with observations on the frog heart(8). It was found that the dilatation of the frog ventricle induced by an increase of OFR, at constant FP, was greater at higher heart rates, which would imply an increase of diastolic compliance. An inverse relation can usually be found between diastolic compliance and volume with most of the changed conditions experimentally imposed on these hearts. If the ventricle behaves according to the Laplace equation (PR = 2T), an increase of its radius (and of its volume) should bring about an increase of the wall tension. This could explain the diminished compliance observed when the ventricular volume is increased under the effect of an elevation of either FP or OFR. This inverse relation does not appear to hold when a change in ventricular volume is brought about by a change in heart rate, at constant FP or OFR. This observation suggests that some factor other than a mechanical effect influences the ventricular compliance in these circumstances. Alterations in elastic modulus due to chemical metabolic changes appear to occur.

Summary. The compliance of the isolated working heart is not constant but varies when physiological parameters are changed. The obvious corollary of this observation is that end diastolic pressure values are not always reliable indices of the diastolic ventricular volume, because the amount of stretch induced by a given filling pressure may be widely different in different conditions of performance. This fact should be kept in mind whenever one is to interpret ventricular function curves.

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Effect of Concomitant Treatment by Cortisone and N-Ethylisatin β-Thiosemicarbazone on Neurovaccinia Virus Infected Mice. (31168)

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N-ethylisatin β-thiosemicarbazone (NEITC) was reported as the most active of a series of related compounds which protected mice against a lethal neurovaccinia virus infection(1). Although NEITC was shown to markedly reduce the formation of elementary or inclusion bodies in mouse brain infected with vaccinia virus, the drug did not suppress the inflammatory response in the meninges(2). In this connection, certain forms of herpetic keratitis were reported to respond to the administration of pyrimidine nucleosides and corticosteroids(3-6). Therefore it was of interest to study the effect of concomitant therapy of NEITC and an anti-inflammatory steroid, cortisone, in vaccinial meningoencephalitis. The results of a preliminary study(7), are presented here.

Materials and methods. Viruses. The passage history of the WR strain of vaccinia virus and vesicular stomatitis virus (VSV) has been described(2). Vaccinia virus was used as the eighth to tenth passage on the chorioallantoic membrane (CAM) of 12-day-old chick embryos to produce 10% stock pools having an LD_{50} mouse brain titer between $10^{6.5}$ to $10^{7.5}$/0.03 ml. VSV was used as the fifth or sixth passage in chick fibroblast tissue cultures to produce stock pools having a plaque-forming unit (PFU) titer of

$10^{7.0}$/ml in L 929 mouse cells.

Virus infectivity titration. Brains from sacrificed mice were ground with sterile RR Alundum (mesh no. 60)* as 10% suspensions in Gey's solution(8) and centrifuged at 1500 rpm for 10 minutes. Serial 10-fold dilutions of the supernates were made in Gey's solution and 1-ml aliquots were tested for the presence of virus by the plaque technique in chick embryo tissue cultures(9). PFU titers were calculated as the negative $\log_{10}$ of the highest dilution yielding an average count of one or more plaques on duplicate plates.

Virus lethality titration. For lethality determination (LD_{50}), serial 10-fold dilutions of supernates from 10% infected brain suspensions were made in tryptose phosphate broth (Difco), and 0.03 ml of a dilution was injected intracerebrally into lightly anaesthetized mice. Deaths were recorded over a period of 14 days and the LD_{50} was computed by the method of Reed and Muench(10).

Mice. Male, albino CD-1 mice weighing 18 to 20 g were supplied by the Charles River Mouse Farms, Inc., Wilmington, Mass.

Drugs. Cortisone-alcohol was prepared at Schering Corp. and was used as a freshly prepared suspension in 0.85% saline. NEITC was also prepared at Schering Corp. according to methods previously described(1). The drug was suspended in aqueous 0.5% methyl cellulose (MC) and stored in amber bottles at 4°C.

Preparation of ultraviolet-inactivated (UV) virus. A 10-ml volume of a 10% stock pool of vaccinia virus ($10^{7.0}$ PFU/ml) and a 10-ml volume of a 10% extract of uninfected CAM from 12-day-old chick embryos was dialyzed 4 hours at 4°C against 20 volumes of 0.85% saline. Each dialysate was diluted 1:2 with saline and 13 ml was placed in a 150 × 25 mm round Petri dish (Falcon Plastic). The dish was placed on a platform† located 4 inches from the UV source‡ and gently rocked over a 1-inch excursion. Each preparation was irradiated for 1 minute, transferred to a fresh plate and again irradiated for 1 minute to assure inactivation of virus. Infectious virus was not detected in the UV fluids as indicated by 3 serial passages in chick fibroblast tissue cultures. All irradiated fluids were stored at 4°C.

Interferon (ITF) assay. Brains were ground as 25% suspensions with medium 199§ and centrifuged at 1500 rpm for 10 minutes. The supernates were dialyzed overnight at 4°C against pH 2.0 followed by overnight dialysis back to pH 7.4(11). L cell monolayers were then treated with brain supernates, challenged with VSV, overlaid with agar, and stained with iodonitrotetrazolium chloride for plaque detection as reported previously(2,9). A significant level of ITF was recorded as the highest dilution which reduced the VSV plaque count to 50% of the control(12).

Histopathologic studies. Brains from drug-treated and control mice were immersed in buffered neutral formalin fixative, embedded in paraffin and sections 6 μ in thickness were stained by a modified hematoxylin and eosin technique(13).

Results. Effect of cortisone on antiviral activity of NEITC. A group of 110 mice was injected intracerebrally with 250 LD_{50} of vaccinia virus. Two hours after infection, groups of 5 mice were injected intraperitoneally with a single dose of 10 mg of cortisone contained in 0.5 ml of saline or saline only. At the same time, the mice received a single subcutaneous injection of 4 mg of NEITC suspended in 0.5 ml of MC or MC alone. At various times after infection, groups of 5 mice were sacrificed and brains were harvested for PFU and LD_{50} determinations. The remaining 5 mice per treatment group were observed for survival over a period of 14 days. The results presented in Table I demonstrated that 10 mg of cortisone did not reduce the antiviral action of 4 mg of NEITC. This was shown by 100% survival and, in general, by similar titers of infectious and lethal virus. Virus was detected in the brains of mice on day 8 but was not demonstrated 10 days after infection. However, ruffled fur and loss of weight was observed in the majority of the mice given simultaneous drug treatment. In

* Norton Co., Worcester, Mass.

† Drummond Scientific Co., Philadelphia, Pa.

‡ G15T8 15 watt General Electric germicidal bulb.

§ Obtained from Microbiological Associates, Bethesda, Md.

TABLE I. Effect of Concomitant Therapy by Cortisone and NEITC in Mice Infected with Neurovaccinia Virus.*

Drug†	Dose (mg)	Day post-infection	Brain virus titers PFU	Brain virus titers LD_{50}	No. surviving/total day 14
Cortisone	10	1	$<10^{1.0}$	$<10^{1.0}$	5/5
NEITC	4	3	$10^{3.3}$	$10^{2.3}$	
		5	$10^{3.0}$	$10^{2.3}$	
		8	$10^{2.0}$	$10^{2.0}$	
		10	$<10^{1.0}$	$<10^{1.0}$	
Cortisone	10	1	$10^{3.0}$	$10^{2.0}$	0/5
MC		3	$10^{5.0}$	$10^{5.4}$	
		5	$10^{5.0}$	$10^{5.8}$	
		8	NS‡	NS	
Saline					
NEITC	4	1	$10^{1.0}$	$<10^{1.0}$	5/5
		3	$10^{2.0}$	$10^{1.0}$	
		5	$10^{3.0}$	$10^{2.4}$	
		8	$10^{2.0}$	$<10^{1.0}$§	
		10	$<10^{1.0}$	$<10^{1.0}$	
Saline		1	$10^{2.0}$	$10^{1.2}$	0/5
MC		3	$10^{5.0}$	$10^{4.2}$	
		5	$10^{5.0}$	$10^{5.0}$	
		8	NS	NS	

* Intracerebral challenge with 250 LD_{50}/0.03 ml.
† Two hours post-infection cortisone or saline injected via intraperitoneal route and NEITC or MC injected via subcutaneous route.
‡ NS = No survivors.
§ One of 5 mice died.

NEITC-treated mice, transitory signs of illness were noted but the mice gained weight. Neutralizing antibody was detected(2) on day 14 in the sera of mice treated with cortisone and NEITC or NEITC alone, and the mice were fully protected when challenged at this time with 5000 LD_{50} of the WR strain of vaccinia virus.

Effect of cortisone on interferon production in mouse brain. Groups of 10 mice were injected intracerebrally with 100 LD_{50} of vaccinia virus or a diluted extract of CAM. Two or 24 hours after infection the mice were treated with 10 mg of cortisone, 4 mg of NEITC or drug diluent. Brains were harvested from mice sacrificed on day 5 after infection and assayed for ITF content. Ten mice in each group were observed for survival over a period of 14 days. The results presented in Table II indicated that treatment with cortisone 2 hours post-infection suppressed the production of ITF, whereas ITF was detected in infected control brains. However, administration of cortisone at 24 hours did not inhibit production of ITF. When a virus challenge of 1000 LD_{50} was employed, treatment with cortisone at 2 hours did not prevent production of ITF. Nevertheless, mice given cortisone or diluent did not survive the lethal virus challenge. In contrast, ITF was not detected in the brains of mice that received cortisone and NEITC or NEITC and the majority of these mice survived the experimental period.

Effect of cortisone or NEITC on production of interferon by UV vaccinia virus. In order to assess the effect of cortisone or NEITC on the production of ITF, groups of 10 mice were treated with 10 mg of cortisone, 4 mg of NEITC, saline or MC 2 hours before intracerebral inoculation with 0.03 ml of UV vaccinia virus or UV control preparation. Brains were harvested 24 and 48 hours following inoculation and assayed for ITF content. The results summarized in Table III

TABLE II. Effect of Cortisone or NEITC on ITF Production in Vaccinia Virus Infected Mouse Brain.*

Drug†	Dose (mg)	Time of treatment post-infection (hr)	Brain ITF titer day 5	No. surviving/total day 14
Cortisone MC	10	2	<1:4	0/10
Cortisone NEITC	10 4		<1:4	10/10
Saline NEITC	4		<1:4	10/10
Saline MC			1:4	0/10
Cortisone MC	10	24	1:4	0/10
Cortisone NEITC	10 4		<1:4	8/10
Saline NEITC	4		<1:4	9/10
Saline MC			1:4	0/10
None		—	<1:4	10/10‡
None		—	<1:4	10/10§

* Intracerebral challenge with 100 LD_{50}/0.03 ml.
† Cortisone or saline injected via intraperitoneal route and NEITC or MC injected via subcutaneous route.
‡ Intracerebral inoculation with 0.03 ml of CAM extract diluted in tryptose phosphate broth.
§ Mouse brain not inoculated.

TABLE III. Effect of Cortisone or NEITC on Production of ITF by UV Vaccinia Virus in Mouse Brain.*

Drug†	Dose (mg)	Brain inoculum	Brain ITF titer 24 hr	48 hr
Cortisone	10	UV virus	1:4	<1:2
NEITC	4		1:8	<1:2
MC			1:8	<1:2
Saline			1:4	<1:2
Cortisone	10	UV CAM	<1:2	<1:2
Saline			<1:2	<1:2

* Intracerebral injection of 0.03 ml of UV virus or UV CAM.

† Cortisone or saline injected via intraperitoneal route and NEITC or MC injected via subcutaneous route 2 hr before intracerebral inoculation.

demonstrated that pre-treatment with cortisone or NEITC did not block the production of ITF in mouse brain. Under these conditions, ITF was detected in mouse brain at 24 but not 48 hours after inoculation with UV virus.

Histopathologic studies. Experiments were carried out to study the effect of concomitant treatment of cortisone and NEITC on the histopathology of viral meningoencephalitis. Groups of 10 mice were inoculated intracerebrally with 250 LD_{50} of vaccinia virus or a diluted extract of CAM. Two hours later, the mice were injected with 10 mg of cortisone and 4 mg of NEITC, cortisone, NEITC or drug diluent. Five days post-infection, at the time of elevated virus content in the brain, the mice were sacrificed and brains were harvested for histologic examination. No pathologic changes were noted in the non-infected control brain from either a cortisone- (Fig. 1) or NEITC-treated mouse. In the section of infected brain from a cortisone-treated mouse (Fig. 2), the finding of meningoencephalitis and numerous elementary and inclusion bodies was similar to that observed in an infected control brain. In the cortisone- and NEITC-treated mouse, the inflammatory response was minimal and virus elementary and inclusion bodies were not observed (Fig. 3). This finding is in contrast to the inflammatory response observed in a section of brain from a NEITC-treated mouse (Fig. 4). Finally, histopathologic changes were not observed in mouse brain inoculated with UV vaccinia virus.

Discussion. The results of the present study are, in general, consistent with the reports of beneficial effects observed in the treatment of certain forms of herpes simplex keratitis with 5-iodo-2′-deoxyuridine (IUDR) and corticosteroids(3-5) or steroid combined with IUDR and 5-fluoro-2′-deoxyuridine(6). The data indicated that treatment with cortisone did not antagonize the antiviral efficacy of NEITC. This was demonstrated by mouse survival and virus titers similar to those in NEITC-treated mice. Concomitant therapy with cortisone and NEITC did not suppress production of neutralizing antibody, and the mice were immune to challenge with vaccinia virus. However, mice given simultaneous therapy showed a loss of weight compared to NEITC-treated mice.

Cortisone was shown to inhibit the production of ITF in virus-infected chick embryos (14) and rat tissue cultures(15). As noted previously(7) and confirmed in this study, cortisone given 2 hours after infection with neurovaccinia virus inhibited the formation of ITF in mouse brain. These findings were extended by a recent report that steroids depressed ITF levels in the sera of mice infected with Sindbis virus(16). However, the inhibitory action of cortisone on the production of ITF in mouse brain was lost when treatment was delayed 24 hours or when cortisone was given 2 hours following a virus challenge of 1000 LD_{50}. Yet, ITF was not detected in mice given delayed treatment with cortisone and NEITC or NEITC alone, and the majority of the mice survived. Cortisone or NEITC did not inhibit the mechanism of ITF production, as demonstrated when the drug was given 2 hours prior to inoculation with UV virus. The brain inhibitor is interferon or interferon-like since it exhibited the physical and biological properties reported for ITF(11,17-20).

Histopathologic studies of brains from infected mice treated with cortisone indicated that the inflammatory response was not inhibited and numerous elementary and inclusion bodies were noted. This finding is in accord with previous reports that cortisone did not affect influenza virus(21) or Newcastle disease virus-induced lung inflamma-

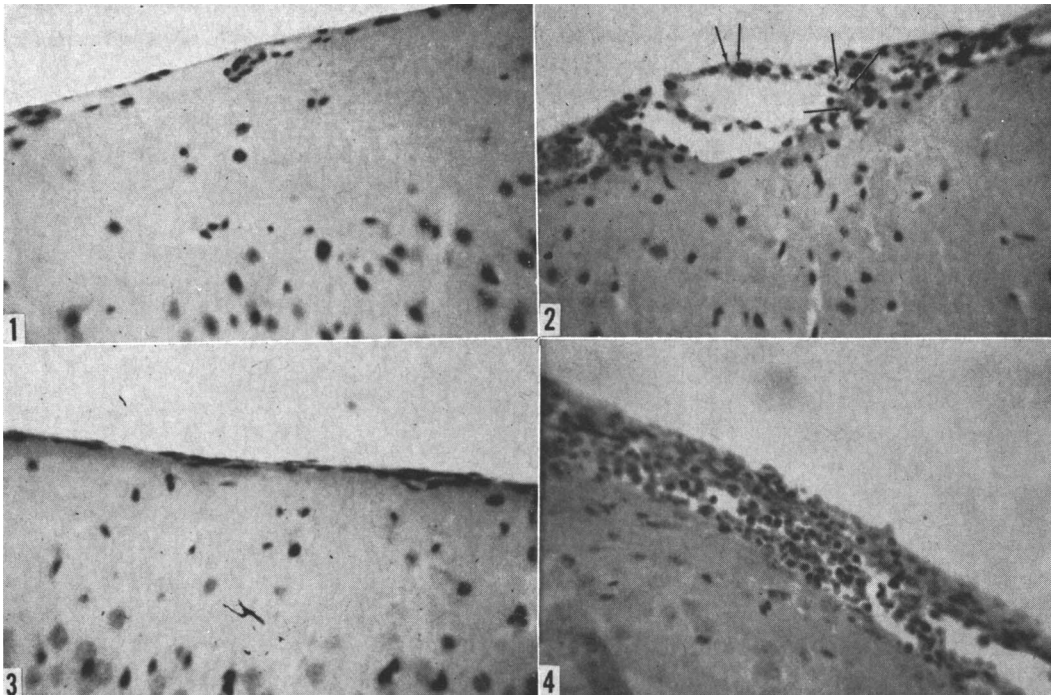

FIG. 1. Normal brain inoculated with an extract of CAM. Cortisone-treated mouse sacrificed 120 hr post-inoculation. Hematoxylin and eosin (× 232).

FIG. 2. Vaccinia-infected brain from a cortisone-treated mouse sacrificed 120 hr post-infection. The meninges show an inflammatory response and the presence of elementary and inclusion bodies. Hematoxylin and eosin (× 232).

FIG. 3. Vaccinia-infected brain from a cortisone- and NEITC-treated mouse sacrificed 120 hr post-infection. The meninges are slightly thickened and elementary or inclusion bodies are not observed. Hematoxylin and eosin (× 232).

FIG. 4. Vaccinia-infected brain from a NEITC-treated mouse sacrificed 120 hr post-infection. The inflammatory changes are not suppressed. Elementary or inclusion bodies are not observed with certainty. Hematoxylin and eosin (× 232).

tion in mice(22). However, in brains from mice given simultaneous treatment with cortisone and NEITC, the inflammatory response was markedly reduced and virus elementary or inclusion bodies were not observed. These experimental findings in neurovaccinia-infected mice suggest that combination therapy of a corticosteroid with an effective antiviral drug may demonstrate a useful therapeutic approach in controlling virus growth and host response to virus infection.

Summary. The effect of concomitant treatment with cortisone and N-ethylisatin β-thiosemicarbazone (NEITC) was investigated in mice infected with neurovaccinia virus. The findings demonstrated that a single dose of cortisone did not reduce the antiviral activity of NEITC. Mice given NEITC and cortisone were fully protected but showed a loss of weight prior to recovery. Cortisone inhibited interferon (ITF) formation when administered at 2 but not 24 hours post-infection. In this respect, cortisone given 2 hours post-infection was ineffective against a challenge of 1000 LD_{50}. ITF was not detected in the brains of infected mice treated with cortisone and NEITC or NEITC. On the other hand, pre-treatment with cortisone or NEITC did not inhibit production of ITF by UV vaccinia virus. Finally, concomitant therapy with cortisone and NEITC markedly reduced the inflammatory response in the brain; elementary or inclusion bodies were not observed and antibody formation was not suppressed.

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Oxytetracycline and Hypoglycemia with Convulsions in Pancreatectomized Dogs.* (31169)

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During an investigation into the source of serum amylase, dogs with vagotomy, pyloroplasty and staged removal of the pancreas and small intestine were prepared. The animals were maintained post-operatively by intravenous infusion of a solution of half normal saline containing 50 g of glucose, 15 units of regular insulin and 200 mg of oxytetracycline per liter. It was found that after several days, the dogs developed hypoglycemia with staggering and convulsions. In these animals the liver glycogen was usually normal and occasionally elevated. It was subsequently determined that neither the enterectomy nor the vagotomy and pyloroplasty was needed for the development of this condition. It was found that pancreatectomy was the only necessary operative procedure if the animal was maintained post-operatively by intravenous infusion of fluid containing oxytetracycline.

This report describes a condition of hypoglycemia with convulsions that can be induced in pancreatectomized dogs when oxytetracycline is added to intravenous fluid used to maintain them post-operatively. The report also describes the response of the blood glucose of these animals to intravenous injection of glucose and of glycogenolytic agents.

Method. Inasmuch as the operative procedures other than pancreatectomy had no influence on the experimental results, the operated animals were pooled for analysis of the influence of oxytetracycline.

Fourteen dogs of both sexes, weighing be-

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