probably accounts for the observation that normal frogs can regain lost body water faster than hypophysectomized frogs. In any case, this observation suggests strongly that the release of hormone from the neurohypophysis can serve a physiologically useful purpose in the frog. In the toad, which is much more sensitive to the antidiuretic effect of pituitrin(10), it would appear likely that the neurohypophysis could exert a significant effect on the rate of water loss as well.

Summary. 1. The hormone content of the frog neurohypophysis is depleted by dehydration of the animal. 2. Normal and hypophysectomized frogs become dehydrated at the same rate. Normal frogs, however, regain the body water lost by dehydration more rapidly than do hypophysectomized frogs. 3. These observations suggest that the neurohypophysis plays a physiologically significant role in the regulation of the water balance of the frog.

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In vitro Effects of Cortisone on Multiplication of Influenza B Virus.* (20090)

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Infections with a wide variety of organisms including protozoa(1), fungi(2), bacteria (3,4), and viruses(5,6) may be increased in severity by the administration of cortisone to the host. This virtually universal effect remains unexplained despite evidence that cortisone suppresses such physiological host reactions as inflammation(7) and antibody formation(8), depresses granulocytosis(9), and induces lymphocytolysis and the dissolution of lymphoid tissue(10). Although many studies of cortisone and infection have been concerned only with host mortality and disease, some have demonstrated increased concentrations of the infecting agent(3,4,11). It appeared of obvious importance to establish whether an increase in an infecting agent might be induced by cortisone in host tissue isolated from the influence of collateral cortisone effects on phagocytic infiltration and antibody reaction. Accordingly, the present study was undertaken of the multiplication of Influenza B virus in isolated sections of chick chorioallantoic membrane.

Materials and methods. Virus. Influenza B virus (Lee strain) was used. Aliquots of the same seed pot, stored in glass at —65°C were used throughout the study. 0.1 ml of $10^{7.9}$ or $10^{6.9}$ E.I.D.$_{50}$ of virus solution (undiluted and 10-fold diluted allantoic fluid) was inoculated into 2 ml of the following medium to give initial virus concentrations in the nutrient fluid of $10^{6.6}$ and $10^{5.6}$ E.I.D.$_{50}$ per ml. *Culture medium.* Equal volumes of modified glucosol solution as described by Fulton and Armitage(12) and phosphate buffer adjusted to 7.3 pH were mixed immediately before use and penicillin and streptomycin were added to yield final concentrations of 10 units and 40 mg/ml as described by Tamm *et al.*(13). This solution was distributed in test tubes (18 × 150 mm) in amounts of 2 ml. Tubes were stoppered to prevent evaporation. *Tissue*

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TABLE I. Distribution of Log_2 of Titer Ratios with t Values and Mean Titers of Cortisone and Control Groups.

Initial EID_{50}	Incubation, hr	Log_2 T ratios* −2	−1	0	1	2	3	Total N	Student's t, ts† vs to‡	Mean titer§ Cortisone	Control
$10^{6.6}$	0									24	24
	12		2	10	2	1		15	.280 < 2.977	35	28
	24		3	7	5			15	.693 < 2.977	55	51
	36		1	8	10	1		20	3.58 > 2.861	71	49
	48			6	9	4	1	20	5.10 > 2.861	83	41
	60			3	11	5	1	20	7.01 > 2.861	76	37
$10^{5.6}$	0									2	2
	12	2	2	9	2			15	.354 < 2.977	17	12
	24			11	4			15	2.25 < 2.977	49	42
	36		1	7	9	3		20	3.90 > 2.861	68	48
	48			3	10	2		15	6.05 > 2.977	62	32
	60			1	10	3	1	15	6.84 > 2.977	52	28

* T ratio: Titer ratio of cortisone and control tubes.
† ts: Sample value of t when $M = 0$.
‡ to: $Pr\{|t| > 2.977\} = .01$ when degree of freedom is 14.
$Pr\{|t| > 2.861\} = .01$ " " " " " 19.
§ Reciprocal of virus, arithmetic dilutions.

specimens. Specimens of chorioallantoic membrane measuring approximately 13 × 13 mm were taken from White Leghorn eggs of 10-12 days incubation. One membrane sample was added to each tube, and 5 tubes containing specimens from as many different eggs were used in each group in each experiment. *Cortisone.* (Free alcohol; 11-dehydro-17-hydroxycorticosterone, Merck†). A saturated aqueous solution of 280 μg/ml was prepared and sterilized by heating at 100°C for 30 minutes. 0.1 ml of this solution was added to the culture medium to give a final concentration of 14 μg/ml of nutrient fluid. An equivalent volume of distilled water was added to control test tubes. *Virus titrations.* Virus concentrations were measured by the conventional hemagglutination technic, employing serial 2-fold dilutions of 0.2 ml volumes of culture media and equal volumes of 1% human "O" RBC. Tubes were usually read independently by both investigators after a one hour period at room temperature. *Incubation of cultures.* Groups of test and control tubes inoculated with virus were incubated in a Warburg water bath adjusted to 35°C and were shaken at a rate varying from 70 to 95 strokes/minute with a stroke amplitude of 5 cm. After incubation for various time periods, as described below, 0.2 ml of the medium was withdrawn for hemagglutination titration and the same amount of fresh medium added to maintain a constant volume.

Design of experiments. Early experiments confirmed the finding of Tamm(14) that great variation in virus production might occur among membrane specimens from different eggs; therefore, care was taken to distribute tissues from each egg used through all experimental groups, and statistical analysis of experimental results has compared only pairs (cortisone and control tubes) containing membranes from the same egg. As 2-fold dilution series have been used in viral titrations, titer ratios of comparable pairs have been expressed as the logarithms (to the base 2) of the arithmetic dilutions in order to expedite analysis of data.

Experimental results. Control studies. To determine experimental error, 2 series of tubes containing the same amounts of virus (and no cortisone) were incubated and virus titrations performed at 45, 60, and 72 hours, and the distribution of the log_2 titer ratios of comparable pairs was examined. Statistical analysis revealed (with less than 1% risk) that both groups ($10^{6.6}$ and $10^{5.6}$ initial $E.I.D._{50}$) belonged to the same population, the mean of which is zero. Therefore, the 2 groups were combined and discardable values calculated

† Cortisone was generously supplied by Merck and Co., Rahway, N. J.

(with 1% risk). Values lay outside the following range: $1.54 \geqq \text{Log}_2$ T ratio $\geqq -1.40$. Thus, under the same conditions, or if no variable (*e.g.* cortisone) is added in one series as in this experiment, the Log_2 titer ratios will not be expected to exceed ± 2.

Effect of cortisone. Comparative viral increases with time in control and cortisone-containing tubes are summarized in Table I, in which mean hemagglutination titers derived from 15-20 separate titrations in 3-4 experiments are tabulated. It is evident that no essential differences in viral concentration are seen until the 36th hour of incubation, at which time and thereafter significant differences in virus titer are observed between control and cortisone-treated groups. Evidence for the statistical significance of the titer differences at 36, 48, and 60 hours is presented in Table I, in which the distribution of the Log_2 of the titer ratios of cortisone to control tubes is illustrated. It is notable that only twice in 110 titrations in the later time periods was the titer ratio less than ½ (indicating the possibility of less virus in the cortisone tube), and in most instances the concentration of virus was 2-fold or more greater in cortisone than in control tubes. Experiments using an initial inoculum of $10^{4.6}$ E.I.D.$_{50}$ have demonstrated results comparable to those described above with larger viral inocula. Preliminary experiments with 10-fold greater concentrations of cortisone have demonstrated slightly less viral increase than observed with the smaller amount.

Egg infectivity titrations. Despite marked differences in the amounts of virus as measured by hemagglutination, egg-infective virus decreased in amount in both cortisone and control groups after 60 hours of incubation. E.I.D.$_{50}$ were $10^{3.5}$ and $10^{3.0}$ at the end of one experiment, although the initial E.I.D.$_{50}$ had been $10^{6.6}$ and $10^{5.6}$. There were no significant differences in the infectivity titers of cortisone and control groups, as might be expected with this less precise method.

Histologic study. After 60 hours of incubation, chorioallantoic membranes were fixed in Zenker's solution and stained with H and E. Microscopic examination revealed no differences between cortisone-treated and control membranes.

Bacterial contamination. In the course of 9 experiments including 198 tubes no instances of bacterial contamination have been revealed by aerobic culture on blood agar plates of culture fluids following the completion of experiments.

Direct effect of cortisone on viral hemagglutinin. Incubation of virus and cortisone in the absence of tissue has shown no evidence of any effect of cortisone on the concentration of hemagglutinating virus.

Discussion. It has been demonstrated that the addition of small quantities of cortisone to a tissue culture medium results in significantly greater final concentrations of Influenza B virus than observed in cultures without cortisone. Studies of virus concentration at varying time intervals have not revealed differences in the *rate* of increase, however, but instead have indicated continued increase of virus in cortisone cultures in the later time periods. Experiments in this laboratory have shown that cortisone in appropriate dosage may increase the survival time of Lee virus-infected chick embryos(15) and decrease the pathological reaction in the chorioallantoic membrane(16) despite a concomitant increase in virus greater than observed in shorter-surviving control eggs. It is suggested, therefore, that the increase in viral concentration induced by cortisone in the present study may result from prolonged survival of membrane cells permitting continued viral multiplication after "death" of control tissue has occurred. Histologic study of membranes at the crucial 36-hour period may adduce further evidence for this hypothesis.

Summary. The addition of cortisone to chick embryo chorioallantoic membrane tissue cultures results in significantly greater final concentrations of Influenza B virus than observed in controls.

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Further Studies on Oral Administration of Living Poliomyelitis Virus to Human Subjects. (20091)

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In a previous study(1) the results of oral administration of living poliomyelitis virus to 20 human volunteers were reported. In most of the subjects the virus was recovered from the stool; 16 subjects who were not immune prior to the feeding developed Lansing type neutralizing antibodies in their blood; none of the subjects showed any apparent untoward effects from the administration of the virus nor was any febrile response noted.

In the present communication, the results are reported of the effects of oral administration of the same type of virus to 61 human subjects, all of whom had no Lansing antibodies prior to the experiment. It will be seen that the results obtained in the first trial have been confirmed and to a certain extent amplified.

Materials and methods. The subjects for experiment were selected in the following way: 181 mentally defective children ranging in age from 8 months to 8 years were bled in April, 1952, and the sera tested for neutralizing antibody content against the MEFl strain, Type II poliomyelitis virus (see below). The children were inmates of a State institution for mental defectives. Of the children who had no antibodies, 61 were selected for the trial after permission of each child's parents had been legally obtained. Each child was tested again for presence of neutralizing antibodies during the last week of June, 1952, and fed the virus on July 1. During the following period of 21 days the children were placed in an isolation unit under constant medical observation and nursing care. Rectal temperatures were taken every 8 hours and search was made daily for development of somatic disturbances with particular attention to the central nervous system. Stools for virus isolation were collected from every treated child on the 5th, 8th, 12th, 15th, 18th and 21st days after feeding. Blood for virus isolation was obtained from each subject by venous puncture. The patients were divided into two groups and members of each group were bled on alternate days for 12 days, starting on the first day after feeding. Thirty days following virus feeding, all patients were bled and the sera submitted to

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