for example that of lactalbumin hydrolysate (7), may be due to presence of ammonia. This may also account for the apparent variations observed in susceptibility of tissue culture cell systems to virus infections. Since ammonia tends to accumulate in the medium during cell growth, the age of culture media becomes a significant factor in cell susceptibility to infection. Preliminary studies indicate that the primary source of ammonia in fresh medium is glutamine. Inasmuch as glutamine has been shown to be required for virus synthesis in some systems(8) and markedly to change the type of CPE observed in others(9) careful consideration must be given to incorporation or exclusion of this material in all systems.

Of particular interest is the mechanism by which this inhibition is effected. Since addition of the ammonium salts must be made prior to or simultaneously with the virus inoculum for maximum protection, one would suspect an interference with virus adsorption to the host cell or a direct virucidal effect. Experimental evidence seems to rule out these explanations and consequently some intracellular mechanism must be suspected. An understanding of this inhibition may shed new light on the process of virus replication with possible applications to viral chemotherapy. Studies are now in progress to explore the mechanism involved as well as the effect of ammonium ions on other virus-cell systems. The effect of various other inorganic ions and the smaller organic amines is also being investigated.

Summary. Ammonium ions in trace amounts were shown to exert a very marked protective effect upon a tissue culture system infected with influenza virus. The ions inhibited viral synthesis as well as viral CPE and the effect was apparently not due to interference with virus adsorption or to a direct inactivation of the virus. It was also shown that the tissue culture system produced sufficient ammonia during growth to render itself insusceptible to the virus.

Addendum. Since the preparation of this manuscript a paper has appeared, Eaton, M. D. and Scala, A. R., *Virology*, 1961, v13, 300, in which inhibition of both influenza and Newcastle disease viruses by ammonia in Krebs 2 cells was reported. The results and conclusions given by these authors are very similar to those presented here.

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Structure-Activity Relationships of Adrenocorticoids. (26654)

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The past 8 years have heralded significant advances in syntheses of cortisone and hydrocortisone analogs possessing quantitative and qualitative differences in metabolic activities. Though it is not possible to predict accurately the qualitative metabolic effects of chemical substituents on hydrocortisone, this communication describes the influence of 1,2 unsaturation, 6α-methylation, 9α-fluorination, 16α-hydroxylation and 16α,17α-ketalization, alone and in combination, on liver glycogen deposition and thymus involution in

TABLE I. Comparative Adrenocorticoid Activities.

Steroid	No. of assays	Relative potency (95% confidence limits)* Liver glycogen deposition	Thymus involution
Hydrocortisone		1.0	1.0
16α-hydroxyhydrocortisone	4	.4 (.3- .6)	.3 (.2- .3)
Hydrocortisone-16α,17α-acetonide	3	2.0 (1.6- 2.5)	2.2 (2.0- 2.5)
Δ^1-hydrocortisone	2	4.1 (2.9- 5.7)	2.3 (1.7- 3.2)
Δ^1-16α-hydroxyhydrocortisone	1	.9 (.5- 1.0)	1.3 (.8- 2.1)
Δ^1-hydrocortisone-16α,17α-acetonide	1	13.0 (7 -22)	17.0 (10 -28)
6α-methylhydrocortisone†	1	9.5 (6.2-14.5)	5.8 (4.5- 7.7)
6α-methyl-16α-hydroxyhydrocortisone	2	1.1 (.8- 1.5)	1.2 (1.0- 1.5)
6α-methylhydrocortisone-16α,17α-acetonide	1	10.0 (5 -18)	14.0 (10 -20)
9α-fluorohydrocortisone	2	6.1 (4.8- 7.6)	5.6 (4.5- 6.9)
9α-fluoro-16α-hydroxyhydrocortisone	2	7.9 (5.5-11.5)	2.4 (1.7- 3.2)
9α-fluorohydrocortisone-16α,17α-acetonide	2	14.0 (10 -20)	14.0 (12 -17)
6α-methyl-9α-fluorohydrocortisone†	2	9.0 (6.8-11.9)	9.8 (8.0-11.9)
6α-methyl-9α-fluoro-16α-hydroxyhydrocortisone	3	2.8 (2.3- 3.4)	4.2 (3.6- 4.8)
6α-methyl-9α-fluorohydrocortisone-16α,17α-acetonide	1	15.0 (9 -24)	29.0 (21 -40)
Δ^1-6α-methylhydrocortisone	2	8.4 (5.3-10.4)	10.0 (8 -13)
Δ^1-6α-methyl-16α-hydroxyhydrocortisone	2	2.0 (1.2- 3.4)	2.2 (1.8- 2.6)
Δ^1-6α-methylhydrocortisone-16α,17α-acetonide	2	24.0 (19 -29)	28.0 (22 -34)
Δ^1-9α-fluorohydrocortisone	2	13.0 (8 -22)	16.0 (13 -20)
Δ^1-9α-fluoro-16α-hydroxyhydrocortisone	9	6.1 (5.5- 6.7)	3.9 (3.6- 4.3)
Δ^1-9α-fluorohydrocortisone-16α,17α-acetonide	5	32.0 (16 -39)	33.0 (29 -38)
Δ^1-6α-methyl-9α-fluorohydrocortisone‡	2	16.0 (13 -20)	25.0 (20 -31)
Δ^1-6α-methyl-9α-fluoro-16α-hydroxyhydrocortisone	4	4.9 (4.1- 5.9)	5.5 (4.8- 6.3)
Δ^1-6α-methyl-9α-fluorohydrocortisone-16α,17α-acetonide	3	21.0 (18 -24)	70.0 (60 -81)

* Relative potency × hydrocortisone. † Steroids kindly supplied by Upjohn Co., Kalamazoo, Mich. ‡ 21-acetate.

an attempt to quantitate the increments and decrements in biological activity induced by these chemical modifications.

Materials and methods. In a 5-day bioassay, liver glycogen deposition and thymus involution were simultaneously determined in adrenalectomized, immature (40-60 g Sherman strain) male rats. Animals were maintained with Purina Lab Chow and 1% NaCl drinking fluid *ad lib.* Six concentrations of test steroids were assayed in parallel with 6 concentrations of hydrocortisone, using 4 rats per group. Twenty-four hours after adrenalectomy the rats were injected subcutaneously with steroid suspended in 0.2 ml of carboxymethyl-cellulose vehicle(1), excluding benzyl alcohol. Control animals received vehicle only. Steroid was administered daily for an additional 4 days. The rats were fasted 15 hours before and 7 hours after last injection to insure maximum depletion of liver glycogen. Animals were then anesthetized with sodium pentobarbital and livers quickly excised, weighed and hydrolyzed in 30% potassium hydroxide(2). Thymi were removed and weighed on Roller-Smith torsion balance. Liver glycogen was measured by the anthrone method of Seifter *et al.*(3). Data were statistically analyzed by fitting straight lines by the method of least squares and computing the corresponding equation. Potency estimates were combined by the method of Wilcoxon and Haynes (unpublished).

Results. Modification of the hydrocortisone molecule by either introduction of a double bond at the 1,2 positions, 9α-fluoro, 6α-methyl, 16α-hydroxy or 16α,17α-isopropylidenedioxy, or combinations of these structural constituents endows the steroid with distinct biological potencies (Table I). For the majority of the steroids studied, the relative potencies calculated for liver glycogen deposition and thymus involution compared favorably, suggesting that potency estimates from various assays of glucocorticoid

activity would be similar if conducted simultaneously. This is by no means a new concept, but has been advocated by Stephenson (4). These potency estimates have been tabulated to show the activity-enhancing capacity of each substituent on both liver glycogen deposition and thymus involution (Table II).

The glycogenic activity of hydrocortisone was increased approximately 2-fold by introduction of a double bond at positions 1,2. This augmentation in activity represents the

TABLE II. Action of Functional Groups on Adrenocorticoid Potency.

Parent steroid (Potency = 1)	Substituent											
	Δ^1		6α-methyl-		9α-fluoro-		16α-hydroxy-		16α,17α-acetonide-	
	Biological potency relative to parent steroid									
	LGD	TI	LGD	TI	LGD	TI	LGD	TI	LGD	TI
Hydrocortisone	4.1	2.3	9.5	5.8	6.1	5.6	.4	.3	2.0	2.2
16α-hydroxyhydrocortisone	2.2	4.3	2.7	4.0	—	8.0	—	—	—	—
Hydrocortisone, 16α,17α-acetonide	6.5	7.7	5.0	6.4	7.0	6.4	—	—	—	—
Δ^1-hydrocortisone	—	—	2.0	4.3	3.2	6.9	.2	.6	3.2	7.4
Δ^1-16α-hydroxyhydrocortisone	—	—	2.2	1.7	6.8	3.0	—	—	—	—
Δ^1-hydrocortisone-16α,17α-acetonide	—	—	1.9	1.7	2.5	1.9	—	—	—	—
6α-methylhydrocortisone	.9	1.8	—	—	1.0	1.7	.1	.2	1.1	2.4
6α-methyl-16α-hydroxyhydrocortisone	1.8	1.8	—	—	2.5	3.5	—	—	—	—
6α-methylhydrocortisone-16α,17α-acetonide	2.5	2.1	—	—	1.5	2.1	—	—	—	—
9α-fluorohydrocortisone	2.1	2.9	1.5	1.8	—	—	1.3	.4	2.3	2.5
9α-fluoro-16α-hydroxyhydrocortisone	.8	1.6	.4	1.8	—	—	—	—	—	—
9α-fluorohydrocortisone-16α,17α-acetonide	2.3	2.4	1.1	2.1	—	—	—	—	—	—
6α-methyl-9α-fluorohydrocortisone	1.8	2.6	—	—	—	—	.3	.4	1.7	2.9
6α-methyl-9α-fluoro-16α-hydroxyhydrocortisone	1.7	1.3	—	—	—	—	—	—	—	—
6α-methyl-9α-fluorohydrocortisone-16α,17α-acetonide	1.4	2.4	—	—	—	—	—	—	—	—
Δ^1-6α-methylhydrocortisone	—	—	—	—	1.9*	2.5*	.2	.2	2.9	2.9
Δ^1-6α-methyl-16α-hydroxyhydrocortisone	—	—	—	—	2.5	2.5	—	—	—	—
Δ^1-6α-methylhydrocortisone-16α,17α-acetonide	—	—	—	—	.8	2.5	—	—	—	—
Δ^1-9α-fluorohydrocortisone	—	—	1.2*	1.6*	—	—	.5	.2	2.5	2.1
Δ^1-9α-fluoro-16α-hydroxyhydrocortisone	—	—	.8	1.4	—	—	—	—	—	—
Δ^1-9α-fluorohydrocortisone-16α,17α-acetonide	—	—	.7	2.1	—	—	—	—	—	—
Δ^1-6α-methyl-9α-fluorohydrocortisone	—	—	—	—	—	—	.3	.2	1.3	2.8
Mean	2.3	2.8	2.4	2.9	3.3	3.9	.4	.3	2.1	3.2

LGD = liver glycogen deposition; TI = thymus involution.

* Acetate.

mean increment for 12 steroids with ranges from 0.8 to 6.5. Substitution of a methyl group in the α position at carbon 6 enhanced the ability of parent steroids to induce glycogen deposition approximately 2-fold (range 0.4-9.5). Fluorination at carbon 9 resulted in a 3-fold increase in activity (range 0.8-7.0). Although the addition of a hydroxyl group at the 16α position decreased the glycogenic potencies of the various corticoids studied by approximately 0.4 (range 0.1-1.3), formation of the 16α,17α-isopropylidenedioxy derivatives increased it some 2-fold (range 1.1-3.2).

The thymolytic potency of the steroids included in this study was increased in all cases by introduction of a double bond in positions 1,2, 6α-methyl, 9α-fluoro and the 16α,17α-acetonide. Data in Table II demonstrate that an α hydroxyl group at carbon 16 depressed the action of corticosteroids by one-third.

Discussion. Though not conclusive, these data suggest a possible means of predicting the effects of structural modifications on the biological activities of corticosteroids. Thus, introduction of a double bond at positions 1, 2 or 6α-methylation, or 16α,17α-ketalization increases glycogenic and thymolytic activity 2- to 3-fold. The presence of a 9α-fluoro group enhances activity 3 to 4 times. 16α Hydroxylated steroids were 0.3 to 0.4 times as efficacious as the nonhydroxylated parent steroids.

In comparing increments of biological activity, one has the impression that they are greater for either Δ^1, 6α-methyl or 9α-fluoro substituents on hydrocortisone and 16α-hydroxy hydrocortisone than on more complex substituted steroids. It is thus apparent that modification in biological activity induced by a specific permutation of the steroid is generally dependent on presence of other activity-influencing groups.

Presentation of enhancement factors for numerous functional groups has previously been outlined by Fried and Borman(5). They have tabulated a series of enhancement factors, which, when multiplied by the biological activity of the parent steroid, results in a numerical figure indicative of activity of the substituted steroid. Some of these enhancement factors(5) are presented in Table III with those recorded in this publication.

TABLE III. Adrenocorticoid Activity-Enhancement Factors.

Functional group	Liver glycogen deposition	Thymus involution	Anti-inflammatory
9α-Fluoro	10 * 3.3	3.9	7–10*
1 Dehydro	3–4 * 2.3	2.8	3–4 *
6α-Methyl	2–3 * 2.4	2.9	1–2 *
16α-Hydroxyl	.4–.5* .4	.3	.1–.2*

* Fried and Borman(5).

With the exception of the enhancement factor for the 9α-fluoro substituent, the data are in agreement.

Application of the indicated enhancement factors to steroids not possessing either 11β, 17α or 21-hydroxyl functions has not been fully studied. However, it has been recognized in this laboratory that ketalization of the 16α,17α-hydroxy groups of 11-keto adrenocorticoids does not potentiate biological activity.

Summary. The influence of 1,2 unsaturation, 6α-methylation, 9α-fluorination, 16α-hydroxylation, and 16α,17α-ketalization alone and in combination on glycogenic and thymolytic activity has been simultaneously assessed in immature adrenalectomized rats. Good agreement between glycogenic and thymolytic relative potencies was observed. Unsaturation at positions 1,2 or 6α-methylation, or 16α,17α-ketalization increased glycogenic and thymolytic activity 2- to 3-fold. Presence of a 9α-fluoro group enhanced activity 3- to 4-fold. 16α-Hydroxylation reduced activity by $\frac{1}{3}$ to $\frac{1}{4}$.

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Activity of α-Phenoxyalkyl Penicillins Against Sensitive and Resistant Staphylococci. (26655)

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While studying the antimicrobial activity of a large number of synthetic penicillins, it became apparent that members of the α-phenoxyalkyl penicillin series were displaying significant activity against penicillinase-producing resistant staphylococci. Preliminary microbiological data and methods of synthesis of a number of such penicillins have been reported(1,2). A detailed study was done on certain members of this series of penicillins using both sensitive and resistant staphylococci in an attempt to delineate the extent of the activity and relate some of the properties of the penicillins to the structure. Comparisons of these penicillins were made with penicillin G and dimethoxyphenyl penicillin, the former being penicillinase sensitive and the latter extremely penicillinase resistant (3). Studies were also carried out to determine the *in vivo* effectiveness of the different penicillins in curing penicillin-sensitive and penicillin-resistant *Staphylococcus aureus* infections in mice.

Materials and methods. The penicillins used in this study were alkyl derivatives of phenoxymethyl penicillin substituted in the α position. The side chains of the penicillins studied are shown in Table I together with the side chains of controls, benzylpenicillin (penicillin G) and dimethoxyphenyl penicillin. The last was used as the monohydrate of the sodium salt. All the other penicillins were potassium salts. Benzylpenicillin (penicillin G), α-phenoxyethyl penicillin (phenethicillin), dimethoxyphenyl penicillin, and phenoxymethyl penicillin (penicillin V) were obtained from commercial sources, the first 3 being Bristol Laboratories production lots, the last one a Lilly product. The other penicillins were synthesized by the Organic Chemistry Dept. of Bristol Laboratories.

Cultures were maintained on porcelain beads(4), on nutrient agar slants, or frozen in milk suspension. The inoculum used for determination of the minimum inhibitory concentration was prepared by overnight incubation of a bead or a loopful from a slant culture in heart infusion broth. Standard 2-fold serial dilution technics in heart infusion broth (Difco) or in heart infusion mixed 1:1 with pooled filter-sterilized human serum were used. Usually, the overnight culture was diluted 10^4, but other dilutions were also used as noted below. Cultures for *in vivo* challenges were grown overnight in veal infusion broth. The challenge dose of *Staphylococcus aureus*, Smith strain, consisted of a dose of organism equivalent to 100 LD_{50} or approximately 8×10^5 viable cells per mouse, suspended in 5% hog gastric mucin. The challenge dose of *S. aureus*, 1633-2 strain, consisted of a dose of organism equivalent to 50 LD_{50} or approximately 1.1×10^8 viable cells per mouse prepared similarly. This latter strain was isolated from clinical material and is a penicillinase producer.

Penicillinase inactivation of the penicillins was determined by the method of Henry and Housewright(5). Acetone-ether treated staphylococcal cells prepared according to Gilson and Parker(6) from cells of the 52-75 strain of *Staphylococcus aureus* were used as the source of penicillinase. The culture was induced for 3 hours at 28°C on a rotary shaker with 100 μg/ml of benzylpenicillin added at 0 time, followed by a similar addi-