tradictory effects observed throw considerable doubt upon the relation of the *in vitro* response observed to the action of these agents in the intact animal. The *in vitro* response, therefore, seems to be related to some property of the steroid which bears no discernible relationship to its physiological action. This conclusion seems to be substantiated by the observation of Jones and Gerarde(14) that cortisone acetate has no effect in essentially the same system used by Layton(4) where the effect of cortisone alcohol was observed.

Summary. An *in vitro* system for the synthesis of acid insoluble sulfate (presumably chondroitin sulfate) has been devised. The synthesis may be influenced (inhibited or stimulated) by the *in vitro* addition of cortical steroids. The influence of the steroids, however, seems to bear no relation to their physiological action. There was no difference in the rate of *in vitro* sulfate synthesis by cartilage from normal or adrenalectomized rats.

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Assay of Influenza Virus Infectivity with Chicken Embryonic Membranes.* (21165)

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(Introduced by Max A. Lauffer.)
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The purpose of this study was to develop an improved assay technic for influenza virus infectivity. The method proposed has several advantages over the titration in chicken embryos in that it requires less labor, less space, and one-twentieth the usual number of eggs. The principal disadvantage is that this new method is not as sensitive. Two animals have proven satisfactory for measuring influenza virus infectivity. These are the mouse(1) and the chicken embryo(2). Bernkopf(3) demonstrated that the virus could be grown on the chorioallantoic membranes of eggs from which the fetus had been removed. There have been references(4-9) to the growth of the virus in cells growing in tissue culture. In view of these findings it was considered likely that small pieces of the chorioallantoic membrane would support influenza virus growth and could, therefore, be used as the host in an assay system for the virus.

Materials and methods. The PR8 strain of influenza virus was used. Stocks had undergone from 17 to 19 egg passages from a pool of preparations A, B, and C described in a paper by Lauffer and Wheatley(10). The individual assay unit consisted of a half-inch test tube containing one ml of nutrient broth, one membrane section, and the appropriate virus inoculum. Membrane sections were prepared by first removing everything except the chorioallantoic membrane from the inside of

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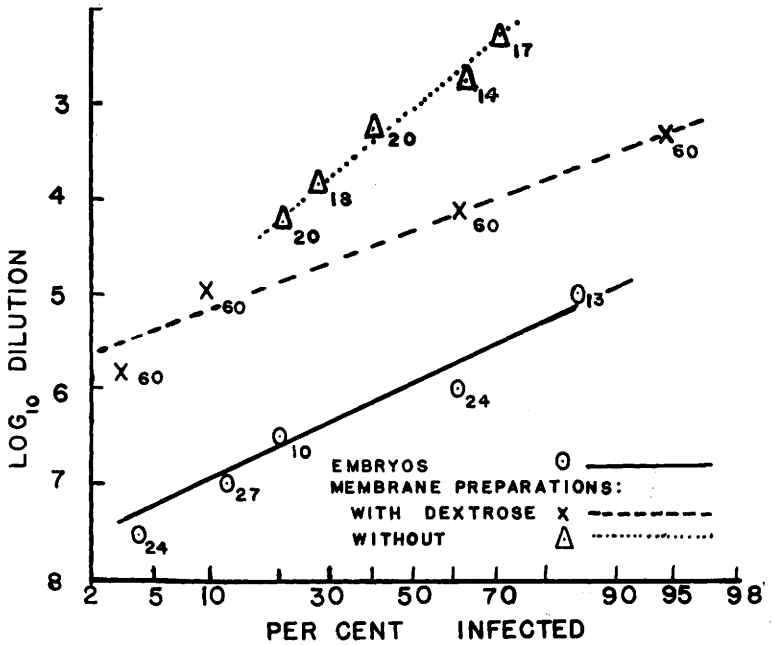

FIG. 1. Comparison of responses with 3 different assay units. Uppermost curve shows distribution of response to PR8 influenza A virus by chicken embryos. The 2 other curves show response of membrane preparations with and without dextrose. Numerals beside the points give number of assay units employed at the given dilution. These points represent the experimental per cent of infection.

9-15-day-old embryonated White Leghorn eggs. The chorioallantoic membranes together with the attached cells and shell membranes were cut into one-quarter inch discs with an ordinary paper punch and were stored from 1-8 days in sterile growth medium at 4°C. The punch and the outside of the shell were sterilized by swabbing with 70% ethyl alcohol. About 16 usable punchings were obtained from an average embryo. The growth medium contained 8 g of nutrient broth, 5 g of sodium chloride, 100,000 units of penicillin, 0.1 g of streptomycin, and, in certain cases, 1 g of dextrose per liter of distilled water. Serial dilutions (usually 10-fold) were made of the virus in broth and the appropriate dilutions were inoculated into the membrane-test tube preparations. The inoculation procedure was to add 0.1 ml of each dilution to each of a given number of these preparations. The inoculated membrane preparations were then incubated at 37°C for 45 hours, or for 112 hours if the broth contained dextrose. The criterion for infection was ability of the incubated broth to agglutinate chicken red blood cells in an 0.8% suspension. The 50% infectious doses were calculated by a modification of the probit method(11). This consists of plotting on probability paper the cumulated percentages of infected membranes against the logarithms of dilution and taking the value of the logarithm of dilution at 50% cumulated response as the endpoint. In certain cases the endpoints were also calculated by an algebraic method(12) equivalent to the method of Reed and Muench(13). Membrane preparation and embryo infectious titers were compared during growth of the virus and during inactivation by urea. The virus was inactivated at 37°C in 1 M urea containing .09 M pH 7 phosphate buffer and 10% broth. During growth, 0.1 ml samples of allantoic fluid were removed at various times from an embryo inoculated with about 10^5 embryo 50% infectious doses of virus The removal was accomplished by inserting a syringe needle through the same hole in the shell that was used for inoculation. Embryo infectious titers were determined in the usual manner (2) using 10- or 11-day White Leghorn embryos.

Results. Two titrations were performed on

TABLE I. Reproducibilities of Endpoints when Membrane Preparations Are Used.

Logarithm of endpoints without dextrose	Logarithm of endpoints with dextrose
3.57	5.43
3.62	6.6
3.40	6.5
3.60	6.5
3.50	5.5
3.03	5.5
4.00	6.9
	6.73
	6.66
	6.42
Avg 3.53 ± .29	6.27 ± .57

the same sample of virus using 14 to 30 assay units per serial 2-fold dilution. The logarithms of the dilutions were plotted as ordinates against the cumulated per cent infections using a probability scale as the abscissa. Straight lines are fitted to this data in Fig. 1. From this figure one can see that the embryos are about 800 times more sensitive than membranes, when the growth media does not contain dextrose.

Reproducibility of these titers was demonstrated by performing 7 independent titrations on the same sample of virus using 5 membrane preparations per dilution and serial 10-fold dilutions. Parallel reproducibility experiments were done in growth media with and without dextrose. A different virus sample was used in each experiment. The results are shown in Table I. The endpoints were calculated by the probit method. The standard error for the probit endpoints is .29 logarithmic units without dextrose and .57 logarithmic units with dextrose in the media. (The differences in standard errors might be attributed to the fact that not all the dilutions were prepared by the same person.) Using 5 animals per serial 10-fold dilution, Lauffer and Miller(1) found a standard error of .260 for mice, and Knight(2) found a standard error of .225 for embryos.

When dextrose is added to the broth, the sensitivity of this assay technic increases about 20-fold. For this reason, although less accuracy is obtained, dextrose was used in subsequent experiments.

When using this technic in growth and inactivation studies, it is desirable to know whether or not the membrane preparations measure the same infectious entity as the embryos. If the same virus preparation is titered by both methods, the embryo infectivity will be different than the membrane preparation infectivity due to a difference in sensitivity of the 2 methods. However, if they both measure the same infectious entity, the ratio of the infectivities, which corresponds to a difference in the logarithm of the infectivity, will be constant during growth or inactivation. The results of experiments performed to test this hypothesis are shown in Tables II and III.

When 4 membrane preparations and 3 embryos per dilution were used, the average endpoint difference was 1.66 ± .24 logarithmic units for growth, and 1.54 ± .25 logarithmic units for inactivation. The ± values are the standard errors of the means. The probability that this difference could be due to errors of random sampling is .76 and, therefore, the difference is considered insignificant. When the data of the 2 tables are pooled, an average difference of 1.60 ± .25 is obtained. In other words, the rate of growth like the rate of inactivation of PR8 influenza A virus infectivity is the same whether it is titered in embryos or in membrane-test tube preparations. These data were also used to plot the response curves of Fig. 1.

Summary. An assay technic has been developed for titrating PR8 influenza A virus infectivity on preparations of chorioallantoic membranes removed from chicken embryos. This technic is simpler and less laborious than the standard assay method involving the use of chicken embryos. It is comparable in ac-

TABLE II. Difference in Growth Values of Virus as Measured in Embryos and Membrane Preparations.

Hr of growth	Logarithm of titer: Embryos	Logarithm of titer: Membrane preparations	Difference
2	3.6	1.3	2.3
4		3.5	
6	5.0	3.6	1.4
8	5.5	4.5	1.0
10	7.5	7.8	1.8
12	9.5	7.0	2.5
24	7.3	9.5	−1.7*
27	8.3	7.3	1.0

* This value was disregarded due to the obvious disagreement with other values.

TABLE III. Difference in Inactivation Values of Virus as Measured in Embryos and Membranes.

Min. of inactivation	Logarithm of titer: Embryos	Logarithm of titer: Membrane preparations	Difference
0	9.5	8	1.5
16	9.2	8	1.2
32	8.2	7.5	.8
48	8.3	5.8	2.5
64	5.5	3.8	1.7

curacy to the embryo technic although appreciably less sensitive. Evidence is submitted to show that both technics measure the same infectious entity.

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A Rapid Pad-Plate Microbiologic Assay for Thymine.* (21166)

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Although several methods(1-4) are available for the microdetermination of thymine, none of these is suitable for the rapid estimation of this material in a variety of biological materials. Williams, Esposito and Pierce(5) have described a microbial plate method for the assay of vit. B_{12}; this makes use of paper pads as originally used in the assay of antibiotics on plates containing a solid assay medium heavily inoculated with microorganisms. As compared with the usual microbiologic methods of assay, this technic affords many advantages, particularly for the assay of large numbers of samples. The present study is concerned with the development of a similar microbial assay method for thymine and thymidine using *Streptococcus faecalis* (ATCC 8043) as the test organism.

Experimental. Materials. Thymine. Material purchased from Nutritional Biochemicals, Inc., Cleveland, Ohio, was further purified by chromatography on Dowex I followed by several recrystallizations from water. *Thymidine* was prepared with the aid of intestinal phosphatase from thymidylic acid; the latter was isolated from calf thymus deoxyribonucleic acid by the method of Hurst, Little and Butler(6). The deoxyriboside was purified by adsorption on Dowex I, hydroxyl form, at pH 11.0; after washing the column thoroughly with 0.01 *N* NaOH followed by water, the thymidine was eluted with 0.04 *M* formic acid. Thymidine was obtained as a dry powder by lyophilization of the formic acid eluant. *Dihydrothymine* was obtained from Dougherty Chemicals, Richmond Hill, N. Y.; *5-methylcytosine* was supplied by G. H. Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y.; *5-hydroxymethyluracil* and

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