of animals. Four fetuses from infected mothers showed the same type of change. Rather similar, if less intense, acidophilia has been found in the pancreas of an occasional newborn mouse born of a healthy, uninoculated mother.

6 mice were sacrificed immediately following birth or were removed at term from the uteri of mothers infected with the pancreatic line. The pancreases and carcasses of these aniamls were ground in a mortar with sand and enough broth-salt solution to make a 10 per cent suspension. The materials were tested for the presence of Group B Coxsackie virus by inoculating each into 2 litters of 0-day-old mice by the intraperitoneal route. In one instance only was virus demonstrated. In this case, although all the 16 animals of the test litters developed signs of Group B Coxsackie virus infection, the starting material had

TABLE II

The Pathogenicity of the Pancreatotropic Line of B-1 Virus for the Young of Mothers Infected with the Brain Line

Week of pregnancy when mother inoculated	Frequency of pancreatic necrosis			
	6 weeks old		10 weeks old	
	Experimental	Controls	Experimental	Controls
1st	8/9	5/5	9/10	Not tested
2nd	8/10	10/10	9/9	" "
3rd	6/9	10/10	9/9	7/7

been a fetus of an infected mother and may have been contaminated with maternal blood during harvest.

Finally, to determine whether or not maternal infection or immunity had been transmitted to the young, groups of mice born of mothers injected with the brain line of Coxsackie B-1 during the 1st, 2nd, or 3rd week of pregnancy were challenged with the pancreatic line 6 and 10 weeks after birth. The challenge dose consisted of 0.1 ml. of a 10 per cent pancreas and carcass suspension injected intraperitoneally. Normal mice of approximately the same age were the controls. It is known that adult mice infected with the brain line are thereafter resistant to subsequent inoculation with the pancreatic strain. The results of challenge can be determined by examining the mice for pancreatic lesions. As will be seen in Table II, the young of infected mothers had only questionable immunity when 6 weeks old and none by the 10th week.

DISCUSSION

Clearly, under the conditions of the tests, adult mice become progressively more susceptible to severe infection with the pancreatic line of Coxsackie B-1

virus as gestation advances. The susceptibility disappears immediately following delivery and mice infected the day before delivery escape the consequences of infection and their young survive. The nature of the susceptibility of gravid mice resembles that following cortisone administration (1) and deserves further study. Large amounts of steroids are excreted during the end of pregnancy but it is not proven that they are of adrenocortical origin (4). The plasma of pregnant women is said to have cortisone activity (5) and the beneficial effect of pregnancy on the course of rheumatoid arthritis has been historically important in the recognition of that hormone (6). Possibly the disproportionately huge size of the fetal adrenal and its involution following birth are responsible for the susceptibility of newborn animals to certain virus infections.

The experiments failed to establish the occurrence of intrauterine infection of the young of diseased mothers or of organic defects in them in consequence of maternal infection. Judging by the evidence we have, the young born alive are capable of the usual rapid growth and maturation, and are free of defects. The single isolation of virus that was obtained from a fetus is inconclusive in its significance since the specimen may well have been contaminated with the mother's blood or body fluids. The finding is outweighed by the failure in 5 other instances to recover virus from the fetuses or newborn young of infected mothers and by the absence of characteristic lesions in them. Since newborn mice are exquisitely susceptible to infections with the Coxsackie viruses and many other agents, the absence of disease at birth seems likely to be due to maternal factors. However, the available criteria are clumsy ones by which to judge such a situation. Perhaps the most significant finding in this relation is the absence of immunity in the young born of infected mothers.

The increasing susceptibility of pregnant mice to a Group B-1 Coxsackie virus is not unlike that reported by Knox (7) who studied Columbia SK virus infection in gravid mice and the well known influence of advanced gestation on the susceptibility of women to poliomyelitis (8, 9). Both murine encephalomyocarditis and human poliomyelitis have other features in common with Coxsackie virus infection. On the other hand our results are quite different from those reported by Berger and Roulet (10) who noted encephalitis in gravid mice inoculated with large doses of either an A-1 or a B-1 strain of Coxsackie virus. They also observed muscle lesions and the death of the young.

The experiments incidentally verified the finding that the pancreatic tropism of the B-1 Coxsackie virus under study has been lost, apparently for good, in the substrain maintained by transfer of brain suspension, the so called *brain line*. Kilbourne found the same substrain of virus incapable of inducing pancreatitis when cortisone was given (11).

Finally, the experiments reveal another respect in which Group B and Group A strains of Coxsackie virus differ, namely, in their pathogenicity for gravid mice.

SUMMARY

Gravid mice become progressively more susceptible to infection with the pancreatic line of Group B-1 Coxsackie virus during the last week of pregnancy. A Group A-8 strain did not have such an effect.

The young that survive despite the fact that their mothers are infected with a B-1 strain appear to be normal in the gross and microscopically, to grow at the usual rate, to be free of demonstrable virus, and to be susceptible on challenge with a homologous strain.

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