FACTORS INFLUENCING HOST-VIRUS INTERACTIONS

I. A COMPARISON OF VIRAL MULTIPLICATION AND HISTOPATHOLOGY IN INFANT, ADULT, AND CORTISONE-TREATED ADULT MICE INFECTED WITH THE CONN.-5 STRAIN OF COXSVACKIE VIRUS*

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The influence of host age on the outcome of viral infections has been studied by a number of investigators (1-5) and it is now recognized that the period of life at which infection occurs often has a marked effect on the progress of the disease. The details of this subject have been reviewed recently by Sigel (6).

There has been much documentation of differences in severity of disease, alteration of the disease pattern, development of overt or latent infection, and duration of the carrier state in animals of various ages. However, few attempts have been made to compare pathogenesis of disease at periods of susceptibility and refractoriness in order to gain insight into fundamental mechanisms involved. It is often assumed that increased resistance developing with maturation is related to inability of the virus to multiply at the site of inoculation, or failure in dissemination caused by regional barriers so that the virus does not reach areas favorable for multiplication or ones in which damage may be lethal. This concept has been based largely upon the extensive and detailed investigations of vesicular stomatitis and equine encephalomyelitis infections by Sabin and Olitsky (2-4) who found that with maturation barriers to CNS invasion appeared at the peritoneum, the myoneural junction, the retina, and the anterior rhinencephalon.

Coxsackie virus infection in mice is a host-virus system in which there are striking changes in resistance from marked susceptibility to relative refractoriness within a

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§ The opinions expressed in this article are solely those of the authors and do not necessarily reflect the viewpoint of the Navy Department.

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period of 1 to 3 weeks after birth (7). That this is not total refractoriness was demonstrated by Pappenheimer et al., who showed (8) that although adult mice seldom die of infection, they regularly develop pancreatitis following inoculation with certain strains, including the Conn.-5 strain. Other lesions observed in the muscle, central nervous system, heart, lungs, and peripheral fat lobules of infant mice were not seen in adults, however. The differences in Coxsackie virus infection in infant and adult mice are the more interesting in view of the demonstration by Kilbourne and Horsfall (9) that infection by the Conn.-5 strain of Coxsackie virus in adult mice can be converted from a non-fatal asymptomatic one to a quite uniformly lethal infection by a single injection of cortisone given at the time of inoculation. They also showed that sufficient virus accumulated in the brains of cortisone-treated and infected animals to indicate that multiplication occurred.

The present study was undertaken to obtain information as to how infection with Coxsackie virus in adult mice differs in pathogenesis from the infection in infants and at what point in the progress of the disease cortisone exerts its influence to change the course in adult mice. It will be shown that virus dissemination is prompt and widespread in all groups, but whereas in infant mice multiplication and cellular injury occur in many organs and tissues, in untreated adults it is largely limited to the pancreas. Treatment with cortisone results in an infection with multiplication and damage in several sites. However, the pattern of the disease areas is not identical to that in 4 to 5 day old infants.

Materials and Methods

Mice.—Albino mice from an inbred colony originally derived from the Webster strain were used throughout this study. They were fed ad libitum a diet of Rockland mouse pellets. The animals referred to as adult mice were 4 weeks of age and 14 to 15 gm. in weight.

Virus.—The Conn.-5 strain, a Group B strain of Coxsackie virus, was originally obtained by Dr. A. F. Rasmussen, Jr., from Dr. J. L. Melnick of Yale University and has been maintained by mouse carcass passage and supplied to us by Dr. Lois Kitze.

A stock 10 per cent bone-muscle-brain suspension of virus was made from infant mice inoculated at 24 to 48 hours of age and killed by etherization 48 hours later. The carcasses were skinned, eviscerated, feet and tail removed, ground in a mortar, and suspended in sterile phosphate buffered saline (pH 7.2) containing 500 units of penicillin and 50 μg. of streptomycin per ml. Large particles were removed by light centrifugation and the supernatant was stored at −40°C. This suspension was free of bacterial contaminants culturable on the usual media. The LD₅₀ doses of virus indicated in the experiments are referable to mice 24 to 48 hours of age.

For control material, a 10 per cent normal mouse tissue suspension (N.M.T.S.)₁ was prepared, as above, from normal infant mice. This was used in a volume and dilution equal to that of the virus suspension.

Injections of virus, virus-serum mixtures, and control suspensions were made intraperitoneally in 0.05 ml. volume.

Cortisone.—Cortone acetate (cortisone acetate, Merck and Co., Inc.) in a saline suspension

₁ N.M.T.S., normal mouse tissue suspension.
containing 25 mg. per ml., plus the suspending agents and 0.9 per cent benzyl alcohol incorporated in the commercial product, was given subcutaneously 2 to 3 hours before inoculations of virus or N.M.T.S. Control injections of 0.9 per cent benzyl alcohol in saline were given in the same manner and volume as those for cortisone.

**Preparation and Titration of Tissues.**—At the time of sacrifice mice were etherized and bled out by partial decapitation. Blood and carcasses were stored at -40°C until such time as titrations could be carried out. The mice were not perfused.

Tissues were removed as aseptically as possible and separate instruments used for each organ to avoid cross-contamination. Blood, brain, liver, pancreas, and muscle were collected for virus titrations. Organs from all of the animals sacrificed within a group were put into appropriate tissue pools. One hind limb from each mouse of a group was taken for the suspension of skeletal muscle. Clotted blood and pooled organs were weighed and ground in a mortar with sterile saline (containing 1000 units of penicillin and 1.0 mg. of streptomycin per ml.) to make $10^{-2}$ suspensions for pancreas and $10^{-1}$ suspensions for all other organs. Suspensions were clarified by centrifugation at 2000 g for 1/2 hour and the supernatant fluid used for the virus titrations.

For titrations serial decimal dilutions were made in saline buffered with 0.01 M phosphate at pH 7.2 and containing 500 units of penicillin and 500 µg. of streptomycin per ml. An average of 14 mice (two litters), 24 to 48 hours of age, were inoculated with each dilution. Mice were observed for 10 days and deaths occurring in the first 48 hours were considered non-specific. Titration end-points (LD$_{50}$) were calculated by the method of Reed and Muench (10).

**Preparation of Tissue for Histologic Study.**—Collected for histologic study were brain, lung, pancreas, skeletal muscle, interscapular fat, mesenteric fat, heart, liver, kidney, adrenal, diaphragm, tongue, urinary bladder, spleen, and small intestine. These tissues were removed from adult mice at the time of autopsy and all except skeletal muscle were fixed in 10 per cent neutral formalin. The specimens of skeletal muscle, which consisted of both intact hind limbs, were decalcified in Zenker's acetate solution and then placed in formalin. Infant mice were fixed whole after opening the cranium, thorax, and abdomen, and individual tissues were dissected out after a period of fixation. Sections were cut at 10 µ and stained with hematoxylin-eosin.

**Neutralization Tests.**—Specific antiserum against the Conn.-5 strain was prepared in adult hamsters. Each animal was given approximately $10^6$ infant mouse LD$_{50}$ doses of fully active virus by intraperitoneal injection each week for 3 weeks. The hamsters were bled prior to and 1 week after immunization.

Tenfold dilutions of virus were mixed with equal volumes of a constant dilution of inactivated serum. Mixtures were incubated at room temperature for 1 hour and then injected into mice 24 to 48 hours of age using eight mice per dilution. Mice were observed for 10 days and deaths occurring in the first 2 days were not considered as caused by the virus.

**EXPERIMENTAL**

Preliminary experiments confirmed the finding of Kilbourne and Horsfall (9) that a single injection of 2.5 mg. of cortisone given to adult mice caused a marked increase in mortality following infection with the Conn.-5 strain of Coxsackie virus. Normal mice, of the strain used in this study, after 8 days of age survived inoculation with as many as $10^9$ infant LD$_{50}$ doses of virus, whereas, following injection with 2.5 mg. of cortisone, infecting doses as small as 500 infant LD$_{50}$ resulted in death in approximately 50 per cent of adult mice, and larger viral inocula regularly resulted in a mortality of 70 to 80 per cent.
The plan of distribution and treatment of 237 infant mice and 240 adult mice used in two experiments is summarized in Table I. After inoculation, at intervals of 1 day for 7 days, mice were killed for collection of tissues for virus titration and histologic study. Infant mice, 4 to 5 days old, were selected for study because their increased survival time, as compared with newborn mice, allowed a better study of the development of lesions as well as virus levels. The susceptibility of 4 to 5 day old mice was such that by the 8th day after inoculation all were dead or showed marked signs of disease. Adult mice were given a dose of virus ten times that given infant mice in order that the dose per gram of body weight would be approximately equal to that of the infants. Even though extensive studies by many investigators have not indicated that the aqueous vehicle of cortisone has any effect upon the ultimate outcome of experimental infections in mice, several control groups were given virus or normal tissue suspension, cortisone, or benzyl alcohol in order to permit differentiation of effects of the several components upon tissue histology. The data on virus levels and histology included in this report are from mice without overt signs of disease at the time of sacrifice.

**Distribution of Virus in Tissues**

The titers of virus found in tissues of infant, adult, and cortisone-treated adult mice are presented graphically in Text-fig. 1.

**Blood.**—24 hours after inoculation virus was present in the blood in all groups: at a titer of about $10^{-4.3}$ in infants and in cortisone-treated adults,
and $10^{-1.3}$ in untreated adults. At 48 and 72 hours virus levels were quite similar in all groups. After the 3rd day virus disappeared abruptly and was not detectable subsequently in the blood of any of the three groups.

**Brain.**—Measurable virus was not recovered from the brain in any group until the 2nd day, at which time, in infant mice the titer was $10^{-2.75}$ and had declined only slightly by the 7th day. Virus was found at very low levels in the brains of untreated adult mice, the maximum being $10^{-1.42}$ on day 4, and no virus was detectable in the brains of this group after the 4th day. In cortisone-treated adults the rise in virus to peak levels was slower than in infants but the maximum titers reached were comparable. High levels were not as sustained as in infants, however, and virus was undetectable on the 7th day.

**Pancreas.**—24 hours after inoculation virus was found in all groups at titers
of $10^{-5.4}$ to $10^{-4.1}$, and it reached high levels in all three groups. The highest titer ($10^{-4.1}$) was found on the 2nd day in untreated adults, but in this group the virus fell to low levels most rapidly, little being found after the 4th day. The slowest rise in virus levels was in the infant mice, but otherwise the levels in this group were quite similar to those found in cortisone-treated adults.

**Muscle.**—24 hours after infection virus had already reached levels of $10^{-4.3}$ and $10^{-4.5}$ in the cortisone-treated adults and infant mice while it was not yet detectable in muscle from untreated adults. In the infant group the titers reached a maximum of $10^{-5.6}$ on the 2nd day and remained at a sustained high level through the 7th day. In both the untreated and the cortisone-treated adult mice maximum titers were found on the 2nd day ($10^{-3.3}$ and $10^{-4.4}$) and at that time the levels in the two groups were not significantly different. However, virus levels in the muscle of untreated adults fell precipitously after the 2nd day and none was measurable on the 4th day. In the cortisone-treated animals the decline was 1 day slower.

**Liver.**—The highest titer in infant mice was found at 24 hours ($10^{-4.4}$), after which the levels gradually declined and disappeared by the 5th day. In untreated adult mice the level was only $10^{-4.3}$ at 24 hours, reached $10^{-4.1}$ on the 2nd day, and then dropped to become undetectable by the 4th day. In this tissue the titers in cortisone-treated adults markedly exceeded those of other groups both for levels of virus and length of time during which virus was measurable. The maximum titer ($10^{-6.4}$) was found on the 2nd day but virus was found at appreciable levels through the 6th day.

**Identification of the Agent Recovered from Mouse Tissues**

Although cortisone has been known to activate latent infections in laboratory animals (11, 12), in this study control groups of mice given various combinations of N.M.T.S., cortisone, and benzyl alcohol, did not develop obvious symptoms or histological signs of disease, and suspensions of their tissues proved innocuous on subinoculation into 24 hour old mice. Blood and organs of cortisone-treated mice were essentially free of bacteria on aerobic culture and in no instance did lesions in any group contain visible bacteria. As further indication that the agent, lethal for suckling mice, recovered from tissues of the test groups (Text-fig. 1) was the Conn.-5 strain of Coxsackie virus inoculated, neutralization tests were carried out with specific antiserum.

Twelve high titer tissue suspensions were selected; several from each type of tissue collected from infant, untreated adult, and cortisone-treated animals. The addition of specific anti-Conn.-5 hamster serum to $10^{-2}$ tissue suspensions (from 50 to 10,000 infant LD$_{50}$, depending on the tissue) resulted, without exception, in complete neutralization of the lethal effects of the suspension on subinoculation into 24 hour old mice while normal hamster serum was without protective effect. This was interpreted as indicating that the test system employed measured virus persisting from the original Conn.-5 inoculum or arising from multiplication of the virus injected.
Distribution and Characteristics of Microscopic Lesions

The evolution and characteristics of lesions of infant mice infected with the Conn.-5 strain have been thoroughly described (13-15), and Pappenheimer (8) has described the lesions found in adult mice. Lesions found in the infant and untreated adult mice in this study were similar in their histologic aspects to the previous descriptions. This presentation, therefore, will deal primarily with the lesions occurring in cortisone-treated adult mice and with comparisons of the appearance, frequency, and distribution of lesions in the different groups of animals.

The relative frequency and magnitude of lesions found in certain tissues of infant, untreated adult, and cortisone-treated adults from one experiment are presented in Table II. It should be emphasized that the data recorded

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<th>Day after inoculation</th>
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* Numbers express degree of severity and extent of lesions in tissue from a single mouse, 4 being most severe.
† Tissue absent from section.
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here were taken from a single section of the tissues of each mouse. It is possible, therefore, that in some of the tissues recorded as normal additional sections would have revealed lesions, or that in those tissues in which lesions were noted, the estimation of extent of changes would have been modified by other sections. However, sections were made to give the maximum area for study, and the single section of each tissue was believed to give a reasonable index of the extent of changes in a particular organ. This belief was supported by a second study in which tissues, as listed in Table II, were again collected and examined. Essentially the same degree of pathological change was found in the various groups.

Brain.—No lesions were found in the brain in any of the three groups of mice.
Lungs.—Beginning on the 3rd day in the infant mice there was a moderate to severe interstitial pneumonitis, occasionally so marked that bronchioles were filled with inflammatory cells. Many of the sections showed emphysema as previously described by others (13). In untreated adult mice also, a moderate interstitial pneumonitis frequently was found. This was less severe than that seen in infants and it was most marked in the first 4 or 5 days and then subsided. Some areas of emphysema occasionally were found. In cortisone-treated adults the situation was reversed; interstitial pneumonitis was absent or mild until about the 5th day, after which it was moderate in degree but present in almost all lungs examined. Suppression of tissue inflammatory reaction by the cortisone may have been operative during the first four days in this group of mice.

Heart.—All sections from infant mice were normal with the exception that one mouse killed on the 4th day had areas of necrosis in the myocardium. Of the untreated adult mice, none included in the tabulation in Table II was found to have myocardial lesions but one mouse from a second experiment, sacrificed on the 6th day, had small areas of necrosis in both ventricular and atrial myocardium. In the cortisone-treated adults the myocardial lesions were extensive areas of necrosis, moderate inflammatory reaction, and marked blue staining, probably due to deposition of calcium salts, which we will term mineralization, in most of the necrotic areas (Fig. 1). Many lesions appeared to have originated directly around blood vessels, but in others the association of the lesion with a vessel was not apparent. The lesions closely resembled those seen in infant mice.

Pancreas.—There was no microscopic evidence of pancreatic disease in infant mice. In untreated adult mice and in those treated with cortisone severe lesions were regularly found after the 1st day. The majority of acini were destroyed in both groups. In the untreated animals destruction of acinar tissue was accompanied by a severe inter- and intralobular inflammatory reaction with mononuclear cells predominating, but in the cortisone-treated mice this inflammatory reaction was much reduced. The islets and ducts remained intact in both groups.

Fat.—In infants there were marked necrosis, inflammation, and mineralization by the 4th day and all sections examined thereafter showed this characteristic lesion. A low grade inflammatory reaction and some necrosis and mineralization were seen in most untreated adults. Necrosis was somewhat more severe in cortisone-treated adults, but in neither of the adult groups did the alterations equal in severity or extent those seen in infant mice.

Skeletal Muscle.—Beginning on the 3rd day most sections from infant mice showed, in moderate to marked degree, the characteristic destruction of myofibrils (14). Of the untreated adults only one mouse had lesions in muscle, but these closely resembled those observed in infants. Muscle lesions were found in several mice from the cortisone-treated group but were mild and of limited extent in comparison to the infant group.
Liver.—All sections from infant mice were normal except for two. In one of these there were diffuse areas of vacuolated cells and in the other small, scattered areas of necrosis of hepatic cells. In about 25 per cent of untreated adult mice there were small foci of inflammatory cells at the periphery of lobules and occasionally some were found around the central vein. There was no necrosis of hepatic cells (Fig. 2). Histologic changes in the cortisone-treated, infected adult mice were extensive and seemed to be due to two factors: the effects of cortisone on liver cells, and the effects of some destructive agent, presumably virus. With the aid of tissue sections from the control groups of mice these two processes could be clearly differentiated. In control animals injected with cortisone and normal mouse tissue, within 24 hours after cortisone administration the hepatic cells become vacuolated to the extent that the cytoplasm seemed almost completely removed and the nucleus appeared suspended in a clear zone surrounded by the cell membrane (Fig. 3). This change has been described previously by others (16). A pink-staining material was noted in some of the sinusoids. After the 1st day the vacuolation progressively decreased and by the 3rd day after cortisone administration had almost totally disappeared leaving the cells appearing somewhat swollen but normal in their staining reactions (Fig. 4). In those mice infected with Conn.-5 virus following cortisone treatment, in addition to these changes, small foci of round cells appeared at the periphery of lobules and around some central veins by the 2nd day. These foci enlarged and the hepatic cells surrounding them developed a foamy appearance in the cytoplasm with dark, intensely staining nuclei, and frank necrosis of hepatic cells appeared by the 4th day. In some areas eosinophilic bodies were seen. These were similar to those described by Pappenheimer et al. (13), as occurring in the livers of 1 to 2 day old mice infected with the Conn.-5 strain. Mononuclear cells accumulated in the necrotic areas and mineralization occurred in some. These lesions, usually situated at the periphery of lobules, were extensive by the 3rd day after infection (Fig. 5).

Other Tissues.—No significant morphological changes were found in intestine, diaphragm, tongue, bladder, kidney, or adrenal of any of the three groups. Spleens were normal in the infant and untreated adults, but all animals receiving cortisone, infected or controls, had spleens much reduced in size and on microscopic examination they were found to have a marked reduction in lymphocytic elements and dissolution of the Malpighian follicles. No lesions were found in spleens which could be attributed to viral infection.

Microscopic Appearance of Tissues from Control Groups.—Adult mice given a preliminary injection of benzyl alcohol instead of cortisone and infected with Conn.-5 virus had lesions comparable in distribution, frequency, and severity to those found in untreated, infected adult mice. One mouse of this group, sacrificed on the 7th day after infection, was found to have two very small areas of inflammation and necrosis in the myocardium similar in appearance to those found in infant and cortisone-treated mice but small and limited in area.

No morphologic changes of significance were noted in sections from infant controls. Tissues from adult mice given benzyl alcohol and N.M.T.S. were normal in appearance. The changes in the livers of cortisone-treated controls are discussed above. Other tissues from these animals were normal with the exception of a single mouse, which, on the 6th day after treatment, was found to have two areas in the left ventricular wall that appeared necrotic and contained collections of mononuclear cells. In the sections of other tissues from this mouse, no evidence of disease was found.

RéSUMÉ AND DISCUSSION

The findings make it evident that there was no serious local barrier to the Conn.-5 strain of Coxsackie virus in the peritoneum or blood vessels of adult mice. The rise in virus level in the circulating blood was slightly delayed in
untreated adults, but after the 1st day virus titers were very similar in all of the three groups studied. The experimental plan employed in this study does not provide information regarding the extent of local multiplication at the site of injection nor does it allow analysis of the exact sequence by which the virus spread in the very early period following inoculation. Repeated sampling during the first 24 hours would have been required to follow the order of dissemination. It is sufficiently clear, however, that early and widespread distribution of the virus occurred, that there was a viremia of several days duration in adult mice as well as in infants, and that tissues of all groups were exposed to similar concentrations of virus.

It is in the consequences of seeding of organs and tissues with virus that the differences in the test groups appear. In infants viral multiplication and tissue damage were widespread and extensive. Although, in this study, the liver, pancreas, and brain of infant mice were spared cellular damage in spite of virus levels indicative of active multiplication, the liver and pancreas were sites of severe injury by the Conn.-5 strain in mice 1 to 2 days of age, and brain lesions appeared in 4 to 5 day old mice if the infection was allowed to progress for 8 to 10 days (13, 14). With maturation, changes occurred in the mouse which resulted in little or no multiplication or cellular injury in tissues other than the pancreas, even though all tissues were exposed to virus. Low titers of virus found in other organs coincided in time and level with those in the blood, and it thus appears that they may have been due largely to contained blood and virus mechanically filtered from the blood rather than to multiplication in the tissue. In adult mice treated with cortisone the infection again became generalized with major injury in the liver and heart in addition to the pancreas and with some indication of increased multiplication in muscle and brain.

In view of the demonstration that virus is widely distributed early in the infection in all three groups, two major possibilities may be considered to explain the subsequent course of the infection in each of the groups.

1. Multiplication and cellular damage in untreated adult mice may be inhibited and controlled by rapidly mobilized defense mechanisms, such as specific antibody production, which are immature and inefficient in infant mice and depressed in cortisone-treated adults. It has been demonstrated that the specific antibody response to antigenic stimulation is slower in rate and smaller in quantity in very young animals than in adults (5, 17, 18), and Morgan has presented correlative evidence (1) suggesting that the increasing resistance to peripheral inoculation of Eastern equine encephalomyelitis virus found with advancing age in mice may be related to the improving capacity to develop specific antibody rapidly. Overman and Kilham have presented particularly strong evidence (5, 19) to indicate that the capacity to produce antibodies before the end of the incubation period is a
factor of primary importance in the resistance of adult hamsters and mice to infection with mumps virus and, furthermore, have shown that following treatment of adult hamsters with cortisone, there was a delay in the appearance of antibody in the serum and an increase in virus in the brain to levels comparable to those found in infants (5). Other investigators have likewise shown that antibody production may be partially suppressed by cortisone but demonstration of significant effect has required large and continued doses of cortisone, and, with antigens such as viruses, inhibition has usually been irregular and of low degree (20-23).

It is to be expected that the host-immune response will be most effective in suppressing those infections in which there is a relatively long interval after virus is introduced before an area of particularly vulnerable cells is reached, or infections by agents relatively slow in their multiplication and spread within vulnerable tissue. In the mumps infections studied by Overman and Kilham the incubation period was 9 days in hamsters and 11 days in mice, even after direct intracerebral inoculation into infants, and 7 days were required before maximal virus titers were obtained (5, 19). In the present experiments with Coxsackie virus, peak titers were reached within 48 hours and lesions appeared in some tissues as early as the 1st day after infection. The fact that virus circulated in the blood at significant levels in all groups until it rather precipitously disappeared between the 3rd and 4th days indicates that antibody did not appear in quantity, in the blood at least, until after the 3rd day. Thus it would appear that with this virus and under the conditions of these experiments, multiplication had begun in most susceptible cells within the first 24 to 48 hours and that the essential events leading to the differences in virus titers and histopathology found in the three test groups were determined before antibody production could exert an important influence. It also seems unlikely that a single island of apparently unlimited viral multiplication and tissue destruction could persist in the pancreas of untreated adult mice if the inactivity of the virus in other tissues were due solely to suppression by immune mechanisms.

2. The observed differences in areas of viral multiplication and tissue damage in mice of different ages may be related to ontogenetic changes in host tissues and cells. While not specifically definable at present, these could be such as to affect access of virus to parenchymal cells, entry into cells, or replication within the cells. The specificity of some viruses for certain tissues is a well known phenomenon although no other virus has been recognized with the marked selectivity for pancreas that is exhibited by this virus in adult mice.

Rowe has presented evidence (24) that Group A Coxsackie viruses will proliferate in adult mouse muscle provided denervation of the muscle is carried out at least 7
days prior to inoculation. Denervation has been reported to result in decrease in phosphorlase and phosphoglucomutase activity in muscle to levels similar to those found in embryonic and infant muscle (25), and the capacity of the denervated adult muscle to support viral multiplication may be related to these changes in metabolic processes although such a relationship has not been directly demonstrated. Cortisone is known to produce marked alterations in metabolism in many tissues (26) and one or more of these changes may result in conditions advantageous to viral replication. That cortisone can alter cells in a way favorable to the infecting virus in a system free of the complicating factors of immune reactions has been shown in experiments with influenza virus in surviving cell cultures (27).

It is of interest that the tissue in which viral multiplication was most active following cortisone treatment was the liver. After cortisone treatment of mice, intravenously injected influenza virus undergoes some degree of multiplication in the liver, but there is no indication of an effect on multiplication in other non-pulmonary tissues (28). Findlay and Howard have also observed a particularly striking increase in the susceptibility of mouse liver to the damaging effects of Rift Valley fever virus following cortisone treatment (29). Evidence in the literature indicates that whereas cortisone has a catabolic effect on protein metabolism in most tissues, in the rat the effect in the liver is anabolic and results in increased protein synthesis (30). While this difference in protein metabolism in various tissues may conceivably be of some significance in the effect of cortisone on the multiplication of certain viruses, it is evident that the influence of cortisone on viral infection is not limited solely to activity in the liver (29, 31, 32). In view of the multiple metabolic effects of cortisone and the uncertainty that exists regarding mechanisms of viral multiplication, further speculation as to the direction in which cortisone might alter tissue metabolism to result in increased virus production seems unwarranted.

After these data were collected it came to our attention that Kilbourne et al. have published, in abstract form (33), information indicating that they have found detectable virus in the blood, liver, spleen, lung, brain, and pancreas of cortisone-treated adult mice infected with another Group B Coxsackie virus, and particular attention was drawn to areas of myocardial necrosis noted in their animals. These workers found microscopic myocardial lesions on first passage in cortisone-treated mice and indications that myocarditis increased in extent with continued passage in cortisone-treated animals. In their uninfected, cortisone-treated control animals, as in ours, small focal areas of myocardial necrosis were occasionally found even though no virus was detectable. It would thus appear that some Group B Coxsackie strains other than Conn.-5 may behave similarly in cortisone-treated adult mice and that large doses of cortisone have effects which result in mild microscopic myocardial lesions in the absence of demonstrable infection. The small myocardial lesions found in two adult mice from infected control groups not given cortisone seem to emphasize the contention of Aronson and Schwartzman (32) that cortisone is effective only in expanding and intensifying viral multiplication and damage in tissues already inherently capable of being infected.
SUMMARY

A study was made of the pathogenesis of infection due to the Conn.-5 strain of Coxsackie virus in 4 to 5 day old infant mice, untreated adult mice, and adult mice treated with cortisone. The quantitative distribution of virus and the evolution of lesions in different tissues were followed for the first 7 days of the infection. Virus dissemination was prompt and widespread via the blood in all groups. In 4 to 5 day old infant mice viral multiplication and cellular injury occurred in many organs and tissues, while in untreated adult mice these processes were largely limited to the pancreas, even though infecting virus appeared to be equally available to other tissues from the blood. Treatment of adult mice with a single injection of 2.5 mg. cortisone resulted in viral multiplication and tissue damage in several sites in addition to the pancreas, the most marked occurring in the liver and heart.

In a consideration of possible mechanisms involved, it was thought unlikely that the differences in the course of the disease in the three groups could be attributed solely to differences in the specific immune response. It is suggested that developmental changes in cells and tissues, perhaps related to cellular metabolism and alterable by cortisone administration, are the major factors determining the location and extent of viral multiplication and tissue injury in this infection in mice.

BIBLIOGRAPHY

FACTORS INFLUENCING COXSACKIE INFECTION


EXPLANATION OF PLATES

PLATE 83

Fig. 1. A section of the ventricular myocardium from a cortisone-treated mouse on the 6th day after infection with the Conn.-5 strain of Coxsackie virus. Extensive destruction and mineralization are evident. × 75.

Fig. 2. A section of liver from an untreated, adult mouse 5 days after infection with the Conn.-5 strain. There is little evidence of damage by the virus. × 300.

Fig. 3. Liver from an uninfected, adult control mouse 24 hours after receiving 2.5 mg. of cortisone subcutaneously. The characteristic foamy and vacuolar appearance seen in the first 24 to 48 hours after cortisone administration are very apparent here. × 300.
(Boring et al.: Factors influencing Coxsackie infection)
FIG. 4. Liver from an uninfected, adult control mouse 72 hours after cortisone treatment. Note that the vacuolar appearance, so prominent in Fig. 3, has largely disappeared by 72 hours. × 300.

FIG. 5.—A section of liver from a cortisone-treated, adult mouse on the 5th day after infection with the Conn.-5 strain of Coxsackie virus. Extensive disorganization, destruction, vacuolization, and round cell infiltration are evident. × 300.
(Boring et al.: Factors influencing Coxsackie infection)