Finally, add 1 ml of 1 N sulfuric acid and return the rods to the centrifuge tubes. Include a blank tube containing 1 ml of sulfuric acid and a standard tube containing 1 ml of working standard with each series of determinations.

Dissolve the precipitate by placing the tubes in a boiling water bath and stirring for 4-5 minutes. Remove the rack of tubes from the bath and allow them to cool for a few minutes. Then add 4.0 ml of ceric sulfate-cerous sulfate reagent, stir, and remove the rods. Finally, mix thoroughly by inversion, using parafilm instead of rubber stoppers, and allow to stand for 15 minutes. Transfer to colorimeter tubes and measure the color in an Evelyn colorimeter with filter 42 at the 6 cc aperture. Obtain the concentration of calcium from the difference in density between the blank and unknown solutions on a standard curve prepared from solutions of known amounts of calcium oxalate. The values obtained for the standard should be close to 10 mg per 100 ml.

In Table I the results obtained by this method in a series of sera are compared with those determined in 2 ml samples by the method of Clark and Collip(7). It is evident that the values agree in every case within 0.3 mg per 100 ml.

TABLE I. Comparison of Results of the New Method for 0.5 ml of Serum with Those Determined by Titration in 2 ml of Serum.

Serum	New method mg per 100 ml	Clark-Collip method mg per 100 ml
1	13.3	13.0
2	13.2	13.4
3	12.4	12.1
4	12.1	11.9
5	10.8	10.9
6	9.7	9.8
7	9.1	9.1
8	9.0	8.9
9	8.8	8.6
10	8.7	8.7
11	8.6	8.6
12	8.5	8.4
13	8.1	8.3
14	7.9	8.0
15	7.6	7.5

Summary. A method is described for the colorimetric determination of calcium in 0.5 ml of serum with ceric sulfate. Data are presented to show that the results agree within 0.3 mg per 100 ml of those determined by titration with permanganate.

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6. Hausdorf, G., *Die Pharmazie*, 1947, v2, 257.
7. Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, v63, 461.

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Increased Virus in Eggs Injected with Cortisone. (18406)

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In the course of investigations of viral infections of the chick embryo, experiments were undertaken to determine the effects of alteration of host physiology upon viral multiplication. Studies by Karnofsky, *et al.*(1), revealing the profound effects of adrenal cortical steroids on the growth and development of the chick embryo, suggested the use of these hormones to alter host metabolism*. Mumps and influenza viruses were employed because of the relative ease and precision with which their concentration may be measured by the hemagglutination reaction.

Materials and methods. Viruses. The PR8 strain of influenza A virus, the Lee strain of influenza B virus, and the Habel strain of mumps virus were employed. 10^4 to 10^5 ID_{50}

1. Karnofsky, D. A., Stock, C. C., and Rhoads, C. P., *Fed. Proc.*, 1950, v9, 290.

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TABLE 1. Comparison of Viral Concentration of Allantoic Fluid Pools from Cortisone-Injected and Control Eggs.

Virus	Control		Cortisone			Cortisone
	Hemagglutination titer*	Infectivity titer	Dose, mg	Hemagglutination titer*	Infectivity titer	Control %
Mumps	158		2.5	744		471
Lee (1)	3810		5.0	10740		282
(2)	1624		2.5	3320		204
(3)	3400	$10^{-6.7}$	1.0	6780	$10^{-8.3}$	200
PR8 (1)	1956		5.0	2406		123
(2)	1866		5.0	2240		120
(3)	814		2.5	1262		155

* As measured by the photometric hemagglutination technic.

was injected into the allantoic sac in 0.1 cc volume.

Cortisone. A saline suspension of cortisone acetate (Merck, 11-dehydro-17-hydroxycorticosterone-21-acetate), containing "suspending agents" and 1.5% benzyl alcohol, was used. 1 to 5 mg in volumes of 0.1 to 0.2 cc was injected into the yolk sac of each egg prior to viral inoculation.

Control material. Benzyl alcohol (1.5%) in 0.85% NaCl solution buffered to pH 7.2 with phosphate was utilized as a control for the cortisone yolk sac injection. (Recent experiments employing the "suspending agents" incorporated in the Merck preparation as a yolk sac injection have disclosed no evidence of increased viral concentration in eggs so injected).

Procedure. Groups of 7 to 10-day old fertile White Leghorn eggs were injected in the yolk sac with cortisone or control material 30 minutes to 48 hours prior to the introduction of virus by the allantoic route. Eggs were incubated thereafter at 35°C for 32 to 52 hours when infected with either influenza virus, and for 131 hours when mumps virus was employed. Embryos were then placed at 4°C for 12 to 18 hours prior to the harvesting of allantoic fluid and embryonic tissues. Embryos were removed, freed from extraneous tissue, and dried with blotting paper before weighing. Hemagglutination titrations were performed with 0.5% suspensions of chicken RBC in the usual manner as well as by a precision photometric technique developed recently in this laboratory. This procedure has a precision of $\pm$ 4% in replicate determinations with each of the viruses employed. Infectivity titrations were carried out *in ovo* using groups of 4 to 6 eggs per dilution.

Results. The results of representative experiments are summarized in Table I. The viral concentration of pooled allantoic fluids from cortisone-injected eggs was 120 to 471% of the viral concentration of control eggs as determined by the photometric hemagglutination technic. Allantoic fluids from eggs injected with cortisone but not with virus caused no agglutination of chicken RBC. In all instances the allantoic fluid volume of cortisone-injected eggs exceeded that of controls, militating against fluid concentration as a cause of increased virus in the former. Further evidence of increased viral concentration was provided by the less precise method of comparative infectivity titrations of allantoic fluid pools.

In all experiments the embryo-stunting effect described by Karnofsky(1) was observed; cortisone-injected embryos averaged 32% less in weight than controls. In addition, marked thickening of the chorioallantoic and amniotic membranes and an increase in trichloracetic acid-precipitable material in dialyzed allantoic fluid were noted. This increase in what is presumably protein was demonstrable in allantoic fluids from eggs injected with cortisone alone, and therefore appears to be a phenomenon unrelated to similar protein increases previously described in connection with viral multiplication in the allantoic sac(2).

2. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 708.

Studies of the distribution of virus in cortisone-injected and control eggs are now in progress. Preliminary experiments have disclosed increased viral concentration in embryo tissue from eggs injected with cortisone. It is therefore unlikely that the increased amount of virus in the allantoic fluid is the result merely of a localization or re-distribution of virus in the altered host.

Discussion. The data presented indicate that under the experimental conditions employed the concentration of influenza or mumps viruses attained in developing hens' eggs injected with cortisone may exceed that in similarly infected control eggs by a significant amount. If the concentration of mumps virus in cortisone-injected eggs is multiplied by a factor expressing the increased allantoic fluid volume of such eggs, it is found that the increased yield of virus per egg may be as high as 688% of controls.

Coincident with the observed increases in the amount of virus formed, profound changes in the host occur which are attributable to the effects of cortisone(1). Because the mechanism of cortisone effects upon the host is obscure, speculation concerning the basis for increased viral concentration is not warranted at present. It should be recalled, however, that the chick embryo is not known to possess immunologic mechanisms so that the suppression of antibody formation observed with cortisone(3) cannot be invoked in explanation of the present phenomenon.

Recent studies by Kalter, *et al.*(4) on the effect of growth hormone and testosterone on influenza virus multiplication in mice revealed increases in the "rate of virus proliferation and . . . the amount of virus present" in lungs of hormone-injected animals. These increases were related by the authors to increased host protein anabolism induced by hormone administration. In contrast, cortisone injection depresses chick embryo growth and may produce weight loss and negative nitrogen balance in the rat(5), but nonetheless results in increased viral concentration in the infected host in which anabolic function is apparently depressed. These findings constitute a paradox only if the dual assumption is made that: (1) viral concentration is directly related to host protein anabolism, and (2) cortisone actually causes depressed anabolism of the developing egg. Present evidence is insufficient to justify either assumption.

Summary. The concentration of Lee, PR8, or mumps virus in the allantoic fluid of eggs injected with cortisone acetate is significantly greater than the concentration of these agents in the allantoic fluid of control eggs.

3. Germuth, F. G., Jr., and Ottinger, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 815.

4. Kalter, S. S., Stuart, D. C., Jr., and Tepperman, J., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 605.

5. Ingle, D. J., *A Symposium on Steroid Hormones*, 1950, The University of Wisconsin Press, p161.

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Non-effect of a Growth Hormone Preparation in the Regenerating Feather.* (18407)

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An acceleration of feather growth normally occurs in the fowl during the juvenile molts when the bird is in the phase of rapid development. Such accelerations occur in association with the appearance of new pigment—and structural traits, as may be seen when the measured axial increments of successively regenerating feathers from given follicles are compared in relation to the respective pat-

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