in man(6). We have but a single experiment on this analog in the climbing rats; 10 mg/kg failed to block the effect of 1 mg/kg of LSD. Further experiments of the effects of this drug in LSD-treated animals are indicated.

Summary. 1) A syndrome resulting from intraperitoneal injection of LSD in rats is described. In rats trained to climb a rope, LSD produces signs of confusion and markedly prolongs climbing time. This effect on climbing time can be objectively and quantitatively measured, and is linearly related to the log dose of LSD. 2) Effects of LSD could be partially antagonized by serotonin or by 5-hydroxy tryptophane administered intraperitoneally. α-4 (piperidyl) benzhydrol did not affect the action of LSD, while reserpine intensified and prolonged the LSD-syndrome.

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Reversible Effect of Hypotonic Solutions on Growth of Influenza Virus in Tissue Cultures.* (22454)

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A previous publication from this laboratory (1) has described the inhibitory effect of potassium deficiency on growth of influenza virus in chorioallantoic (CA) membrane suspended in a medium containing the usual amounts of other inorganic salts and glucose or pyruvate. Although there was some evidence that K^+ may play a part in penetration of virus into cells, it was suggested that deficiency of this ion causes inhibition of intracellular enzyme systems concerned in virus syntheses. Previous investigations have emphasized the necessity of NaCl or other electrolytes at concentrations from 0.01 M to 0.15 M for adsorption of influenza virus to red cells(2), to mucoid hemagglutinin inhibitors, and for enzymatic action of virus on mucoid inhibitors(3). Absence or a low concentration of electrolytes resulted in diminished adsorption of influenza virus hemagglutinins to CA membrane *in vitro*(4) and similar observations had been made earlier with mouse lung and pneumonia virus of mice(5). Salt is also necessary for adsorption of phage or influenza virus to cationic ion exchange resins (6).

In most of the work just cited little effect on virus adsorption was observed unless the concentration of electrolyte in the solution was reduced to values of 0.01 M or less. In the observations to be described partial and reversible inhibition of virus growth occurred in suspensions of CA membrane with Hanks' balanced salt solution diluted to contain electrolyte between 0.04 and 0.08 M. Addition of NaCl to return the salt concentration to normal allowed growth of virus to proceed. It is believed that the inhibition of virus growth may be attributed more to reversible effects on tissue metabolism, as suggested by measurements of O_2 consumption, than to prevention of adsorption through purely electrostatic effects at the cell membrane. The relative roles of electrolyte (NaCl) and osmotic effects produced by various concentrations of glucose on growth of virus and its dissociation from tissue were investigated.

Materials and methods. Tissue suspensions of minced CA membranes from 11 or 12 day

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chick embryos were prepared as previously described(7) using shaken flasks or roller tubes. *Virus*—The PR8 strain of influenza A was added in amounts representing about 100 tissue culture infectious doses. Infectivity titrations were done in embryonated eggs, hemagglutinin titrations (HA) by the Salk method. *Balanced salt solution (BSS)*—Hanks' salt solution (without bicarbonate) was prepared at the usual concentration (100%BSS) and diluted 1:2 (50%BSS), 1:4 (25%BSS) with water. As energy sources 1 mg/ml of sodium pyruvate and 0.2 mg/ml of glucose were added to both diluted and undiluted BSS. In certain experiments with diluted BSS, NaCl was added in amounts (6 mg/ml of 25%BSS) necessary to return the electrolyte concentration to "normal." Glucose at a concentration of 25 mg/ml (0.14M) was also used for readjustment of the osmotic pressure of the 25% BSS.† Removal of excess electrolytes from the surface of the tissue was accomplished by washing the minced tissue in the concentration of BSS to be used in the particular experimental group. Tissue was suspended in about 10 volumes of solution and packed in a graduated 15 cc centrifuge tube at 1,500 rpm for 15 minutes, the supernatant removed and the processs repeated. The final volume of packed tissue‡ was used as the measure of the amount added to tissue cultures or Warburg vessels. *Viability of tissue* was evaluated by observing the extension of epithelium and fibroblasts on the glass wall of the roller tubes and by measurements of oxygen consumption in the Warburg apparatus. Unless otherwise noted the latter were done in 100%BSS with 1 mg/ml glucose for periods of 3 to 5 hours and the results expressed as μl O_2 per ml wet tissue per hour. Possible alterations of the volume of tissue by osmotic effects will be considered later.

† 25 mg/ml of glucose is taken as equivalent to 4.0 mg/ml NaCl in osmotic effect. Normal concentration of NaCl in BSS is 8 mg/ml (.138 M). KCl is 0.4 mg/ml (.018 M) and other inorganic constituents are less than 0.4 mg/ml.

‡ Measured in protein determination centrifuge tube with graduations in 0.01 ml.

Results. To confirm that no effect on adsorption occurs under the conditions of our experiments, a test similar to those previously described(4) was done with minced tissue in the presence of 25%BSS and 100%BSS both containing 0.3% serum albumin as a stabilizing agent. Tissue washed in the respective concentrations of BSS was added in amounts of 0.4 ml per 4 ml of medium and virus to make approximately 100 HA units per ml in one series and 20 per ml in another was incubated with the tissue for periods of 1, 3 and 5 hours. Virus controls were set up without tissue. After 3 hours the titers of hemagglutinins were reduced to an equal degree in 25% and 100%BSS reaching a level ½ of the controls with an inoculum of 100 HA units, while with the smaller inoculum of 20 units 90% of the hemagglutinating virus disappeared from the supernatant. At 5 hours, due to growth, hemagglutinating units in excess of those originally present appeared in the preparations with 100%BSS, while in the 25%BSS the titer was still below that at the zero hour. It appears from the results of this experiment that concentrations of electrolytes must be below those present in 25%BSS before effects on adsorption of hemagglutinins by minced tissue become measurable.

Effect of 25%BSS on early virus growth. When tissue is suspended in 25%BSS and inoculated with 100 infectious doses of virus, hemagglutinins may not become detectable before 48 or 72 hours, while they appear in the control with 100%BSS at 18 hours. Infectivity titrations on washed ground-up tissue indicate that slight multiplication of the virus has occurred by 6 hours, but the amount in 25% BSS is more than 2.5 logs below the controls. The results of infectivity titrations on tissue and supernatant fluid are shown in Fig. 1. In both 25% and 100%BSS the titer of the fluids tends to lag behind that of the tissue. Between 6 and 18 hours the tissue: fluid ratio of titers is lower in 100%BSS than in the hypotonic preparations. This may represent greater inactivation of free virus in the 25%BSS, or it may be due to delay in liberation of virus from tissue. By 24 hours the amount of virus in the tissue with 25%BSS

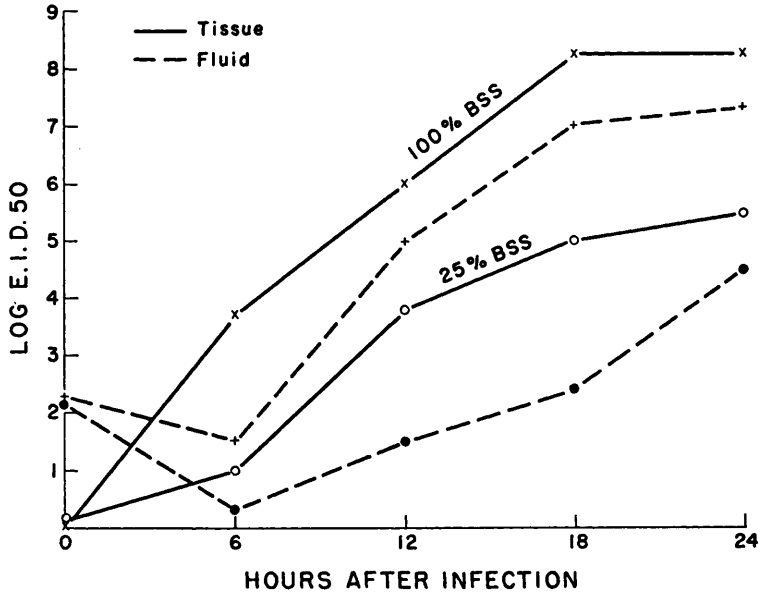

FIG. 1. Effect of 25% BSS on infectivity titers of virus in tissue and fluid. Roller tube tissue cultures.

had reached the level of the controls at 11 or 12 hours and the ratio of infectivity titers for tissue:fluid had returned to a value of approximately 10, similar to that in 100%BSS. The difference in titer of tissue between the groups with 25%BSS and the controls was maintained at about 2.5 logs.

Reversal of inhibition by late addition of NaCl. Titration of hemagglutinins at periods after 18 hours indicated the same difference in titers between 25%BSS and controls as those measured by infectivity. By 72 or 96 hours, as shown in Fig. 2, the HA titer of the controls had reached a plateau while that of

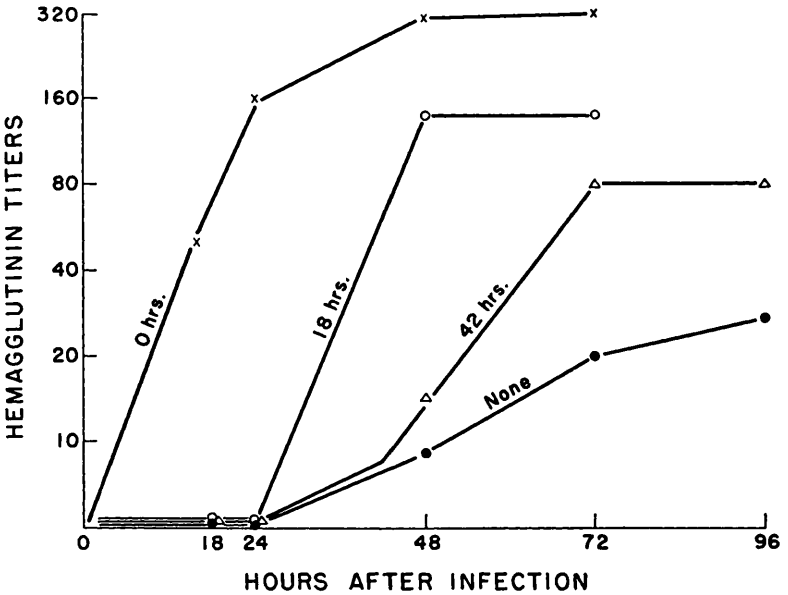

FIG. 2. Reversibility of inhibition of viral growth with NaCl. Additional amount of 6 mg/ml added to roller tube cultures in 25% BSS at times indicated on curves.

TABLE I. Effect of Medium on Growth of Virus and Tissue: Reversal by NaCl.

BSS, %	NaCl at hr	HA titer at hr			Tissue extension‡					
					Epithelium			Fibroblasts		
		24	48	72	24	48	72	24	48	72
25; 1.0 P; 0.2 G*	—	2	3	15	1+	1+	1+-±	0	0	±-0
"	18	2	90	150	±	±	1+-±	0	±	1+-±
50; 1.0 P; 0.2 G	—	12	80	150	2+	1+-2+	2+	1+-0	2+	2+-3+
"	18	38	140	280	2+	1+-2+	2+	±	2+	2+-3+
100; 1.0 P; 0.2 G	—	100	180	220	2+	2+	2+-3+	2+	2+-3+	2+-3+
25; 25 G†	—	2	32	40	2+-3+	2+	1+-2+	±	1+	1+
50; "	—	16	80	120	3+	2+	2+	1+-2+	2+	1+
100; "	—	32	80	160	2+	2+	3+	1+	2+	2+

* 1.0 mg/ml pyruvate and 0.2 glucose.

† 25 mg/ml glucose (.14M); also contains .7 mg/ml $NaHCO_3$.

‡ Relative amounts (1+, 2+, 3+) of epithelium or fibroblasts seen to migrate or grow out from the original fragments. ± indicates degeneration, round cells only.

the hypotonic preparations was still rising so that eventually a value about one-tenth of the controls was reached. With 50%BSS there was some initial delay in virus production but this was mostly overcome by 72 hours (Table I). When NaCl was added to the preparations with 25%BSS in amounts necessary to make the solution isotonic, increased titers of hemagglutinins appeared quite promptly in the supernatant fluids (Fig. 2 and Table I). Partial reversal of the effects of 25%BSS on virus growth could be obtained by adding NaCl as late as 42 hours after infection. Microscopic examination of the tissue revealed marked inhibition of the extension of epithelium and fibroblasts from the tissue fragments in the 25%BSS and growth was not appreciably stimulated by the addition of NaCl. The increased rate of virus multiplication after late addition of NaCl occurred, therefore, without observable change in the condition of the host cells. With 50%BSS the extension of epithelium and fibroblasts was somewhat delayed but not much less than in the controls and late addition of NaCl again caused little detectable change.

Partial substitution of KCl or glucose for NaCl. Use of BSS with 6 mg ml of KCl and 2 mg/ml of NaCl resulted in growth with virus and tissue about equal to that with BSS having a normal concentration of NaCl. Higher relative concentrations of K^+ poisoned the tissue. By adding glucose to a concentration of 25 mg/ml in the 25%BSS, the osmolarity of the medium was restored to a value approximately ¾ that of normal BSS. In these experiments it was essential to add $NaHCO_3$ (0.7 mg/ml) and keep the roller tubes loosely capped in order to balance excessive acid formation. The initial pH above 8.2 gradually fell to values between 7.4 and 8.0 by the end of the experiment. The addition of glucose resulted in somewhat better virus growth as compared to that in 25%BSS alone, but inhibition was very definite at 24 hours. With high glucose, extension of epithelium was equal to that of the controls during the first 48 hours. The result suggests that osmotic effects play an important part in growth of the host cell while growth of virus is not effectively stimulated by adjustment of the osmolarity without adding more electrolyte.

Addition of calcium chloride or inorganic phosphate to 25%BSS in amounts necessary to make the concentration of these substances equal to their concentration in 100%BSS had no effect. Magnesium was present in sufficient quantity as determined by unpublished experiments of other investigators.‡

Inhibition of growth and loss of virus from tissue by changing from 100% to 25%BSS. CA membrane was infected in 100%BSS and incubated in shaker flasks until hemagglutinins became detectable in the supernatant fluid. The appearance of virus in the fluid required 18 to 24 hours. At this time the tissue

‡ R. S. Gohd personal communication.

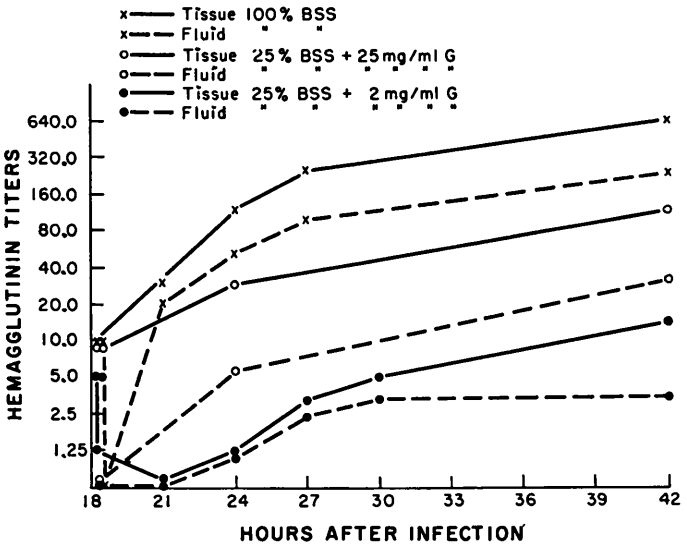

FIG. 3. Infected tissue incubated for 18 hr in 100% BSS, washed and changed to fresh solutions as indicated. In this experiment 100% BSS contained 1 mg/ml pyruvate and 0.2 mg/ml glucose.

washed with 100%BSS and ground in buffered normal saline to make a 10% suspension gave an HA titer approximately equal to that in the fluid. When infected tissue was washed twice with 25%BSS and then ground in buffered normal saline the HA titer was found to have dropped to 1/2 to 1/16 that in tissue washed with normal BSS. Measurements of the volume of tissue revealed no significant change such as might be expected from possible cytolysis or osmotic swelling. Further incubation of the samples of washed infected tissue with 100% and 25%BSS was then done in flasks or roller tubes. The resulting effects on HA titers are depicted in Fig. 3. Tissue washed with 100%BSS and returned to this medium showed a continued increase in HA titer and there was also a prompt recovery of HA in the supernatant fluid. When the tissue was placed in 25%BSS there was a loss of virus from the tissue followed by a little increase, and accompanied by a partial recovery of hemagglutinins in the fluid. When the washed tissue was returned to 25%BSS containing 25 mg/ml of glucose the virus continued to increase, but at a rate intermediate between that of plain 25%BSS and 100%BSS. Correction of the osmotic effects by means of glucose instead of NaCl did therefore favor the continuous growth of virus and prevented its initial loss from tissue. In another experiment all infected tissue was first washed in 100%BSS and then samples were placed in 100, 50, 35 and 25%BSS. The results of HA titrations on the tissue are shown in Fig. 4. It may be seen that the loss of hemagglutinating virus from the tissue was a progressive process which was related to the concentration of salt. In tissue pre-incubated for 18 hours and then placed in 25%BSS the HA titer drops promptly to less than 2. With 35% and 50%BSS the titers remained below the controls for 3 hours and then showed a drop. The remaining lines in Fig. 4 illustrate a similar course of events in tissue pre-incubated for 24 hours in 100%BSS so that the virus titer was approaching a maximum at the time of change to lower concentrations of BSS. In all instances a loss of virus was evident in tissue incubated with the hypotonic BSS.

Effect of hypotonic BSS on tissue respiration. Measurements of O_2 uptake were done in the Warburg apparatus with fresh tissue in 25% and 50%BSS, and also on tissue which had been pre-incubated for various periods of time in hypotonic BSS and then placed in 100%BSS for the O_2 measurement.

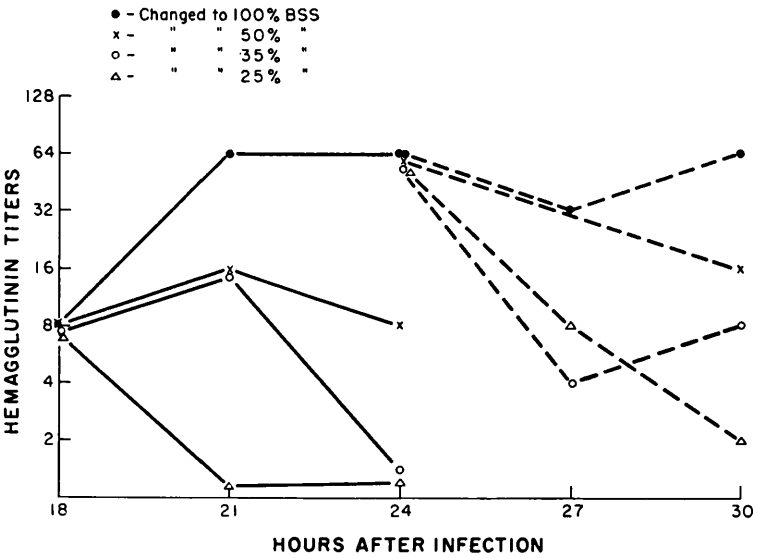

FIG. 4. Hemagglutinin titers of virus in tissue after pre-incubation in 100% BSS and change to diluted BSS as indicated at 18 hr (solid lines) or 24 hr (broken lines).

In general 50%BSS produced little change in the rate of O_2 consumption either with fresh tissue or with tissue incubated in this solution for 48 to 72 hours. On the other hand, the respiration of fresh tissue placed in 25%BSS was reduced almost immediately to about one-third of the rate with control tissue in 100%BSS. Since the volume of tissue was measured before placing it in the Warburg vessels these differences cannot be attributed to errors introduced by osmotic swelling during the course of the measurement of O_2 consumption. The reversibility of this inhibition of respiration is indicated by several lines of evidence based on the data in Table II. Addition of NaCl from the side arm of the Warburg vessel in amounts necessary to reestablish isotonicity at 3 hours after the start of the experiment caused a partial reversal within a short period of time. Although in a 24 hour period the controls showed a descending rate of respiration(8), the group with added NaCl reached 65% of the controls at this time. Warburg measurements on tissue

TABLE II. Oxygen Consumption after Treatment of Tissue with Hypotonic Solutions.

Pre-incubation	Medium in Warburg vessels	Rate O_2 uptake as % of controls at age of tissue in hr: 3	6	24	48
None; fresh tissue	25% BSS; 1.0P; .2G*	30	30	33	—
Idem	25% BSS; " " + NaCl at 3 hr	30	50	65	—
"	25% BSS; 1.0P; 25G*	52	49	—	—
"	50% BSS; 1.0P; .2G*	80–95†	—	—	—
25% BSS, 24-48 hr‡	100% BSS; 1.0G	—	—	70–100	60–90
25% BSS + 25G, 24-48 hr	*Idem*	—	—	90	70
50% BSS, 24-48 hr‡	"	—	—	70–100	60–90
100% BSS, 24 hr; then 25% BSS§	"	—	—	50–75	10–20
100% BSS, 18 hr; then 25% BSS + 25G	"	—	—	75	60
100% BSS, 24 hr; then 50% BSS	"	—	—	100	90

* P = pyruvate as mg/ml, G = glucose as mg/ml, corresponding controls with 100% BSS and equivalent glucose concentration.

† Double figures indicate low and high of range in several experiments.

‡ Controls pre-incubated with 100% BSS; 1.0 P + 0.2 G in all series.

§ Tissue washed with 25% BSS, and further incubated in 25% BSS. Corresponding controls washed and reincubated in 100% BSS.

in 25%BSS containing 25 mg/ml glucose revealed a marked depression of respiration. In this medium the osmotic pressure was intermediate between that of 50%BSS and normal but as shown in Table II the depression of O_2 uptake with 50%BSS was much less. In the controls with 100%BSS and 25 mg/ml glucose some substrate stimulation of respiration, as compared with the O_2 uptake of tissue in BSS containing only pyruvate, was observed. When fresh tissue was put in 25%BSS in roller tubes or shaken flasks and incubated for 24 to 48 hours, Warburg measurements done with this tissue in 100%BSS with 1 mg/ml glucose showed rates in some instances were equal to but usually slightly less than those for control tissue pre-incubated in 100%BSS. Since tissue kept in 25%BSS in the Warburg vessels for 24 hours showed a markedly depressed respiration these results suggest that return of the tissue to normal conditions at 24 to 48 hours results in reversal of inhibition of respiration. Another possible interpretation would be that the fresh tissue is able to adapt itself to the hypotonic solution after a period of incubation. Indeed some evidence for this is found in the observation that small spherules of epithelium (9) often were formed in the hypotonic BSS and this might allow some concentration of electrolytes in the interior medium of these structures. Measurements of the volume of tissue recoverable by packing in graduated centrifuge tubes revealed no difference between the effects of incubation with 25%BSS and those with 100%BSS until 3 days when the tissue volume in 100%BSS had decreased by autolysis to about half of that originally present, while the volume of tissue in the 25% BSS was about ¾ of the original. Combined autolysis and swelling might account for this result.

Tissue pre-incubated for 24 hours in 100%BSS and then washed with 25%BSS and reincubated in the hypotonic solution showed definite signs of irreversible damage. The washing with 25%BSS in itself resulted in a decrease in rate of respiration as measured in 100%BSS although no swelling of the tissue was observed during this treatment. Further incubation resulted in a very marked depression of respiration as contrasted with the oxygen consumption of tissue which was placed in 25%BSS when fresh. Tissue incubated with 25%BSS from the beginning then washed at 24 hours and reincubated in this same medium or in 100%BSS did not show the depression of respiration; another observation which indicates that fresh tissue can show a certain degree of adaptation to the hypotonic medium.

Infection with virus seemed to have no relation to the observations on tissue respiration just described. The depression of respiration by 25%BSS was about the same in degree in normal and infected tissue.

Irreversible damage was less marked when tissue pre-incubated in 100%BSS was placed in 25%BSS containing 25 mg/ml glucose (next to last line of Table II). This suggests that osmotic effects rather than lack of NaCl *per se* play an important part in the tissue deterioration under these conditions. However, pre-incubated tissue placed in 50%BSS showed less depression of respiration than that put in 25%BSS with 25 mg/ml glucose.

Discussion. At the concentrations of electrolyte in the range 0.04 M to 0.08 M used in these experiments there was no directly measurable inhibition of the adsorption of virus to tissue and this factor was probably negligible in the observed retardation of growth. An indirect role of NaCl or other electrolytes in aiding irreversible attachment or penetration of the cell membrane by virus seems possible. For example, Garen and Puck(10) found that when T_2 bacteriophage was attached to *E. coli* B in the presence of 0.04 or 0.02 M NaCl the virus could be eluted with distilled water and the host cells survived. Attachment in the presence of 0.1 M NaCl, on the other hand, was not reversible and the host cells were killed. Furthermore, absence of electrolyte apparently prevented multiplication of virus because nutrient agar without NaCl permitted growth of *E. coli* B, but not of the associated phage. The authors expressed the opinion that the irreversible attachment of T_2, as a step in infection, repre-

sented an enzymatic establishment of covalent linkages, while the reversible attachment was probably electrostatic in nature. The curves in Fig. 1 indicate that multiplication of influenza virus in the tissue is retarded from the start of the experiment. This could be attributed either to unsuccessful penetration of the tissue by a large proportion of the adsorbed virus particles or to delayed reproduction of the intracellular virus.

Dissociation of virus from heavily infected tissue as the result of exposure to hypotonic medium is clearly indicated by the data in Fig. 3 and 4, but it is not known that a similar effect occurs during the initial stages of infection. The results are reminiscent of the dissociation of pneumonia virus of mice from lung tissue or red blood cells in the presence of distilled water or 0.25 M dextrose respectively(5). Davenport and Horsfall found that the pneumonia virus failed to dissociate from red blood cells in 0.25 M glucose, only when the pH was below 7.0. In our own experiments loss of influenza virus from tissue occurred in diluted BSS with electrolyte concentrations ranging from 0.04 to 0.08 M and this dissociation (Fig. 3) was apparently prevented by adding 0.14 M glucose to 25% BSS buffered at a pH near 8.0. Since the osmolarity of the solution was increased by this addition but not the electrolyte content, it seems evident that the observed dissociation of virus from tissue in this case is at least in part due to osmotic effects. Gross plasmolysis was not observable in 25% BSS.

Although reduction of the salt concentration to half of the normal level produced little significant change in the respiratory metabolism of chorioallantoic tissue, solutions more hypotonic than this caused definite inhibition of O_2 uptake. This effect with fresh tissue was for the most part reversible, but with tissue pre-incubated in 100% BSS for 24 hours, irreversible changes occurred on exposure to 25% BSS. Neither of these effects was completely overcome by the addition of 0.14 M glucose (Table II), and although the resulting osmolarity was greater than that of 50% BSS, the tissue respiration was not preserved as effectively. It will also be noted (Table I) that virus growth in 0.04 M electrolyte (25% BSS) plus 0.14 M glucose was slower than in 0.08 M electrolyte. These observations suggest that lack of electrolytes (as NaCl or KCl) by itself and apart from the osmolarity of the solution has a depressive effect on tissue metabolism and probably virus synthesis. There was no clear-cut relation between metabolism, proliferation of tissue, and growth of virus.

The relation of these observations to the effect of electrolytes and the osmotic pressure of solutions on mitochondria should be considered. Isolated mitochondria maintain their normal morphology only when the solution is hypertonic(11,12) with 0.88 M sucrose, while physiological NaCl results in rounded swollen forms not only with free mitochondria, but also with mitochondria in suspended intact liver cells(11). Respiration of free mitochondria is greatly depressed in 0.15 M NaCl and slightly in 0.15 M KCl. It may be, therefore, that in simple salt solutions such as those used in our tissue cultures the intracellular mitochondria are already abnormal and may undergo additional changes in hypotonic solutions. This might result in depressed respiration or oxidative phosphorylation, such as accompanies morphological changes in isolated mitochondria exposed to hypotonic solutions or distilled water(12). More detailed cytochemical studies would be required to establish this, but in addition there is the possibility that other unknown changes may be occurring in the cell membrane or in other components of the cytoplasm.

It is evident that effects on virus growth such as those revealed in the present study cannot be attributed simply to factors of adsorption or elution, but that reversible effects of electrolytes or osmotic pressure on tissue metabolism must also be considered.

Summary. 1. Growth of virus is inhibited by exposure of infected chorioallantoic membrane to Hanks' balanced salt solution diluted 1:2 or 1:4. This occurs either at the beginning of the experiment or after a 24 hour period of virus multiplication. Restoration of isotonicity with NaCl up to 42 hours after in-

fection results in reversal. 2. NaCl when added late, although it stimulates virus growth, does not increase tissue proliferation. Glucose 0.14 M in 25%BSS permits almost normal tissue proliferation, but does not significantly stimulate virus synthesis during the first 24 hours. 3. Loss of virus from heavily infected tissue occurs on exposure to 50, 35, or 25%BSS. This is prevented by adjusting the solution to near normal osmolarity with glucose. Tissue respiration is markedly inhibited by 25%BSS but not by 50%BSS. 4. The relative roles of electrolytes and osmotic properties of the solution in virus attachment, penetration, and synthesis and on tissue respiration are considered.

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Anesthesia: LIV. Effects of Viadril and Some Water-Soluble Steroids on Brain Oxidative Phosphorylation.* (22455)

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Although claims of "anesthesia" produced by steroids have been made previously(1,2-5), Viadril, a new water-soluble steroid, represents the first compound of this type to evoke anesthesia with a safety margin adequate for human use(6). A survey of the effects of various hypnotic and anesthetic drugs on the coupling between oxidative phosphorylation and respiration in rat brain mitochondria(7) prompted the extension of this study to Viadril. Other water-soluble steroids lacking anesthetic action were tested for comparison.

Methods. The methods used for measurement of the ratio of oxidative phosphorylation to respiration have been described more fully elsewhere(7,8). Briefly, the ratio is measured as the inorganic phosphorus uptake in micromoles of phosphorus divided by the microatoms of oxygen consumed by rat brain mitochondria which were isolated in 0.25 M sucrose containing 0.001 M disodium Versenate. The final concentrations in the medium used were: pH 7.4 phosphate buffer 0.020 M; potassium chloride 0.050 M; potassium fumarate 0.002 M; sodium pyruvate 0.013 M; ATP 0.0025 M; glucose 0.028 M; hexokinase 6.25 mg/cc; disodium Versenate 0.001 M; sodium fluoride 0.012 M; magnesium chloride 0.008 M; and was incubated at 20° for 30 minutes following a 10 minute equilibration. Viadril (21-hydroxy pregnanedione), hydrocortisone, and pregnenolone were all employed in the form of their sodium succinate salts. The sodium salt of dehydrocholic acid was used.†

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† These compounds were kindly supplied by Dr. Michael Carlozzi of Pfizer Laboratories.