amounts of reaction products. Conversion of 11-deoxycorticosterone was not significantly accelerated by norepinephrine. It thus appears that catecholamines accelerate hydroxylations at C-21 and, perhaps, C-17 but not at C-11. This may explain Kahnt's(7) failure to detect an effect of medullary homogenates or epinephrine on 11β-hydroxylation of substance S by bovine adrenocortical homogenates. Firmer information on site and mechanism of action of catecholamines will require separation of the 3 hydroxylase systems.

Summary. 1. Conversion of progesterone to 17α,21-dihydroxycorticosteroids by homogenates of bovine adrenal cortex is markedly stimulated by addition of small amounts (15 mg/100 mg cortex) of medullary homogenate. Larger amounts produce additional, though relatively smaller increments. 2. The soluble fraction of medullary homogenates equivalent to 15 mg of medulla stimulates as much as the corresponding amount of whole homogenate. Larger amounts of this supernatant fraction do not produce additional increments. 3. Increments similar to those obtainable with 15 mg of medullary homogenate or medullary supernates are produced with 100 μg or less of D(−)epinephrine, D(−)norepinephrine, or D(−)isopropyl-norepinephrine. No further increment results from larger additions. 4. Total catecholamine content of 15 mg of medullary homogenate or corresponding supernatant fraction ranged from 80-115 μg, indicating that the stimulatory action of small amounts of medulla can be ascribed mainly to catecholamines. Additional increments produced by larger amounts of medulla are apparently due to contamination with cortical remnants and, possibly, an additional factor of unknown nature. 5. Exploratory experiments suggest that catecholamines accelerate C-21 and, perhaps, C-17, but not C-11 hydroxylations.

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Parainfluenza 3 — Assay and Growth in Tissue Culture.* (25725)

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Parainfluenza 3 (HA1) causes human(1) and possibly bovine disease(2,3), and is a virus which may produce persistent infection in tissue cultures(4). The process of formation of multinucleated giant cells(5), and a plaque assay technic dependent on these cytopathic changes have been reported(6). We describe a plaque assay system based on hemadsorption characteristics of HA1. Data are presented on thermal stability, rate of adsorption to cells, growth characteristics, and variation in cytopathic response of different cell systems to HA1.

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Materials and methods. Virus. Mill's strain of HA1 was obtained from Dr. Wallace Rowe. Virus pools of passages 11 through 13 in our laboratory were used. Hemadsorption tests were performed by exposing washed tissue cultures to 0.4% suspension of thrice washed guinea pig red cells in phosphate buffered saline, pH 7.3. In hemagglutination

TABLE I. Variation in Response of Different Cell Systems to HA1.

Cell strain	Species origin	Growth medium	Characteristics cytopathic changes	Hemadsorption
Lohi	Human	BME 90, Calf 10	—	+
"	"	Homologous serum 20, Yeast .1	+	+
CRP	Rabbit	*Idem*	—	+
CRE	"	"	—	+
ERK-1	?	"	+	+
ERK-2	"	"	—	+
Amnion	Human	"	—	+
HeLa	"	"	+	+
EE	"	"	+	+

and hemagglutination inhibition tests, 0.25 ml of virus plus 0.25 ml of saline or serum were mixed and incubated at room temperature 1 hour. Then 0.25 ml of 0.4% suspension of washed guinea pig red cells was added, the tubes placed at 4°C overnight and read by pattern. Rabbits were immunized for 7 weeks with 4 weekly I.V. injections of virus and 2 I.M. injections of virus with adjuvant (Arlacel A, 1 part, Bayol F, 4 parts). Lohi cells (5) were grown either in Eagle's basal medium (BME) 90%, calf serum 10%, or Hanks BSS 80%, yeast extract 0.1%, human serum 20%(7). Growth conditions for cells tested for susceptibility have been published (8). After virus inoculation all cells were maintained in BME 75%, tryptose phosphate 20%, and rabbit serum 5%. All media contained penicillin 100 units and streptomycin 100 μg/ml. *Plaque assay.* Two-ounce tablet plaque bottles containing cell monolayers were washed 3 times with BSS and shaken free of residual fluid. The bottles were inoculated with 0.1 ml of suitable dilution of virus and rocked intermittently 1 hour at room temperature. Monolayers were overlaid with maintenance medium containing 0.6% nutrient agar and incubated 3 days; then the agar was poured off, 5 ml of 0.4% suspension of guinea pig red cells applied to each plaque bottle and settled at 4°C for 30 minutes. Red cells were rinsed off gently leaving small 1 to 2 mm "hemadsorption plaques." Plaque reduction by antibody was measured by adding 0.1 ml of serum to 1 ml of virus containing approximately 5 x 10^4 pfu. After incubation at room temperature for 1 hour, plaque bottles were inoculated with 0.1 ml of 1:10 dilution of this mixture. As pointed out by Holland(9) an important point in procedures in which the agar is poured off is the inclusion of calf serum in growth medium to prevent easy detachment of cells.

Results. Tissue cultures infected with HA1 may produce multinucleated giant cells, the centers of which become detached from the glass. This sequence of changes is the basis of plaque technic described by Deibel(6). Lohi cells grown in BME with 10% calf serum showed less marked cytopathic change than Lohi cells grown in BSS 80%, yeast extract 0.1%, and human serum 20%. Cells grown in calf serum medium were not satisfactory for plaque assay systems dependent on development of characteristic cytopathic changes, but were as sensitive as cells grown in the human serum medium for "hemadsorption plaque" procedure. Increasing dilutions of virus resulted in a linear decrease in number of plaques.

Variation in cell response. Four tube cultures of each cell strain shown in Table I were washed and inoculated with approximately 100 pfu of HA1 in 1 ml of maintenance media. After 3 days incubation 2 tubes of each set were stained with 1% crystal violet and examined for characteristic multinuclear giant cells. The remaining 2 tubes were used for hemadsorption tests. Results (Table I) show that although virus was detected in each instance by the hemadsorption test only 4 lines showed marked cytopathic changes. In many instances infected cultures were not easily distinguishable from control cultures.

Thermal stability. HA1 in maintenance medium was rapidly inactivated at elevated

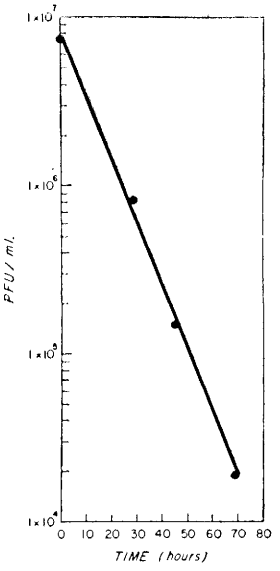
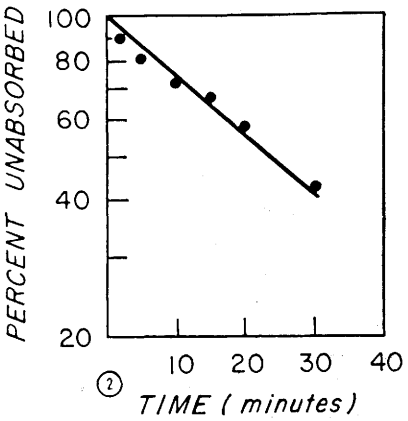


FIG. 1. Thermal inactivation 37°C.

temperatures. Less than 1% of infectivity survived 56°C for 15 minutes and approximately 50% remained after exposure to 45° for one hour. Fig. 1 shows decrease in titers during 39 hours incubation at 37°C.

Adsorption rate. Plaque bottles were shaken free of residual fluid and inoculated with 0.1 ml of suitable dilution of HA1 to yield 30-100 plaques/bottle for each adsorption period. At the end of each period, unadsorbed virus was removed by washing each bottle twice with 10 ml of cold BSS. Monolayers were overlaid with 5 ml of 0.6% agar in maintenance medium, incubated 3 days and number of plaques counted. Percent unadsorbed virus (Fig. 2) was calculated by subtracting plaque count for each adsorption period from count after adsorption for one hour and dividing by 100. Since plaque counts after 2 hours adsorption were less than 10% greater than counts after one hour, the latter were used routinely.

Evidence of antibody in sera used in tissue culture. Reports of antibody to HA1 in humans(1), cattle(2,3), and guinea pigs(10) necessitate pretesting of all sera with this virus. Data in Tables II and III show that sera from each of 8 calves, from pooled calf and from pooled human sera showed evidence of viral inhibition. All sera were trypsin treated and heat inactivated. The possibility that viral neutralization by serum in these tests is due to antibody is suggested by the wide range of titers, lack of decrease in titers after trypsin treatment, heat inactivation, or acetone extraction, and presence of inhibitor in the 20% sodium sulphate precipitated "gammaglobulin," prepared as described by Thurston *et al.*(11). We have not encountered inhibitors in our rabbit sera. However, each of 3 guinea pigs given a single intranasal injection of 2 x 10^6 pfu of HA1 developed significant levels of antibody, suggesting the possibility of natural undetected infection of laboratory animals with this virus. Table III shows hemagglutination inhibition titers, neutralization titers, and plaque reduction results



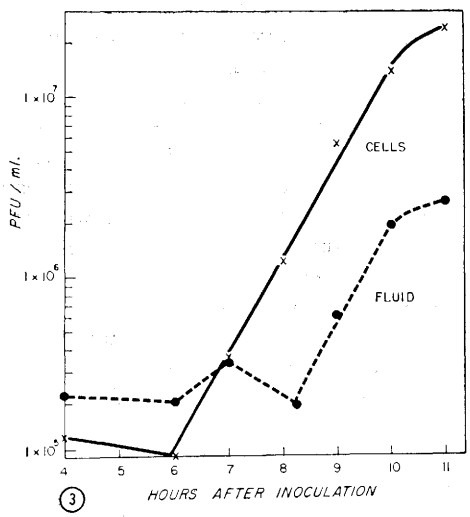


FIG. 2. Adsorption of HA1 to Lohi cells.

FIG. 3. HA1 growth curve. --●-- in supernatant fluid. —×— in cells disrupted in 5 ml fluid.

with individual sera from 8 calves all under 6 months of age. Tube dilution neutralization results were obtained by mixing 0.5 ml of 4 fold dilutions of serum with 0.5 ml of virus containing 10^3 TCD_{50}/0.1 ml. After one hour incubation at room temperature, 0.2 ml of the mixture was added to cultures of Lohi cells. After 3 days incubation infected controls showed confluent hemadsorption when guinea pig red cells were added. The result with serum virus mixtures varied from no evidence of hemadsorption (complete neutralization) to complete coverage of monolayers by red cells. Those serum dilutions limiting hemadsorption to less than 50% of area of the monolayer are arbitrarily shown as exhibiting partial neutralization. Although the 3 methods are in general agreement in measuring viral inhibition, the sensitivity of plaque reduction method is demonstrated in results with serum from calf number 4.

Growth curve. Plaque bottles containing 2 x 10^6 Lohi cells grown in BSS 80%, yeast extract 0.1% and human serum 20% were washed 3 times and inoculated with 0.1 ml of virus containing approximately 6 x 10^6 pfu. After adsorption period of 1 hour, 5 ml of maintenance medium were added to each bottle and cultures incubated at 37°C for various intervals. Fluid was then removed from the bottle (fluid sample) and replaced by 5 ml of cold maintenance medium (cell sample) and both samples frozen and later assayed simultaneously by hemadsorption plaque technic. Fig. 3 shows results of this experiment. Starting 6 hours after inoculation or, after 5 hours incubation, titer of virus in cells increased logarithmically. Titer in the fluid, including any detached cells or cell debris, began to increase 2 hours after that in the cells. By 11 hours total virus in the fluid was only 1/10 that in cells. Thus concentration of virus per unit volume of packed cells was of the order of a thousand times that in the fluid. In this experiment an average of 50 pfu of virus was produced/cell.

TABLE II. Inhibition by Pooled Sera.

Serum	Hemagglutination inhibition	Plaque reduction No.	Plaque reduction % reduction
Calf	1:160	0	>99
" "gammaglobulin"	"	0	"
" "acetone extracted"	"	0	"
Human	1:40	0	"
Horse	1:10	>300	None det.*
Rabbit	<1:10	"	*Idem*
Expected (from control titration)		450	

* None detected.

TABLE III. Inhibition by Individual Calf Sera.

Neutralization titer Complete	Neutralization titer Partial	Hemagglutination inhibition	Plaque reduction No.	Plaque reduction % reduction
1:8	1:128	1:80	0	>99
1:32	"	1:40	1	"
1:8	1:32	1:20	0	"
<1:8	"	<1:10	19	95
"	"	1:40	8	98
1:512	>1:2048	>1:320	0	>99
1:8	1:128	1:40	2	"
1:32	"	1:160	4	99
Expected (from control titration)			480	

Rate of virus synthesis is a sensitive indicator of cell-virus relationship. Preliminary experiments suggest that the pattern shown in Fig. 3 may not hold for all other cell systems.

Summary. Various parameters involved in assay and growth of HA1 virus in tissue culture have been examined. Variation in occurrence of cytopathic changes in different cell systems led to development of a new plaque assay technic utilizing hemadsorption properties of the virus. HA1 is adsorbed to cell monolayers rapidly from small volumes (0.1 ml) so that 1 hour adsorption is adequate for routine plaque work. Thermal inactivation curve showed a halflife at 37°C of 10 hr. Presence of antibody to HA1 in human and calf serum, and in serum from guinea pigs inoculated intranasally confirms work by others and emphasizes the need to pretest all sera used in work with this virus. Growth curves showed that at end of logarithmic increase, concentration of virus in the supernatant fluid was less than 0.1% that in cells. An average of approximately 50 pfu was produced/cell.

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Mineralocorticoid Effects of 9α-Fluorodeoxycorticosterone in Adrenalectomized Rats.* (25726)

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A large number of 9α-halogenated derivatives of hydrocortisone, corticosterone, 11β-hydroxyprogesterone and 11β,17α-dihydroxyprogesterone and of corresponding 11-oxo steroids have been prepared from 11α-hydroxy intermediates(1,2). In addition to glucocorticoid effects, many of these compounds have shown high levels of mineralocorticoid and survival activity(2-7). So far, however, no biological data seem to be available for 9α-halogenated steroids lacking oxygen functions at carbon 11. From this standpoint, our tests with 9α-fluorodeoxycorticosterone acetate for mineralocorticoid effects in rats are believed to be of interest. The steroid was synthesized directly from corticosterone acetate by Bergstrom and Dodson(8).

Materials and methods. Male Sprague-Dawley rats (150-200 g) fed Purina Chow for about a week were adrenalectomized and maintained with sucrose cubes and tap water overnight. Approximately 24 hours following operation, the animals were injected subcutaneously with deoxycorticosterone acetate (DCA) or with various doses of 9α-fluorodeoxycorticosterone acetate (9α-F-DCA) in Mazola oil. Brisk heating readily dissolved 9α-F-DCA in oil. All animals received in addition a subcutaneous injection of 2.5 ml isotonic saline. Four-hour samples of urine were collected in metabolism cages and analyzed for Na and K content. The DCA-like property of 9α-F-DCA was evaluated by its influence on Na excretion and the ratio of Na/K. After preliminary tests, 9α-F-DCA and DCA were tested simultaneously in a 4-point comparative assay with 14 rats/point, and relative potencies were calculated by the method of Irwin as discussed by Pugsley(9). All measurements of urinary electrolytes have been converted to logarithms. Repeated standardization tests have revealed that this adjustment gives statistically suitable responses, with relatively constant standard error values over a wide dosage range of DCA. Analyses for urinary Na and K were done on Beckman flame photometer.

Results. Data from several initial tests with 9α-F-DCA are summarized in Table I, together with those of 65 untreated animals for convenient reference. DCA as a reference standard at 10 μg/rat in each test produced Na retention and a reduction of ratio of Na/K. A small K excretion accompanied these urinary changes. In these data, 9α-F-DCA produced greater effects on electrolytes over the range of 1-500 μg/rat than did the standard dose of DCA. However, 9α-halo steroid was less effective at dose of 0.3 μg than 10 μg of DCA, to suggest a potency of at least 10 but less than about 30 times rela-

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