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Effect of Gonadectomy on Susceptibility of the Adult Mouse to Coxsackie B1 Virus Infection.* (30273)

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Susceptibility to viral infection may vary with age. For example, Coxsackie B virus disease in the suckling mouse is frequently lethal with widespread lesions in muscle, heart, brain and other tissues(1) as compared to the asymptomatic infection in the adult with lesions localized to the pancreas(2). This acquired resistance of the adult mouse may depend on hormonal changes that occur with sexual maturation. Supportive evidence for such an endocrine effect on resistance to virus infection has been described for the adult male hamster(3) and for the adult female monkey(4). Gonadectomy substantially enhanced the susceptibility of these animals to poliovirus. If the insusceptibility of adult mice to the Coxsackie B viruses is dependent upon similar endocrine influences, castration should depress their resistance to these agents. To test this hypothesis, we have studied the effects of gonadectomy on the resistance of adult mice to Coxsackie B1 virus.

The Coxsackie B1 virus used in our experiments was particularly virulent for the adult mouse. More than 14 per cent of virus-inoculated normal animals died within one week. Following orchiectomy or ovariectomy the death rate in similar groups of infected mice decreased to approximately one-third rather than increasing as might be expected. Thus susceptibility was depressed rather than enhanced by gonadectomy. This protective effect of castration was no longer present in animals infected 30 days post-surgery. The influence of castration on the acquired resistance of the adult mouse to infection with Coxsackie B1 virus is described here.

Study plan. During each castration experiment, no more than 5 mice of either sex were caged together. Room temperature was maintained at approximately 24°C, but no attempt was made to control the daily hours of darkness or light. *Experiment I*: Cages containing male or female "Swiss albino" animals were assigned without known bias to one of 2 test groups: (1) castrates (102 males, 97 females), and (2) sham-operated controls (102 males and 102 females). Ten days following surgery, both groups of mice were inoculated with the test virus. Body weights were determined frequently throughout the 30-day observation period that followed. *Experiment II*: Two test groups similar to those described above were studied: (1) castrates (60 males, 62 females), and (2) sham-

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operated controls (58 males, 60 females). However, in this experiment the virus inoculum was withheld until the thirtieth day following surgery. *Experiment III:* Three groups of male animals were used for this study: (1) 24 castrates, (2) 26 castrates, and (3) 25 sham-operated controls. Each castrated animal in the first group received an intramuscular injection of 0.5 mg of the 17 (beta)-cyclopentylpropionate ester of testosterone 7 days after castration. Ten days following surgery, all 3 groups of mice were inoculated with the test virus. Careful observations were made throughout the 30-day period that followed.

Materials and methods. Mice. Random bred "Swiss albino" mice (Webster strain) were used throughout this study. Each mouse was at least 2½ months old and weighed 20 g or more.

Virus. The Coxsackie B1 virus used was recovered in monkey-renal cell cultures from stool obtained from a patient with acute infectious myelitis. The agent was passed once in suckling mice. A torso-brain suspension was used to inoculate a group of 24 adult animals; 7 of the 24 died on the third post-inoculation day. A 10% suspension of their pancreases was prepared in a mortar by grinding with an appropriate volume of Hanks' balanced salt solution. One-tenth ml of this suspension was inoculated into each of a number of monkey-renal cell cultures maintained with mixture 199 with antibiotics but without added serum. When cytopathic effects were maximal, the culture fluids were pooled and spun at 1500 rpm for 15 minutes. The clear supernatant, used as the inoculum for all the experiments, was repeatedly assayed in monkey-kidney cell cultures. Each experimental animal received an intraperitoneal inoculation of 0.1 ml of this virus preparation with an infectivity of from $10^{5.5}$ to $10^{6.0}$ $TCID_{50}$.

Virologic studies. Virus titrations and serum antibody determinations were performed in monkey-kidney cell cultures by standard techniques. Ten per cent suspensions of the tissues for assay were prepared by grinding with an appropriate volume of Hanks' balanced salt solution. Virus titers are expressed as log to the base 10 per g of wet tissue.

Neutralizing antibody was determined with 2-fold dilutions of serum and 100 $TCID_{50}$ of the test virus per tube.

Surgical procedures. Drop ether and/or intraperitoneal ethyl alcohol (1 ml of a 15% dilution/30 g of body weight) was used for anesthesia. Ovariectomy or orchiectomy was done through a midline abdominal incision. The wound was closed with interrupted black silk sutures. There was little morbidity or mortality following this operative procedure.

Results. Clinical and laboratory observations. Within the first 10 days following intraperitoneal inoculation of the Coxsackie B1 virus, the test animals, both operated and non-operated, lost approximately 20% of their pre-infection weight. This bodily change coincided with a marked decrease in the usual intake of both food and water. By the third day post-infection, the animals were lethargic; their fur was ruffled, and they maintained a hunchback-like posture even when walking. During this acute phase, an occasional animal became dyspneic or was found convulsing. For some, death occurred between the third and eighth day after infection. Thereafter, the surviving animals showed rapid improvement; their intake of food quickly returned to normal.

Virologic studies were done on a representative group of 26 infected animals. By the third or fourth day post-infection, a suspension of pancreas obtained from either a castrated or control animal yielded virus in tissue culture. The virus titers in pancreas harvested from dead animals ranged from $10^{6.5}$ to $10^{7.5}$ or more as compared to $10^{5.0}$ to $10^{6.5}$ in tissue from sick animals. One week after infection, the agent could still be recovered from pancreas obtained from survivors. After 2 weeks, the remaining live animals had significant levels of serum neutralizing antibody to the test virus (approximately 1:64). Pancreatic tissues tested then were free of virus. Virus concentrations in stool samples contained approximately 10,000-fold less virus than was recovered simultaneously from pancreas. The stool specimens tested were still positive 2 weeks following the inoculation of virus.

TABLE I. Deaths Among Adult Mice Inoculated with Coxsackie B1 Virus.*

	Castrated			Sham-castrated			Normals		
	No. of mice	No. of deaths†	Death rate (%)	No. of mice	No. of deaths†	Death rate (%)	No. of mice	No. of deaths†	Death rate (%)
Male	102	6	5.9	102	18	17.6	95	18	17.9
Female	97	4	4.1	102	15	14.7	101	15	14.9
Total	199	10	5.5	204	33	16.2	196	32	16.4

* Operated animals in 2 of the 3 groups compared were infected 10 days after surgery.
† All deaths occurred from 3rd to 8th day post-infection.

TABLE II. Deaths Among Adult Mice Inoculated with Coxsackie B1 Virus 30 Days After Surgery.

	Castrated			Sham-castrated		
	No. of mice	No. of Deaths*	Death rate (%)	No. of mice	No. of deaths*	Death rate (%)
Male	60	16	26.7	58	11	19.0
Female	62	19	30.6	60	12	20.0
Total	122	35	28.7	118	23	19.5

* All deaths occurred from 3rd to 8th day post-infection.

Within 72 hours, the pancreas in infected animals, whether castrated or normal, was soft, mushy, and whiter than usual. Necrotic areas were apparent in omental and mesenteric fat. The histopathology in the pancreas was the same for all the infected animals. The exocrine portion of the gland was necrotic; whereas, relative to the former, the islets were spared.

Effect of gonadectomy. The deaths that occurred among large numbers of gonadectomized and control mice following Coxsackie B1 virus infection are tabulated in Table I (Exp. I). It is apparent that the death rate for castrated animals inoculated with virus 10 days after operation was almost one-third less than the proportion computed for the comparable, sham-castrated control group or the normal control group. To test the significance of this difference, the relative deviate was computed and compared with the tabulated values for the normal curve. The difference in death rate between the infected castrates and their sham-castrated controls was found to be significant at the 0.01 level. Death rates for the males and females in each group were not significantly different.

The protective effect of castration observed among animals infected 10 days after operation (Table I) was no longer apparent in animals infected 30 days post-surgery (Table II, Exp. II). At that time, the difference between the death rate in gonadectomized and sham-operated groups was no longer significant but was probably due to chance fluctuation.

The death rate for testosterone-treated, gonadectomized, male animals inoculated with virus 10 days after operation was virtually the same as the percentage computed for a group of castrated, untreated, controls (Table III, Exp. III). The proportion of deaths in both groups was appreciably less than observed in a comparable group of sham-castrated, untreated mice. In this experiment, the protective effect of orchiectomy was not modified by testosterone replacement therapy.

Discussion. All of the adult mice used in our study became severely ill, and approximately 15% of the animals died following inoculation of the Coxsackie B1 test virus. The animals that succumbed as well as the survivors had widespread lesions in the pancreas; brain, striated muscle and cardiac muscle were not similarly involved. This histopathologic picture is not unusual and has been described in asymptomatic mice infected with Coxsackie B1 virus(2). The question arises, therefore, as to what was responsible for the increased morbidity and mortality ob-

TABLE III. Deaths Among Testosterone Treated and Untreated Groups of Adult Male Mice Inoculated with Coxsackie B1 Virus 10 Days After Surgery.

Castrated Testosterone replacement*			Castrated No testosterone			Sham-operated No testosterone		
No. of Mice	No. of deaths†	Death rate (%)	No. of mice	No. of deaths†	Death rate (%)	No. of mice	No. of deaths†	Death rate (%)
24	1	4.2	26	1	3.8	25	4	16.0

* Each animal received an intramuscular injection of 0.5 mg of the 17 (beta)-cyclopentylpropionate ester of testosterone 3 days before inoculation of virus (7 days after castration).
† All deaths occurred from 3rd to 8th day post-infection.

served among our infected animals. Perhaps a relatively sensitive, variant strain of mouse was selected for study, or the virulence of the Coxsackie B1 test virus for the mouse was intensified by passage in monkey renal-cell tissue culture and in both immature and mature mice. To evaluate one of these factors, the same virus inoculum used in our experiments was administered to groups of normal CBA black and Charles River CD-1 mice. The death rate and signs and symptoms observed among these dissimilar strains were similar to those detected in infected animals from the Webster strain. Since the pathogenicity of other strains of Coxsackie B1 virus was not similarly evaluated, no clear cut conclusion can be drawn from the studies done.

The interrelationship between castration and susceptibility to virus infection has been investigated by others as well. Aycock(4) studied 15 immature female monkeys. The entire group was infected by intranasal instillation of a suspension of poliovirus given 32 days after 10 of the animals were castrated. Five of the castrates received repeated intramuscular injections of an estrogen preparation before and after infection; 5 others were not treated. The remaining normal females served as controls. Only one of the 5 hormone-treated animals became paralyzed; whereas, 4 of the 5 untreated castrates succumbed. These results suggest that castration exerted an adverse effect which was reversed by estrogen therapy. However, 3 of the 5 normal control animals also became paralyzed. Teodoru and Shwartzman(3) inoculated several hundred adult male hamsters intracerebrally with poliovirus at different intervals following orchiectomy. Susceptibility was significantly increased during the first week following surgery; thereafter, the enhancing effect of castration was lost. In the hamster, the return to normal paralleled the observed hypertrophy of both the thymus and the reticularis zone of the adrenal. Moreover, injection of exogenous gonadotrophins or testosterone also negated the adverse effect of castration.

By contrast, we found that the death rate among gonadectomized mice of both sexes infected with our test strain of Coxsackie B1 virus 10 days after surgery was significantly lower than the percentage of deaths among comparable controls (Table I). Castration rather than a peril, therefore, was protective for these virus infected mice. However, the virus, the route of inoculation, the time interval between castration and infection and the species of animal were all different from those used by other investigators who have previously studied the effects of castration. These variables could account for the disparate results obtained. Yet the enhancing effect of castration in the hamster(3) and the protective effect observed in our mice were both short-lived phenomena.

Possible mechanisms involved in the protective castration effect are of interest. Since gonadectomy increased the resistance of mice of both sexes to Coxsackie B1 infection (Table I), the common factor following castration could be the sudden withdrawal of gonadal secretions rather than any specific sex effect. A return to normal susceptibility 30 days after castration (Table II) might then be attributed to compensatory secretion of sex hormone by other glands, primarily the adrenal. In the hamster, for example, changes do take place in the adrenal cortex following

castration, primarily hypertrophy of the reticularis(3). This area does elaborate sex hormones. However, histologic preparations from adrenal glands harvested from our infected and uninfected mice 2 to 3 weeks after castration revealed no hypertrophy. Furthermore, administration of testosterone following orchiectomy did not increase the susceptibility of experimental male animals to the test virus (Table III). The dose of the hormone preparation used for this experiment was adequate for replacement therapy. It is unlikely, therefore, that in the male the withdrawal of testicular androgen initially or the compensatory secretion of male sex hormone by the adrenal played an important part in the protective effect observed or in the delayed return to preoperative susceptibility.

Lastly, since sham-operated animals remained normally susceptible to Coxsackie B1 virus infection (Table I), it does not appear that the stress of operation alone influenced resistance. However, one cannot state unequivocally that the sham procedure was as debilitating to the experimental animal as true gonadectomy.

The following conclusions are consistent with our present knowledge: (1) gonadectomy decreased the susceptibility of the adult mouse to Coxsackie B1 virus infection and (2) the altered resistance was short lived and unrelated to the sex of the experimental animal. These findings make it unlikely that the sudden withdrawal of sex hormones following castration or the compensatory secretion of similar materials by the adrenal played an important part in the response observed.

Summary. The Coxsackie B1 virus used in this experiment was unusually virulent for adult "Swiss albino" mice; approximately 15% of the infected animals died and the survivors were severely ill. However, following gonadectomy the death rate fell significantly. This protective effect of castration was not sex-linked and was no longer present 30 days after surgery. Possible hormonal mechanisms responsible for this altered susceptibility are discussed.

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Calcium and Phosphorus Content of Ash of Bones and Teeth of Human Fetuses in Relation to Fluoride Content of Drinking Water.* (30274)

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We have presented data(1,2) on the fluoride content of ash of bones and teeth from 4-9-month-old fetuses obtained from areas in which the concentration of fluoride in the drinking water was low (about 0.1 ppm), medium (0.5-0.6 ppm) or higher (about 1 ppm). The results revealed that the fluoride content of fetal bones and teeth increased with increasing age of the fetus when the fluoride concentration in the drinking water was medium or high, but did not increase with age when the fluoride concentration in

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