of such small quantities.

Discussion. The studies of Zaimis(6) have indicated that the immunosympathectomized animal shows a sensitization to both epinephrine and norepinephrine, comparable to the effect of surgical denervation. Brody(7) has also found increased sensitivity to norepinephrine and in addition noted that urinary excretion of catecholamine was depressed in immunosympathectomized rats. This preparation appears to be well suited for studies of performance of various organs deprived of sympathetic innervation.

The failure of the brain and the adrenal glands to show any decline in norepinephrine content after administration of the antiserum to the sympathetic nerve growth promoting factor might suggest that the catecholamine-producing cells in those organs react either quantitatively or qualitatively differently from peripheral sympathetic neurones. Berk *et al*(2) have reported that under antiserum treatment conditions which lead to virtual disappearance of sympathetic ganglion cells the adrenal medullary cells remained intact.

Summary. In immunosympathectomized mice at one month after specific antiserum treatment there is almost complete (87-95%) disappearance of norepinephrine in heart, spleen and kidney. There is no change in brain content, but there is a probably significant increase in adrenal norepinephrine content. Values for epinephrine after immunosympathectomy are not significantly altered from control levels, except perhaps in the kidney.

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Received May 25, 1965. P.S.E.B.M., 1965, v119.

Interferon Production in Powassan Virus Infections of Chickens. (30423)

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Interferon appears rapidly in the serum of several experimental hosts following intravenous inoculation of large doses of certain viruses, bacteria and bacterial endotoxins(1,2,3). In mice, Baron *et al*(4) found that interferon was not detectable during later stages of nonfatal infection with influenza and lactic dehydrogenase viruses despite continued viral multiplication. However, a close relationship between interferon and virus titers persisted throughout infection with encephalomyocarditis virus. The role of interferon in recovery from viral infections has recently been reviewed(5,6,7).

Powassan virus(8), a group B arbovirus transmitted by ticks(9), produced inapparent infections in young chicks inoculated subcutaneously with one to 100 mouse LD_{50} of virus(10). Viremia reached maximum titers on the third or fourth day, falling rapidly thereafter. The present study reports the production of interferon following intravenous (IV) inoculation of massive doses of Powassan virus into chickens aged 6 days and 3 months.

Materials and methods. Tissue cultures. Primary cultures of chick embryo fibroblasts (CEF) were prepared by trypsinization of 11-day-old embryos from which the beak, eyes and limbs had been removed. The growth medium was a modified Gey's balanced salt solution(11) containing 0.25% lactalbumin hydrolysate and 0.1% yeast extract (GLY) together with 5% bovine serum

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previously heated at 56°C for 30 minutes. Ten million cells in 5 ml of growth medium were dispensed in 60 mm plastic tissue culture dishes† and incubated at 37°C in a humidified atmosphere containing 4% carbon dioxide. Cultures were inoculated on the following day at which time monolayers covered the entire surface of the dishes.

Primary cultures of swine kidney epithelial cells (SK) were prepared by trypsinization of kidneys removed aseptically from specific pathogen-free pigs aged 8 weeks. Cells were grown in stoppered roller tubes in a medium consisting of Earle's balanced salt solution, 0.5% lactalbumin hydrolysate and 0.1% yeast extract (ELY) together with 5% bovine serum. Following virus inoculation, cultures were maintained in medium ELY with 2% bovine serum.

Viruses. A seed lot of Powassan virus (Byers strain) (8) in its 10th suckling mouse brain passage was prepared by intracerebral (IC) inoculation of suckling mice aged 3 to 4 days. Mouse brains were harvested when signs of encephalitis developed 4 days later, ground up and extracted with saline containing 10% bovine serum to give a 20% suspension. Amounts of 0.1 ml were stored in sealed ampoules at —70°C. Large stocks of Powassan virus for single experiments were prepared by inoculating weaned mice aged 3 to 4 weeks with seed virus propagated in suckling mice. Harvested brains were ground with medium ELY to give a 20% suspension. Infectivity of virus seed lots was assayed by IC inoculation of groups of 5 weaned mice with 0.03 ml aliquots of serial 10-fold dilutions of virus in bovine serum-saline.

Eastern equine encephalomyelitis (EEE) virus (original strain) (12) was obtained from Dr. P. J. G. Plummer, Animal Disease Research Institute, Hull, P.Q. In Toronto it had been passaged 5 times in suckling mouse brain. Seed virus contained $10^{11.0}$ plaque-forming units (PFU) per ml by inoculation of primary CEF cultures with serial 10-fold dilutions of virus in medium GLY.

Vesicular stomatitis virus, Indiana strain, (VSV) was obtained from Dr. S. Baron, National Institutes of Health, Bethesda, Md. Seed virus was propagated in primary CEF cultures and contained $10^{7.6}$ PFU per ml.

Chicken inoculations. Serum was obtained from each bird one day before virus inoculation. White Leghorn chickens of both sexes, aged 3 months and weighing 1.8 to 2.1 kg, were each injected into the wing vein with $10^{10.1}$ mouse LD_{50} of Powassan virus contained in 3 ml. At 1½, 2½, 3½, 6, 8, 24 and 96 hours after inoculation 2 or 3 birds were bled. Blood samples were held overnight at 4°C. After sera were separated from clots by centrifugation at 1500 RPM for 10 minutes, the clots were stored regularly at —70°C to await titration for infectivity. The virus content of sanguinous fluid expressed from thawed clots from individual birds was determined by inoculating 0.1 ml aliquots of serial 10-fold dilutions into each of 4 primary SK monolayer cultures. Pronounced cytopathic effects were observed in infected cultures after 4 to 7 days' incubation in stationary racks at 37°C(13).

Chicks aged 6 days, weighing 50 to 52 g, were each injected into the wing vein with $10^{9.3}$ mouse LD_{50} of Powassan virus contained in 0.3 ml. This inoculum provided a virus dose per unit of body weight comparable to that injected into 3-month-old chickens. Proportions of plasma (6.6%) and total blood volume (9.6%) are essentially constant when expressed as percentages of body weight, regardless of age and weight of the chicken(14). Other chicks aged 6 days were inoculated IV with $10^{2.9}$ mouse LD_{50} of virus contained in 0.03 ml. At intervals of 1, 2, 3, 4, 6, 8, 24, 72 and 96 hours after injection, 3 chicks were exsanguinated by cardiac puncture. The 3 blood samples taken at each time interval were pooled prior to assay for virus and interferon.

Interferon assay. The reactions of sera were brought to pH 2 by addition of 6N hydrochloric acid. The sera were held at 4°C for 2 to 5 days to eliminate infectious virus (1). The pH of each specimen was then returned to 7.2 by addition of 1N or 5N sodium hydroxide. Treated sera were stored at 4°C pending assay. All pre-inoculation sera were similarly treated.

Preparations were titrated for inhibitory

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TABLE I. Viremia in 6-Day-Old Chicks Inoculated with Powassan Virus: Comparison of Titrations in Swine Kidney Tissue Culture and Mice.

	Hr after inoculation								
Assay	1	2	3	4	6	8	24	72	96
S.K.*	5.5	5.3	4.3	3.8	3.3	3.8	4.0	0	1.8
Mouse†	6.2	5.2	n.t.	4.2	2.9	3.1	2.5	1.9	1.6

* Log TCD_{50} per ml clotted blood.
† Log mouse LD_{50} per ml clotted blood.
n.t. = not tested.

substances using a modification of Wagner's plaque-reduction technique(15). Serial 2-fold dilutions of treated sera beginning at 1:6 were made in medium GLY, and 0.5 ml amounts were added to drained, washed, duplicate CEF cultures. Following adsorption for 2½ to 3 hours at 37°C, plates were washed twice with 5 ml Hanks' balanced salt solution and challenged with 30 to 50 PFU of EEE virus. Cultures were drained after a one-hour adsorption period and overlaid with 0.9% agar (Difco, Noble) in medium GLY plus 5% bovine serum. A second agar overlay containing 1:20,000 neutral red (final dilution) was added 24 hours later. Virus plaques were scored after 48 hours' incubation. Interferon titers, expressed as units per 0.5 ml of serum, were recorded as the reciprocal of the highest dilution of serum which reduced by 50% the number of plaques counted in controls.

To eliminate the effects of possible nonspecific viral inhibitors, controls included, for each dilution of test serum, a similar dilution of pre-inoculation serum from the corresponding bird or group of birds. A stock reference preparation of chick interferon(16) was used at known titer on each day's assay to detect any changes in sensitivity of the system.

Results. Infectivity titrations in tissue culture and mice. Virus titers in blood samples following inoculation of 6-day-old chicks with $10^{9.3}$ mouse LD_{50} (Table I) usually showed, at most, 2- to 5-fold differences by simultaneous inoculation of mice and SK cultures, but somewhat greater discrepancies were observed in samples collected at 24 and 72 hours. In all subsequent infectivity titrations for Powassan virus, primary SK cultures were employed.

Viral inhibitor in serum. Several specimens of serum contained a factor which reduced both the size and total number of plaques present in control cultures. This inhibitor had the following characteristics: (a) stable at pH 2 for 5 days, (b) not inactivated at 56°C for one hour, (c) not sedimented at 105,000 *g* for 2 hours, (d) did not reduce the titers of EEE virus preparations upon direct contact in a cell-free medium for one hour at room temperature, (e) caused similar plaque reduction in CEF cultures challenged with VSV in place of EEE virus and (f) did not protect primary SK cultures from virus challenge. These properties are in keeping with chicken interferon described by other investigators(5). Preparations tested contained no infectious material since mice survived following IC inoculation with undiluted serum. Intravenous injection of a 20% suspension of uninfected normal mouse brain did not stimulate interferon production in 6-day-old chicks.

Course of viral infection. Following intravenous injection of $10^{10.1}$ mouse LD_{50} of Powassan virus into chickens aged 3 months, interferon was detected in serum at 1½ hours, reached maximum levels by 3 hours and was undetectable after 8 hours (Fig. 1). Viremia diminished rapidly during this interval and was absent by the 4th day.

Circulating interferon was present in 6-day-old chicks at 4 and 6 hours, but was not detected subsequently, following IV inoculation of $10^{9.3}$ mouse LD_{50} of virus (Fig. 2). Viremia diminished over the first 3 hours, remained at moderate levels until 24 hours, then fell to low titers by the 3rd and 4th days.

Sera of chicks inoculated with $10^{2.9}$ mouse LD_{50} of virus contained no detectable interferon during the 4-day period (Fig. 3). Viremia increased from negligible amounts to high titers on the 3rd and 4th days, approximating those reported for 2-day-old chicks(10).

No bird developed any sign of illness when observed up to 3 weeks following inoculation with Powassan virus.

Discussion. The phenomenon whereby a large virus inoculum multiplies to a titer lower than that resulting from a small dose of the same virus has been extensively reviewed by Schlesinger(17). The present ex-

periments further exemplify this paradoxical relationship and support recent evidence(18) that interferon production may be closely related to some types of viral interference. Interferon, detectable in the serum of young chicks 4 to 6 hours after injection of a large dose of virus, may have been rapidly taken up by a large proportion of host cells early in the course of infection(1,7). These cells would be unlikely to participate in virus replication since the antiviral activity of adsorbed interferon may persist for 2 days or longer(15,19,20).

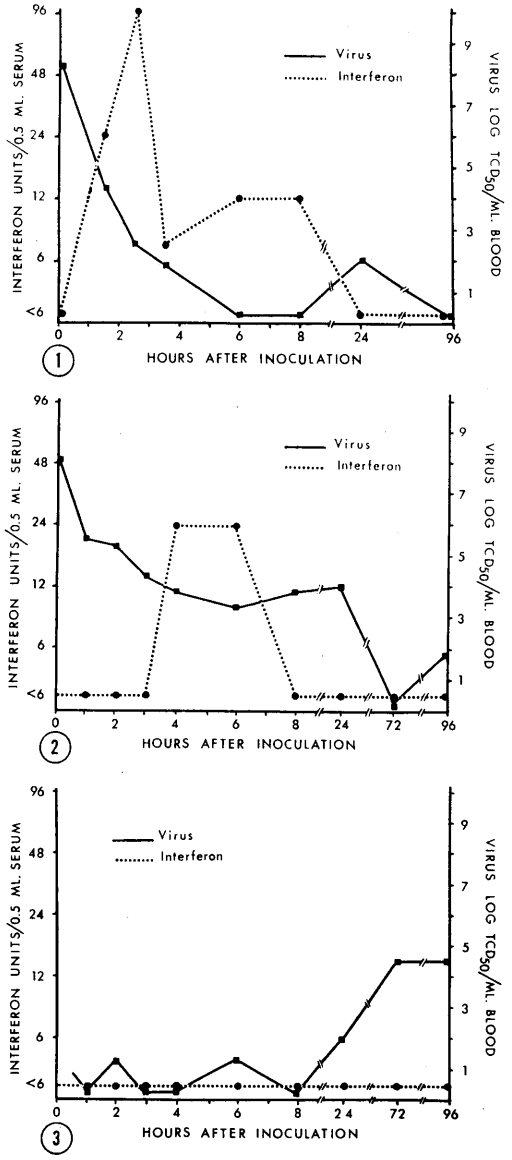

FIG. 1. Titers of circulating virus and interferon in 3-month-old chickens inoculated IV with a large dose of Powassan virus.

FIG. 2. Titers of circulating virus and interferon in 6-day-old chicks inoculated IV with a large dose of Powassan virus.

FIG. 3. Titers of circulating virus and interferon in 6-day-old chicks inoculated IV with a small dose of Powassan virus.

The clearance of virus from the blood following a large inoculum was rapid during the first 2 to 3 hours, then became more gradual (Fig. 1 and 2). Viremia present during the initial 24 hours (Fig. 2) may have resulted partially from virus replicated by cells exposed at time of inoculation and therefore not protected by interferon. However, Mims(21) has shown that ectromelia virus not rapidly adsorbed from the circulation in mice following IV inoculation was present in leukocyte and platelet fractions of peripheral blood. The proportion of uncleared virus was small when less than 10^6 to 10^8 ID_{50} were injected but increased in direct relationship to larger doses. Failure to detect rapidly-appearing interferon in serum following inoculation of small doses of virus(1) suggests that a certain critical number of virus particles must enter the vascular system simultaneously. It is possible that virus which exceeds this amount is not readily adsorbed but rather becomes associated with circulating leukocytes which are capable of interferon production(22,23).

The development of lower titers of interferon in 6-day-old chicks, approximately 2 to 3 hours after maximum levels were reached in older birds, supports other evidence(24,25, 26) that interferon production is influenced by the age of host cells. Heineberg *et al*(26) showed that, with increasing age of mice, the ability to form interferon increased, resulting in decreased multiplication of Coxsackie B1 virus and a corresponding reduction in mortality.

Summary. Levels of circulating virus and interferon were followed in chickens inoculated intravenously with Powassan virus. A massive dose of virus ($10^{9.3}$ mouse LD_{50}) given to 6-day-old chicks resulted 3 to 4 days later in virus titers lower than those developed by chicks of the same age receiv-

ing only $10^{2.9}$ mouse LD_{50}. Circulating interferon was detected in the former group at 4 and 6 hours following inoculation but at no time was it found in birds given the smaller dose. Interferon appeared more rapidly and in higher titer in the serum of 3-month-old chickens inoculated with $10^{10.1}$ mouse LD_{50} of virus.

The advice and encouragement of Dr. D. M. McLean throughout this project is gratefully acknowledged.

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Received May 28, 1965. P.S.E.B.M., 1965, v119.

Effect of Hypophysectomy on Feed Intake in Rats.*† (30424)

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The variation in normal feed intake of mature female rats of the Sprague-Dawley-Rolfsmeyer strain has been reported(1). It has been suggested that feed intake is primarily under neural regulation. The literature has been reviewed recently by Anand (2). That the endocrine glands and their hormones may also play an important role in the regulation of feed intake has been recognized but only limited research of a quantitative type has been reported.

Study of the endocrine control of feed intake may involve the administration of graded levels of the hormones to normal animals or the influence of endocrine gland removal on feed intake may be observed with subsequent replacement therapy.

The effect of thyro-parathyroidectomy and of adrenalectomy separately and together on feed consumption has been reported from this laboratory(3). In addition, the effect of alloxan diabetes on feed consumption has been observed(4).

It has been recognized for many years that hypophysectomy of the rat reduces feed intake but quantitative studies have not been reported(5). Since the pituitary is a key

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2954. Approved by the Director.
† Aided in part by research grants from USPHS.