of ACTH and secondarily adrenocorticosteroids with their modulating effect on uterine metabolism.

Summary. Uterine response to estradiol in immature female mice is inhibited significantly by concurrent injection of estriol. Extent of inhibition is proportional to amount of estriol added. Impeding interaction occurs along the ascending segment of the estradiol dose-response curve and with higher doses as well. In no case does a combination of estrogens have a positive additive action. These conclusions are based on both wet and dry uterine weight observations. Day by day analysis shows that uterine weight gain of each 24 hour period during 3 day estradiol treatment is inhibited when estriol is given concurrently. These studies negate the possibilities that estriol-estradiol interaction is a masked-summation effect in which time of maximum effect has shifted, or that an additive effect occurs in which two estrogens combined exceed maximum response dose. Rather, it appears that estriol competes actively at a physiologic site essential in development of usual estradiol stimulation of the uterus.

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Factors Influencing Host-Virus Interactions. II. Alteration of Coxsackie Virus Infection in Adult Mice by Cold.* (22730)

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Coxsackie virus infections in infant and adult mice are remarkably dissimilar(1,2,3). Many tissues, such as brain, muscle, liver, and heart, develop extensive lesions in very young animals but become refractory to the virus as mice reach 1 to 3 weeks in age. In infections with Conn. 5 strain of Coxsackie virus in adult mice the pancreas appears to be the only regular site of virus multiplication and histopathology. Even though, as a result of initial viremia, virus is available to other tissues, viral multiplication and tissue damage remain localized and non-fatal. A single injection of cortisone alters the infection with Conn. 5 virus in adult mice in such a manner that the infection becomes lethal (4) with multiplication of the virus and histopathology occurring in tissues that are unaffected in untreated animals of the same age (3). This report presents data indicating that the natural resistance of adult mice to disseminated infection with this virus can be altered by another factor, namely a cold environment, to result in rapidly and uniformly fatal disease.

Materials. Virus. The Conn. 5 strain of Coxsackie virus was used and methods for

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preparation of virus suspensions, normal mouse tissue suspensions, and general methods of virus titration and neutralization tests have been described(3). In this study all infant mice 24-48 hours of age employed in a set of virus titrations were mixed and redistributed to mothers at random, and equal numbers, usually eight, were inoculated with each dilution. Unless otherwise stated, mice referred to as adult mice were 4-6 weeks of age and averaged 14-18 g in weight. Sexes were mixed at random. Immune serum was prepared by injecting mice once weekly for 3 weeks with 0.2 ml of a 1% suspension of infected infant mouse tissue. The mice were bled one week after the last injection. Normal serum was obtained from similar, but uninoculated, mice. A room varying in temperature between 23° and 27°C was used to house mice designated as held at 25°C. There was no special control over humidity which varied with atmospheric conditions. Mice referred to as held at 4°C were placed in a refrigerated room which was maintained at 3-5°C and approximately 60% relative humidity while those designated as held at 36°C were kept in room maintained at 35-37°C and approximately 12% relative humidity. Mice were housed in wire cages open to the atmosphere except for the top, bottom and the lower two inches of the sides, and were caged in groups of 8 to 12 without restrictions on huddling or activity. Food and water were available *ad libitum*.

Results. Although adult mice of strain used in this study seldom suffer apparent ill effects from even very large doses of the Conn. 5 strain of Coxsackie virus, experiments indicated that if infected mice were kept at 4°C following inoculation, the infection became uniformly lethal. A representative experiment is presented in Table I. Adult mice previously housed at 25°C were inoculated i.p. with 4000 infant LD_{50} of virus and then maintained at test temperatures along with control mice injected with normal mouse tissue suspension. By the end of fourth day all infected mice at 4°C were dead, while infected mice at 25°C, with one exception, survived the 10 day observation period. The

TABLE I. Lethal Effect of Coxsackie Virus (Conn.-5 Strain) Infection in Adult Mice at 4°C.

Inoculum (i.p.)	Environmental temp., °C	Deaths†/No. inoculated
Virus*	25	1/12
	4	10/10
	36	0/ 8
Normal mouse tissue suspension (.05 cc)	25	0/12
	4	0/ 8
	36	3/ 8

* 4000 LD_{50} (.05 cc) for infant mice.
† No. of mice dying during 10 days of observation.

one mouse dying in the 25°C groups represents the rare death seen in adult mice infected with the Conn. 5 strain. Although the infection was lethal at 4°C, infected mice placed in another stressing environment, 36°C, did not succumb. Indeed, more animals died in the control than in the test groups at this temperature. This is in keeping with numerous experiments which showed that mice of the strain employed thrived at 4°C for periods up to 3 weeks but that 36°C was a more unfavorable environment and occasional deaths occurred after one week of exposure.

To determine size of viral inoculum required to produce a lethal infection in adult mice at 4°C, comparative titrations of a virus suspension were made using adult mice held at 4°C and 24-48 hr old infant mice at 25°C. The inoculum for both groups was 0.05 ml given i.p. The LD_{50} for adult mice at 4°C was $10^{-4.5}$ and was 10 times the $LD_{50}(10^{-5.5})$ for 24-48 hr old infant mice.

Additional experiments showed that lethal infection followed subcutaneous as well as intraperitoneal inoculation of virus in mice held at 4°C and that alteration of Coxsackie virus infection by a cold environment was not limited to mice 4-6 weeks of age since mice 11 weeks of age were also susceptible. The relative susceptibility of adult mice of various ages was not examined in detail.

Specifically to relate this phenomenon to an activity of the virus in infected mice held at 4°C it was important to determine whether the lethal effect could be neutralized *in vitro* with specific antiviral serum and whether pas-

TABLE II. Specific Neutralization of Coxsackie Virus and Passive Immunization of Adult Mice against Lethal Infection at 4°C.

Preliminary* inj.	Inoculum (i.p.)	Environmental temp., °C	Deaths/ No. inoc.
None	Virus† + antiserum	4	1/10
"	" + normal serum	"	9/10
Antiserum	Virus, 140 LD_{50}‡	"	0/15
"	" 280 "	"	0/15
Normal serum	" 140 "	"	13/13
" "	" 280 "	25	0/15

* 0.5 cc mouse serum given i.p. 3 hr before inoculum.
† 280 LD_{50} of virus in 0.1 cc of mouse serum.
‡ Determined in infant mice.

sive immunization of mice with specific antiserum would confer immunity to the lethal effect. Data are presented in Table II which show that death at 4°C was prevented by mixing the viral inoculum with specific immune serum prior to inoculation, and by passive immunization of mice with specific antiserum previous to viral infection.

Since it appeared possible that the lethal effect on infected mice at 4°C might have been due only to inability of mice with pancreatitis to survive in the cold, measurements of levels of virus reached in these animals were made, and the pathological lesions produced were compared with those seen in adult mice at 25°C. In Table III are presented the virus levels found in the blood and liver on the 2nd and 4th days after adult mice were inoculated with Conn. 5 virus and then maintained at either 4°C or 25°C. It is evident that in mice at 4°C a viremia persisted through the 4th day although the blood was cleared of virus by that time in mice at 25°C.

TABLE III. Levels of Virus in Tissues of Adult Mice Infected with Coxsackie Virus (Conn. 5)* and Held at 4°C or at 25°C.

Tissue	Day of infection	Virus titers† in mice at: 4°C	25°C
Blood	2	$10^{-4.5}$	$10^{-4.3}$
	4	$10^{-4.0}$	$<10^{-1.0}$
Liver	2	$10^{-4.0}$	$10^{-3.8}$
	4	$10^{-5.1}$	$<10^{-1.0}$

* Inoculum = 200 infant LD_{50} of Conn. 5 virus.
† Titers in pooled tissues of 5 adult mice as measured in suckling mice.

In the livers of mice in the cold the virus titer increased from the 2nd to the 4th day and was found at a high level on the 4th day of the infection while in mice at 25°C the virus had been reduced to a low level in the liver by the 4th day. Virus recovered from the livers of mice at 4°C was identified as the Conn. 5 strain of Coxsackie virus by neutralization tests in infant mice.

On gross examination of mice dying of Coxsackie infection at 4°C marked inflammation of perinephric, mesenteric, and pelvic fat was noted in many. In approximately 25% of the animals there appeared to be gross bleeding into the small intestine. Similar abnormalities were not found in infected mice held at 25°C nor in uninfected control mice maintained at 4°C. Microscopic examination of tissues revealed the following: *Lungs.* A slight to moderate interstitial pneumonitis was found in infected animals at both 4° and 25°C. *Heart.* In 7 of 9 infected mice held at 4° lesions were found in the myocardium. These consisted of focal areas of fiber degeneration and some of these foci contained basophilic granules. Occasionally small inflammatory granulomas consisting of fibroblasts, macrophages, and lymphocytes were seen. In contrast to these lesions observed in mice at 4°C, no myocardial lesions were found in infected mice held at 25°C. *Pancreas.* A severe pancreatitis was found in infected mice in both 4°C and 25°C groups. *Fat.* Marked enzymatic necrosis was present in the perinephric and peripancreatic fat in both the 4°C and 25°C groups of animals. Inflammatory lesions in the interscapular fat pad were similar in extent and kind in infected mice at both temperatures. *Liver.* In animals at 25° there were occasional small intralobular and portal inflammatory foci. In mice at 4° similar but more extensive lesions were found and with greater frequency. Additional features in the animals at 4° were considerably cloudy swelling, single or small groups of balloon cells, small areas of parenchymal cell necrosis and, in one animal, marked nuclear pleomorphism associated with slight to moderate numbers of mitoses. *Skeletal Muscle.* No specific abnormalities were found in skeletal mus-

TABLE IV. Relationship of Time of Exposure to 4°C and Lethal Effect of Coxsackie Virus (Conn. 5) in Adult Mice.

Environmental temperature: Before inoculation*	After inoculation†	Deaths/No. inoculated
4°C for 2 days	25°C	0/12
" " 1 day	"	0/12
25°C	4°C, 1 day, then 25°C	0/12
"	" 2 days, "	0/12
"	4°C	12/12
"	25°C, 1 day, then 4°C	12/12
"	" 2 days, "	9/12
"	" 4 days, "	9/12
"	" 6 days, "	0/12
"	" 8 days, "	0/12

* Inoculum = 1500 infant LD_{50} given i.p. in 0.05 cc.

† Observation period = 10 days after mice were placed at 4°C.

cle at either 25°C or 4°C. *Intestine.* In all mice at 25° and most animals at 4°C no abnormalities were found in the piece of small intestine obtained for histological examination. In 2 of 9 mice at 4°C large quantities of blood were visible in the intestinal contents, confirming the gross examination finding of intestinal hemorrhage. The intestinal mucosa in one of these mice was markedly disrupted suggesting that the site of bleeding was in the small intestine. *Other tissues.* No lesions were found in the brain, kidneys, adrenal, or spleen at either temperature. No abnormalities were found in the tissues of control groups of mice injected with normal mouse tissue extracts and held at 4°C or at 25°C.

Relationship of time of exposure of mice to 4°C and lethal effect obtained with the Conn. 5 Virus. Since an environmental temperature of 4°C was effective in influencing the infection when continued exposure was begun immediately after inoculation, it was desirable to determine the time, relative to inoculation, during which cold would alter the infection and the duration of exposure necessary to result in lethal infection. In Table IV data from one of several experiments designed to explore these points are presented. Exposure of mice to 4°C for periods up to 48 hours either before or after virus inoculation was insufficient to result in fatalities. However, continued exposure of the animals to cold begun as late as 4 days after virus inoculation resulted in the death of the majority of the mice while mice survived if cold exposure was delayed until 6 or more days after infection.

Discussion. The experiments described indicate that although adult mice possess a natural resistance to the Conn. 5 strain of Coxsackie virus such that the disease is limited to a non-fatal infection of the pancreas, this refractoriness is lost when the animals are maintained in a cold environment and a lethal infection results which is characterized by a persisting viremia, high levels of virus in the liver, and lesions demonstrable in other organs as well as in the pancreas. The mechanisms by which cold reduces resistance to this virus are not yet clear although the fact that cortisone causes a similar loss of resistance suggests that cold may act through its capacity as a stress agent to cause excessive secretion of adreno-cortical steroids. That the change in resistance is due only to the non-specific effects of stress might be questioned, however, since heat, also a severe stress, does not reduce resistance. Another possibility presents itself in the well known stimulation of thyroid activity by a cold environment and depression of that activity by elevated temperatures. Evidence that changes in thyroid activity can influence resistance to bacterial and viral infection has been obtained by several investigators(5,6,7) although the direction of the change has not been constant with the several agents studied.

Reports in the literature(8,9,10) indicate that a cold environment may affect other host-virus systems although none so sharply as that studied in the experiments reported here. Cold has usually intensified the results of the infection but in some instances has afforded protection to the host. Because the effects of cold on the Conn. 5 strain Coxsackie virus infection in mice are so sharp and unequivocal we are continuing the study of this infection in an effort to further clarify the mechanisms involved.

Summary. 1. Exposure of adult mice infected with the Conn. 5 strain of Coxsackie virus to a cold environment resulted in an infection with a persisting viremia, high levels

of virus in the liver, pathologic lesions in other organs as well as in the pancreas, and a uniformly fatal outcome. 2. A lethal infection was prevented by neutralization of the virus or passive immunization of adult mice with specific antiserum. 3. The LD_{50} for adult mice at 4°C was approximately ten times that for suckling mice at 25°C. 4. It was demonstrated that to initiate a fatal infection exposure to cold had to be continuous and begun within a few days after virus inoculation.

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Effects of Short Chain Fatty Acids on Hepatic Acetate Metabolism in Cold Exposure and Fasting.* (22731)

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We reported that liver slices prepared from rats fasted for a period of 24 hours at an environmental temperature of 0-2°C ("cold-fasted" rats) showed severe depression in oxidation of acetate-1-C^{14} to $C^{14}O_2$(1). This defect in acetate oxidation could be restored to normal by addition of glucose(1) or pyruvate(2) to the incubation medium. The evidence suggested that this effect of glucose and pyruvate must be related to a metabolic event prior to entry into the Krebs cycle because succinate was much less effective than pyruvate and glucose in reversing this metabolic defect while citrate and α-ketoglutarate were without effect at all.

Since phases of carbohydrate metabolism other than the Krebs cycle are involved, it is important to know whether acetate oxidation is specifically linked to carbohydrate metabolism or whether other substances can serve as energy sources capable of reversing the block in acetate oxidation imposed by cold exposure and fasting. The latter possibility was tested by adding short chain fatty acids to the incubation medium. The results of these experiments are reported below.

Methods. Adult male rats of Wistar strain were allowed to eat, *ad libitum* for 7 to 10 days, a diet composed of 25 g casein, 10 g fat, 51 g dextrose, 3 g salt mix(3), 5 g cellulose, 6 g Brewer's yeast, 0.04 g cod liver oil concentrate and 0.01 g α-tocopherol. Following dietary stabilization the rats were either given the same diet, *ad libitum*, at 25°C or were fasted for 24 hours at 0-2°C. The animals were killed by blow on head. The liver was rapidly excised and placed in a beaker containing ice-cold Krebs-Henseleit bicarbonate buffer solution(4). Tissue slices were prepared free-hand with razor blade and these slices were collected in petri dish containing ice-cold buffer. The liver slices were blotted free of excess solution on filter paper. Approximately 250 mg of tissue were weighed out and then transferred to specially designed

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