though the reaction does require hydrogenases which are steroid-specific(10). Moreover, 11-desoxycortisol is metabolized by such *in vitro* preparations at a "considerably faster" rate than cortisol(11). With respect to 5-alpha reductase activity, rat liver microsomes catalyze 11-desoxycortisol approximately twice as rapidly as cortisol(10), a ratio which agrees well with the relative clearance of the 2 steroids from plasma in the present *in vivo* study in man.

These observations suggest that the more rapid removal of 11-desoxycortisol compared to cortisol from the peripheral circulation should be taken into account in estimating total adrenal secretory response to the 11-beta hydroxylase inhibitor SU-4885(12,13) based on periodic plasma levels of free 11-desoxycortisol.

Summary. The biologic half-life of free 11-desoxycortisol in circulating plasma of man, estimated as Porter-Silber chromogens, was 42 min. This may be prolonged to as much as 80 min. in the presence of liver disease or to 108 min in hypothyroidism. Absence of an 11-beta hydroxyl group accelerates clearance of 11-desoxycortisol from plasma, its removal rate being twice as rapid as that of cortisol. 11-Desoxycortisol is rapidly conjugated to a glucuronide. Concentration of its Porter-Silber chromogen metabolites is 5 times greater than that of cortisol at comparable peak levels; the glucuronide conjugates of both steroids disappear from plasma with a half-time of 102 min.

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Viral Growth and Oncolysis in Krebs 2 Cells as Affected by Substrate.* (25687)

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In roller tube suspensions of Krebs 2 cells (K2) infected with influenza virus and with glutamine and lactate as the only substrates high titers of hemagglutinins were obtained, but when glucose was added in place of lactate HA titers were significantly lower(1). The newly formed hemagglutinins remained in or on the cells, and infectivity titers were very low. The K2 ascites tumor has such high virulence that it is possible to quantitate tumor producing capacity of a cell suspension by inoculating mice with serial 10-fold dilutions. Application of this technic to roller tube suspensions of surviving K2 cells has made possible a quantitative study of the relation between substrate, growth of virus, and reduction of tumor producing capacity. Results with influenza and Newcastle disease virus (NDV) will be compared.

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Materials and methods. Sources of tumors and virus strains (PR8, WS, and NDV Cal) have been detailed(1,6). The Francis neurotropic WS strain of influenza was received through the courtesy of Dr. R. R. Wagner(4). The medium consisted of Hanks balanced salt solution (BSS) in 2 different modifications as follows: a. Lactate medium contained, besides inorganic constituents of BSS, 0.1% glutamine, 0.1% sodium lactate, 0.1% bovine albumin, and 0.05% more of sodium phosphate than in the original formula. $NaHCO_3$ was omitted. b. Glucose medium contained BSS plus 0.1% glutamine, 0.1% glucose, 0.1% bovine albumin, additional phosphate as above, and 0.18% $NaHCO_3$. Glutamine and bovine albumin prolong *in vitro* survival of ascites tumor cells(7). Cells obtained from 6 or 7 day tumors in mice were washed twice and suspended in medium to give final cell concentration of about 1.3 x 10^6/ml. These suspensions were incubated at 35° with virus at a known initial hemagglutinin (HA) concentration (8 to 32 HA units/0.5 ml) for periods of 4 to 72 hours. The roller tubes with caps (tightened) were rotated at 12 rpm. *Titrations of virus* were done by Salk's method for HA, and by chorioallantoic membrane tissue culture for infectivity. Degree of absorption of virus by cells was estimated by comparing HA titers of fluids at 0 hours with those at 4 hours (Table I). Cells were prepared for titration of their viral content by washing twice, then freezing and thawing them in hypotonic BSS as previously described(1). *Tumor producing capacity* of samples of cell suspensions was titrated by inoculating mice intraperitoneally with serial 10-fold dilutions made in plain BSS containing 0.1% bovine albumin. Two mice received 0.2 ml of each dilution. Mice were observed for one month when survivors were sacrificed and examined for presence of solid and ascites tumors.

Results. K2 cells were incubated with influenza virus in lactate or glucose medium. At 4, 24, 48, and 72 hours extracts were made from pooled cells of 4-6 tubes and viral HA and infectivity were titrated. Measurements of tumor-producing capacity were done on suspensions incubated with and without virus. The results of HA and infectivity titrations are presented in Fig. 1. In contrast to results with glucose medium, where maximum titers were 10-15, much more replication occurred in cells incubated with glutamine and lactate. HA titers increased to 300-400 by 48 hours. Infectivity titers of cell material showed no increase in either medium.

It should be emphasized that diminished formation of HA in K2 cells was associated with glutamine, glucose and $NaHCO_3$ combined under aerobic conditions(1). Addition to the medium of either substrate produced significantly higher HA titers than when both were present. The mechanism of this unusual phenomenon is being investigated.

Average results of several measurements of tumor-producing capacity of cells incubated in the 2 kinds of medium, with and without virus, are graphed in Fig. 2. Considering first the results with glucose it will be seen that after 4 hours, virus had reduced the tumor-producing capacity only one log below that of cells incubated without virus. For 3 days, a gradual fall in tumor-producing capacity occurred, but this one log difference from controls remained constant. Increasing amount of virus to 160 HA units resulted in further reduction of tumor-producing capacity.

Addition of PR8 virus to cells suspended in lactate medium caused a marked reduction of tumor-producing capacity as early as 4 hours, and by 3 days the difference from the control was about 3.5 logs. Relative slopes of the 2 curves, virus + lactate and no virus + lactate, suggest that rate of loss of tumor-producing capacity was only slightly augmented between 4 and 48 hours by presence of virus.

Hemagglutinin production in K2 cells by neurotropic and non-neurotropic WS strains of influenza virus and by NDV was also investigated. Results of HA titrations in cells and supernatant fluids from the 2 media are presented in Table I. The WS strains behaved in the same way as PR8 in that much more HA was obtained when lactate was present. This difference is not obvious from HA titers in the fluids except with the FWS strain where a value of 128 was obtained in the lactate medium as compared with 3 in glucose medium.

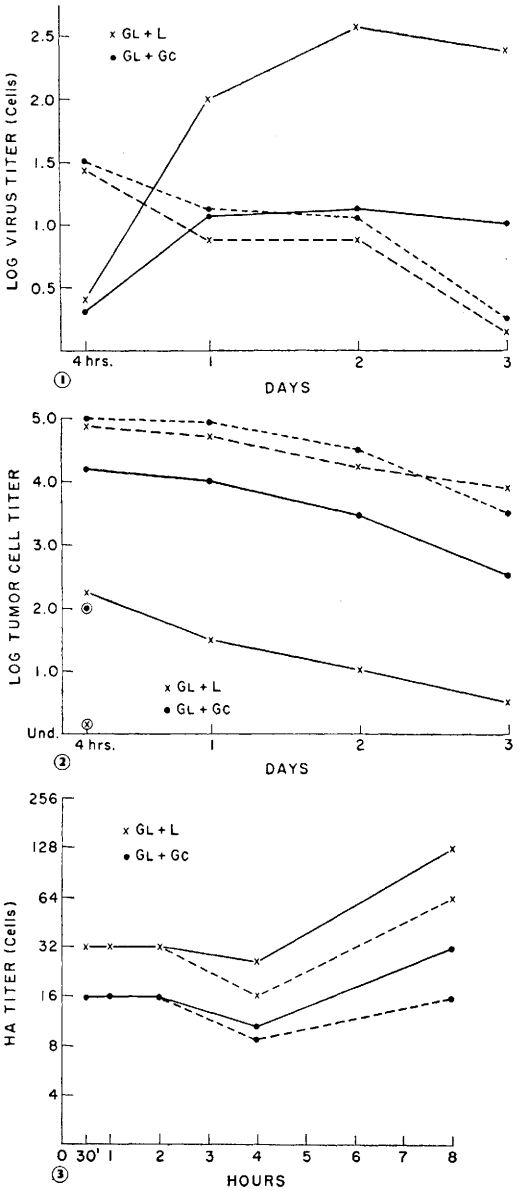

FIG. 1. Effect of substrate on HA titers (solid lines) and infectivity titers (broken lines) as measured in extracts of washed K2 cells. Dose of virus (PR8) adjusted to give initial HA titer in medium of 1:16. GL = glutamine; L = lactate; Gc = glucose, all at 0.1% concentration. Bovine albumin 0.1% also included.

FIG. 2. Results of titration of tumor producing capacity of K2 cell suspensions incubated under conditions identical with those described for Fig. 1 (solid lines). Broken lines represent control suspensions incubated without virus. Circled points at 4 hr ordinate indicate tumor producing capacity of suspensions incubated with virus at 10 × concentration (160 HA).

FIG. 3. Early HA titers in extracts of washed K2 cells which had been incubated with high concentrations of influenza virus (initial HA fluid 500) in glucose or lactate medium. Broken lines represent result when cells incubated for 2 hr with virus were then centrifuged and resuspended in 0.5% anti-PR8 serum in the same media.

No significant differences in titer were found with NDV in these 2 media and in other experiments using lactate, glucose, or glutamine singly as the sole source of energy results similar to those shown in Table I were obtained.

Since growth of NDV in K2 cells did not seem to be affected by the substrate it was of interest to study its oncolytic activity. At 4 and 24 hours tumor-producing capacity of cell suspensions incubated with virus was 0.5 to 1.5 logs below that of suspensions similarly incubated without virus. At 48 hours, the oncolytic effect was irregular but averaged 2 or 3 logs. Comparison of results with the 2 media did not show differences great enough to indicate an effect of substrate on reduction of tumor-producing capacity by NDV.

Experiments designed to compare rate of absorption of viral HA by cells in lactate and glucose media gave inconclusive results. The data in Table I indicate that between 50% and 75% of the added HA units had disappeared from the fluids by 4 hours and similar results were obtained at ½ and 1 hour. No effect of substrate was observable.

Early measurement of HA titers in cells required an inoculum 20 times as great as that routinely used in previous experiments. After 30 minutes incubation with this amount of virus, cells contained hemagglutinins at titers of 16 to 32. A consistent but slight difference between cells on lactate medium and those on glucose medium was demonstrable (Fig. 3). The increase of HA titer at 8 hours probably represented viral replication.

Hemagglutinins on K2 cells could be reduced to undetectable amounts by treatment with PR8 antiserum. Cells incubated 4 hours with virus were centrifuged and resuspended in the same medium with 5% anti-PR8 rabbit serum (HI titer 1:8000) for an additional 4 hours. After washing these cells, extracts made in the usual manner contained no demonstrable hemagglutinins. With lower concentrations of antiserum partial neutralization of cell-associated HA was observed. Broken

TABLE I. Effect of Substrate on Hemagglutinin Formation in K2 Cells.

	Glutamine + lactate					Glutamine + glucose				
	Fluid			Cells		Fluid			Cells	
Virus	0 hr	4 hr	48 hr	4 hr	48 hr	0 hr	4 hr	48 hr	4 hr	48 hr
WS*	8	2	40	10	320	8	2	16	10‡	40
FWS†	8	2‡	128	10	640	8	2	3	10	20
PR8	24	8	20	4	400	24	12	10	4	15
NDV	8	4	64	4	24	8	8	128	6	24

* Non-neurotropic WS strain of influenza virus. † Francis' neurotropic WS strain.
‡ Trace of hemagglutination at dilution indicated.

lines in Fig. 3 represent effect of 0.5% serum in an experiment similar to that described above. Virus was neutralized to a similar degree in lactate medium and glucose. Replication of cell-associated hemagglutinins occurred on further incubation for 24 hours of suspensions in 0.5% antiserum. Tests for absorption of antibody by uninfected K2 cells were negative.

Discussion. In ascites tumor cells the behavior of influenza virus differs from that of unadapted strains of NDV. Elution of influenza virus at room temperature does not occur(2) and HA formation can be demonstrated both in Ehrlich ascites and K2 cells (1). The studies of Prince and Ginsberg(3, 8) indicate that destruction of Ehrlich ascites cells by NDV is accompanied by an incomplete cycle of virus replication which stops before hemagglutinins are produced. Oncolysis by the neurotropic WS strain of influenza is, however, associated with formation of infectious virus(4).

Incubation of NDV and EA cells in roller tube suspensions at multiplicities similar to those used by Prince and Ginsberg leads to death of cells within 3 days as determined by stainability with eosin and loss of respiration (5). At lower multiplicities used in the present experiments, neither NDV nor the PR8 strain of influenza caused such rapid *in vitro* death of ascites cells as judged by above mentioned criteria(1).

It is apparent (Fig. 2) that events determining growth of virus and fate of cells in mouse peritoneum have already occurred by the 4th hour after mixing K2 cells with influenza virus. After the cells are in the peritoneum their failure to multiply and their destruction are presumably caused by viral replication *in vivo*. Between 4th and 24th hour, when the most active replication of hemagglutinins had occurred, changes in tumor-producing capacity were minor. In lactate medium at 4 hours only 0.1% of cells treated with virus remained capable of producing tumors as compared with 10% in a medium containing glucose and bicarbonate in place of lactate. It appears therefore that in the latter medium fewer cells become infected and that the important factor is adsorption, penetration, or early stage of virus synthesis. Attempts to demonstrate differences in viral adsorption in glucose or lactate media by measuring disappearance of hemagglutinins from the fluid did not yield significant results and only a slight substrate effect was observable when cell-associated HA were measured at ½ to 4 hours. During the first 4 hours complete inhibition of HA in or on the cells by 5% immune serum could be demonstrated. There was, however, no evidence that accessibility of virus to antibody differed significantly in the 2 media. The observation that HA titers actually increased in cells incubated with antibody in excess, suggests that these hemagglutinins were intracellular.

In the absence of convincing evidence that the substrate affects adsorption or penetration of virus we should consider the possibility that an early phase of viral synthesis is involved. As indicated by previous studies with NDV (3) formation of viral antigen demonstrable with fluorescein-labeled antibody may occur without appearance of HA, complement fixing antigen, or infectivity. Possibly it is the formation of this virus antigen or virus precursor *in vitro* which makes the ascites tumor cells incapable of multiplication after inoculation into mice. In glucose medium synthesis of influenza virus precursor would seem, on the basis of this hypothesis, to be less uniformly

distributed among individual cells of a suspension than is lactate medium. To explore this idea further studies with fluorescein-labeled antibody are in progress.

Summary. With 3 strains of influenza virus formation of non-infectious cell-associated hemagglutinins in Krebs 2 ascites cells was much greater in a medium containing lactate and glutamine than when glucose was substituted for lactate. Reduction in tumor-producing capacity of cell suspensions by action of influenza virus was correlated with the effect of substrate on HA formation. A marked oncolytic effect was evident in lactate medium at 4 hours, or before the increase in HA. Although Newcastle disease virus was oncolytic at the same concentration as influenza, growth of NDV and its action on tumor-producing capacity were not affected by the substrate.

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Importance of Particle Size in Aerosol Therapy.* (25688)

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It is known that retention of airborne particulate matter in the respiratory tract is chiefly influenced by size of particles inhaled; the large particles settle in the tracheobronchial tree and smaller ones penetrate deeper into the lungs. When smaller than 0.5 μ in diameter, particles are nearly exclusively deposited upon walls of alveolar ducts and alveolar sacs(1). To emphasize the importance of particle size in administration of medicated aerosols, a small series of experiments was undertaken using 2 different sources of airborne particles: a standard nebulizer (commercially available) producing particles ranging from 0.8 to 7.4 μ, averaging 3.1 μ, according to Grau(2) or from 1.6 to 14.8 μ averaging 6.2 μ according to Barach(3) as measured by optical microscopy and an aerosol generator called D-30, previously described(1) producing particles all below 0.5 μ diameter as measured by electron microscopy. Medicated aerosol was produced from a solution containing 0.1% isoproterenol, 2% phenylephrine in medium composed of equal parts of USP propylene glycol and water. At 5 p.s.i. head pressure the standard nebulizer delivered 9 g of this solution in 10 minutes while the D-30 aerosol generator, during same time and same pressure, delivered 0.3 g only. Four normal subjects (30, 32, 45 and 65 years in age) breathed the medicated dispersates alternately as produced by standard nebulizer and by aerosol generator, duration of inhalation varying from 5 to 20 slow, deep, sub-maximal breaths. Airway resistance was measured according to plethysmographic method of DuBois, Botelho and Comroe(4), and blood pressure, heart rate, and electrocardiogram were recorded in serial fashion.

Results were as follows: 1. Airway resis-

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