TABLE I (a). Hemolysis of Red Cells from Vit. E-Deficient Rats by Dialuric Acid.

Exp. No.	Diet	Days after diet started	Hemolysis in 4 hr
1	E-deficient	8	0
		21	0
		46	complete
2	Purina Lab. Chow	46	0

Four animals were used in each experimental group.

TABLE I (b). Resistance of Red Cells to Dialuric Acid of Premature Infants with Retrolental Fibroplasia. No hemolysis in 4 hr.

Subject	Birth wt, g	Age when tested, days	Wt when tested, g	Retrolental fibroplasia
Ma.	880	88	2250	e*
Mi.	1008	84	2310	a
C.	1035	79	2600	a
G.	1148	52	1990	e
G.	1148	75	2370	e
A.	1150	86	2250	a
J.	1268	76	2560	e
L.	1277	64	2400	0
Al.	1305	51	2130	0
F.	1365	28	1460	e
F.	1365	63	2450	r
W.	1417	75	2240	a
Jo.	1470	15	1540	0
R.	1644	57	2860	a
Z.	1750	52	2950	0
Re.	2197	20	2095	0

* e = early; a = advanced; r = regressed.

blood from guinea pig embryos obtained by Caesarian section. No hemolysis was detected in the three litters examined. The guinea pigs had been fed a stock diet of Purina Rabbit Pellets supplemented with lettuce.

Discussion. We have found no correlation between the occurrence of retrolental fibroplasia in premature infants and a deficiency of vit. E as measured by the hemolysis test. It should be mentioned, however, that even if a vit. E deficiency existed in the premature infant, it might not be detected by the procedure employed, which has been validated for the rat only. Mason(6) has summarized the differences in pathology of vit. E deficiency in different species.

Summary. 1. The erythrocytes of 5 normal premature infants and of 9 with retrolental fibroplasia have been examined for a deficiency of vit. E by the hemolysis test of Rose and György. The red blood cells from all of the infants were resistant to hemolysis by dialuric acid. 2. We have confirmed the validity of the hemolysis test for a deficiency of tocopherol in the rat.

6. Mason, K. E., *Vitamins and Hormones*, 1944, v2, 107.

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Lethal Infection with Coxsackie Virus of Adult Mice Given Cortisone. (18702)

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Coxsackie viruses are characterized by virulence for immature mice or hamsters and avirulence for adults of either species. In fact, this unique property is the single biological characteristic which defines this large group of viruses.

Recent studies in this laboratory which demonstrated increased influenza A, B, and mumps virus multiplication in embryonated eggs injected with cortisone(1), and observations of Shwartzman(2) on enhancement by cortisone of poliomyelitis infection in mice and hamsters, suggested the use of this hormone to essay an alteration in the insusceptibility of the adult mouse to Coxsackie viruses.

Materials. Viruses. The Conn. 5 strain of Coxsackie virus (previously obtained through the courtesy of Dr. Joseph L. Melnick) and the RB strain of virus, recovered in this laboratory from the stool of an 8-year-old boy

1. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 116.

2. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

suffering from epidemic pleurodynia, were employed in the present investigation. Preliminary cross-neutralization tests have shown no antigenic relationship between the Conn. 5 and RB viruses. *Sera.* Sera were heated at 56°C for 30 minutes before use in neutralization tests. *Cortisone.* Cortisone acetate (Cortone, Merck) was injected subcutaneously in the dosage indicated. *Control materials.* Pfanstiehl peptone broth and 0.85% NaCl solution buffered to pH 7.2 with phosphate were used as control injections. *Mice.* Rockefeller Institute Swiss mice were used in all experiments. Those referred to as "suckling mice" were less than 1 day old, and "adult mice" were sexually mature, weaned animals averaging 16 g in weight and 3-4 weeks in age, unless otherwise specified.

Procedure. One subcutaneous injection of cortisone was administered to mice 1-2 hours prior to the inoculation of virus. Light ether anesthesia was used when mice were injected by the intracerebral route. Mice were observed at least twice daily for signs of illness or death. In all experiments groups of control mice were injected with both cortisone and broth in quantities and by routes identical with those employed with virus-inoculated animals in order to define the incidence of non-specific mortality in mice not given virus. Viral suspensions were administered in amounts approximately proportional to the size of the mice inoculated (0.05 cc virus/1.5 g of mouse) when the intraperitoneal route was used. All viral inocula contained penicillin and streptomycin, and were bacterially sterile on ordinary culture media. Tissues for passage were obtained from moribund or newly dead animals, except in the case of control animals, which were killed while in apparent good health.

Results. Preliminary experiments indicated that most 16-g adult mice survived the subcutaneous administration of 2.5-5.0 mg cortisone for at least 8 days. Accordingly, these dosages in single injections have been employed. In all experiments to date the concomitant administration of cortisone and Coxsackie virus has resulted, in less than 8 days, in the death of all animals so inoculated. A representative experiment with Conn. 5 virus is summarized in Table I. Three days following inoculation all mice given cortisone and virus were found dead, while control mice, given either virus or cortisone singly, were alive after 8 days when the experiment was terminated.

TABLE I.
Lethal Effect of Coxsackie Virus (Conn. 5) in Adult Mice Given Cortisone.

Preliminary injection (s.c.)		Inoculum		
	mg	(i.p.)	D/t†	Day
Cortisone	5.0	Virus*	5/5	3rd (dead)
"	2.5	"	5/5	" "
Saline		"	0/10	8th (killed)
Cortisone	5.0	Broth	0/5	" "
"	2.5	"	0/5	" "

* 40,000 LD_{50} (0.2 cc), determined in infant mice.

† D = No. of mice which died; t = total mice in group.

It was important to ascertain: (1) whether viral multiplication had occurred, (2) whether the lethal effect was serially transmissible, and (3) whether viral multiplication and the lethal effect could be neutralized by specific antiviral serum. Data answering each of these questions in the affirmative are presented in Table II. No virus was demonstrated in the brains of mice injected with virus alone. On 2nd passage virus titers in cortisone-injected mice exceeded concentrations possible on the basis of mere carry-over of non-multiplying virus. Both viral multiplication and death were prevented by specific immune serum in mice given virus and cortisone.

Additional experiments to determine the age limit of cortisone-induced susceptibility have established that 35-g mice 17 weeks of age are subject to lethal infection if injected with 7.5 mg of cortisone.

To establish whether infection of the adult mouse was possible with Coxsackie virus not previously passed in infant mice, and derived directly from a human source, the experiments outlined in Table III were carried out. A 10% suspension of stool from which virus (RB) had been previously recovered in 1-day-old mice (but *not* in 10-day-old mice) was inoculated by the intraperitoneal of intracerebral route into adult mice pretreated with cortisone. All mice inoculated by the intra-

TABLE II. Serial Passage and Specific Neutralization of Coxsackie Virus (Conn. 5) in Adult Mice.

Passage No.	Preliminary injection (s.c.)	mg	Inoculum: Virus	Inoculum: Serum	D/t	Titer of adult brain in suckling mice
1	Cortisone	5.0*	Suckling mouse susp. 10^{-2}†	—	5/5	$10^{-3.3}$
2	,,	,,	Brain 10^{-1} +	N.S.‡	5/5	$10^{-2.5}$
,,	,,	,,	,, ,, +	I.S.§	0/4	0‖
3	,,	,,	,, ,,	—	2/2	—
1	Saline		Suckling mouse susp. 10^{-2}		0/5	0
2			Brain 10^{-1} +	N.S.	0/5	—

* Followed in 48 hr by additional 2.5 mg, in first passage only.
† 6,000 LD_{50} (0.03 cc).
‡ Normal mouse serum.
§ Conn. 5 antiserum (mouse).
‖ Lowest dilution tested, 10^{-2}.

TABLE III. Recovery and Serological Identification in Adult Mice of Coxsackie Virus (RB) from Human Feces.

Passage No.	Prelim. injec. (s.c.)	mg	Inoculum: Virus	Inoculum: Serum	Inoculum: Route	Age of mice, days	D/t	Day	Titer of adult brain in suckling mice
1	—		10^{-1} stool	—	i.p.	1	13/15	8th (dead)	—
,,	—		,, ,,	—	,,	10	0/8	,, (killed)	—
1	Cortisone	5.0	10^{-1} ,,	—	i.p.	21+	6/6	3rd (dead)	$>10^{-3}$
,,	,,	,,	,, ,,	—	i.c.	,,	6/6	7th ,,	$>10^{-3}$
2	,,	,,	10^{-2} brain	N.S.*	i.p.	,,	5/5	4th ,,	$10^{-4.3}$
,,	,,	,,	,, ,,	I.S.†	,,	,,	0/5	8th (killed)	—
3	,,	,,	10^{-3} ,,	—	,,	,,	4/4	8th (dead)	—
1	Saline		10^{-1} stool	—	i.p.	21+	2/5	5th, 9th (dead)	(see text)
,,			,, ,,	—	i.c.	,,	0/5	9th (killed)	$<10^{-2}$
2			10^{-2} brain	—	i.p.	,,	0/5	8th ,,	0‡

* Normal mouse serum.
† RB antiserum (mouse).
‡ Lowest dilution tested, 10^{-1}.

peritoneal route were dead in 3 days, while mice receiving virus by the intracerebral route succumbed more slowly, but all had died 7 days after inoculation. It should be noted that animals inoculated intracerebrally received one-tenth the volume of inoculum introduced by the intraperitoneal route. Brains of mice inoculated by either route titered more than 10^{-3} in suckling mice. A 10^{-2} dilution of brain tissue from mice which had been inoculated intraperitoneally with stool promptly produced death in all animals when passed with normal mouse serum, but no evident disease or death in the presence of specific immune serum against the RB virus. No deaths occurred in a group of mice pretreated with saline and inoculated with this brain suspension, reaffirming dependence of viral virulence upon cortisone administration. Titration in suckling mice of pooled brains of mice which died in the second passage disclosed a viral concentration of $10^{4.3}$. A comparable titration of this material in adult mice pretreated with cortisone resulted in death by the 8th day of all mice inoculated with the highest dilution employed (10^{-5}). This latter experiment thus simultaneously demonstrates: (1) the serial transmissibility through 3 passages of Coxsackie virus in cortisone-treated adult mice; and (2) that susceptibility of such adult mice is at least as great as that of infants. When the stool inoculum employed in the preceding experiments was injected into control mice not given cortisone, deaths occurred in none of the animals injected intracerebrally and in only 2 of 5 mice injected by the intraperitoneal route.

In the latter group residual virus was demonstrable in the brain of a healthy-appearing mouse killed 3 days after inoculation, but was not detected in brains of survivors killed on the last day on which death occurred in this group. It is therefore improbable that the 2 deaths in these mice were related to viral multiplication. Passage of the 3rd day brain tissue known to contain virus failed to kill saline-injected mice, and virus was not demonstrated in the brains of animals so inoculated.

In adult mice dying of infection with either Coxsackie virus employed, paralysis, tremor, and convulsions were not perceived. Conjunctivitis was noted occasionally in mice given cortisone alone, but was almost universally present in animals inoculated with virus as well. Two to 3 days following inoculation some animals became briefly hyperexcitable, but in most mice disease was manifested by huddling, ruffled fur, arched backs, labored respirations, progressive unresponsiveness and lethargy which proceeded to stupor and sudden death. Gross examination of abdominal viscera post mortem has disclosed splenic atrophy and hepatic enlargement and congestion. Histologic studies are in progress.

Discussion. The experiments described show that adult Swiss mice may be lethally infected with Coxsackie virus if such mice are administered a single dose of cortisone prior to the inoculation of virus. Furthermore, viral multiplication in the tissues of the adult mouse, as well as serial passage and specific neutralization of virus have been demonstrated in this ordinarily insusceptible host. Although focal muscle lesions have previously been noted in 10-12-g mice, attempts to propagate Coxsackie virus either in series or by alternate passage through suckling and weaned mice have met with failure in the past(3).

It is a matter of great interest that the administration of a chemically defined substance may convert a state of apparent complete insusceptibility to one of marked susceptibility to a viral infection. It will be important to determine the range and specificity of this phenomenon by the study of allied steroids and other viruses. Elucidation of the mechanism of the phenomenon will obviously be dependent upon the acquisition of further knowledge concerning the metabolic action of cortisone in the murine host.

Summary. 1. Adult mice, ordinarily insusceptible to Coxsackie virus infection, may be lethally infected if preliminarily administered cortisone. 2. Multiplication, serial passage, and specific neutralization of Coxsackie virus in adult mice have been demonstrated. 3. Recovery of Coxsackie virus from a human source has been accomplished in adult mice.

3. Gifford, R., and Dalldorf, G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 589.

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Nature of Multiple Forms of the *Lactobacillus bulgaricus* Factor (LBF).* (18703)

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In previous reports, the discovery(1,2) and purification(2,3) of a growth factor (designated LBF) required by *Lactobacillus bulgaricus* and many other lactic acid bacteria (4,5) was described. Bioautographic studies

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