larities between the two as regards their general physical and chemical properties. They are almost identical insofar as their effect on the blood pressure is concerned, as shown in Fig. 1, which reproduces typical experiments not shown in Table I. Both are effective orally, manifest their action slowly and exert a relatively prolonged effect (a week or more) compared to the evanescent action which follows the depressor action of other substances.

The relatively high potency and ready availability of crude fish oils render these more satisfactory than kidneys as a source of the active principle effective in hypertension. Further experiments on the nature of the active substance are in progress. It is possible that a quinone analogous to those found to be effective by Friedman *et al.*[2] is formed by the oxidation of marine oils and that this is the agent responsible for the observed reduction in blood pressure.

Summary. Fish body and liver oils contain a substance which is effective in reducing the blood pressure of hypertensive rats. The effectiveness of these oils is increased by oxidative procedures which destroy vitamin A. The observed activity appears to be associated with some substance, other than vitamin A, present in fish oils. Fish oils offer greater promise than kidneys as a possible source of a therapeutic agent effective in reducing the blood pressure in hypertension.

14072

Precipitation and Concentration of Influenza Virus With Alum.*

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Influenza vaccines in which the virus has been concentrated by various methods produce a better antibody response than unconcentrated preparations.[1,2] Concentration of virus from the allantoic fluid of infected chick embryos has been accomplished by lyophilizing,[1] by adsorption on precipitates which result from thawing frozen extra-embryonic fluids,[2,3] by adsorption on and elution from chicken erythrocytes,[4] and, with mouse lung preparations, by adsorption on a precipitate of calcium phosphate and solution in sodium citrate.[5]

Alum has been used in the preparation of vaccines from bacteria and bacterial toxoids especially to increase the immunizing efficiency. This paper will describe a method for alum precipitation of the virus of influenza grown in the allantoic fluid of chick embryos.

Preparation of Virus Suspensions. Virus suspension diluted to 10^{-4} was inoculated into the allantoic cavity of 12-day-old chick embryos. The embryos were then incubated at 37°C for 2 days, candled, and the live eggs placed at 4°C overnight. Chilling the embryos facilitated collection of the allantoic fluid, and admixture of blood cells was avoided. The strain PR8 of type A influenza virus and the strain Lee of type B virus were used.

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1 Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1942, **75**, 495.

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TABLE I.
Alum Precipitation of Influenza Virus in Allantoic Fluid of Chick Embryos.

Strain used	Vol. of fluid, cc	Amt of 10% $NaHCO_3$ added, cc	Amt 10% alum added, cc	pH	Titer of supernatant	Titer of ppt. conc. 10X
PR8	15	—	.3	6.7	160	1280
Titer before	15	.05	.4	6.7	80	3800
treatment = 640	15	.10	.5	6.7	40	5120
Lee	10	—	.2	6.7	480	5
Titer before	10	—	.3	6.3	320	320
treatment = 480	10	—	.4	6.0	160	1280
	10	.10	.5	6.3	40	2560

Titration of the Virus. Quantitative estimations of the virus content of allantoic fluid and of the redissolved alum precipitates were made by a modification of the chicken cell agglutination technic.[6]

Precipitation of Influenza Virus with Alum. Infected allantoic fluid was treated with varying amounts of a 10% solution of $Al_2(SO_4)_3\ K_2SO_4 \cdot 12H_2O$ in order to determine the least amount needed to produce maximum precipitation. The precipitate, which formed within a few seconds after the alum was added, was centrifuged at about 3,000 r.p.m. for 20 minutes in the horizontal head of the centrifuge. The supernatant was decanted and the precipitate washed 3 times in sterile saline. Tests on the first washing revealed only insignificant amounts of virus probably contained in the fluid adhering to the precipitate. Second and third washings did not agglutinate chicken cells in dilutions greater than 1:10 and many contained no demonstrable virus. The washed precipitates were resuspended in a volume of saline equal to 1/10 the volume of the original extra-embryonic fluids. Merthiolate at a dilution of 1:10,000 was used as a preservative.

The alum-precipitated virus readily dissolved in 20% sodium citrate and the resulting solution, restored to the original volume in saline, agglutinated chicken erythrocytes to about the same titer as the original fluids. The mouse infectivity of the redissolved precipitates was only slightly diminished.

The results of experiments on precipitation of strains PR8 and Lee are shown in Table I. Strain PR8 was precipitated with less alum than was required for strain Lee and generally a smaller loss of virus occurred with PR8 than with Lee. Of 14 attempts to concentrate PR8 tenfold, yields of 80% or over were obtained in 10 experiments. With strain Lee, however, an 80% yield was obtained in only 2 out of 8 preliminary experiments. The unsatisfactory yields, particularly with strain Lee, can be explained, in part at least, by the acidity which developed during the precipitation. pH measurements were made with indicators. At pH below 5.0 the antigenicity of influenza virus is destroyed.[7] When .5 cc of alum was added to 15 cc of fluid the pH reached 6.0 or below, and unless the acid was neutralized the agglutination titer was reduced. The addition of sodium bicarbonate brought the pH near to 6.5 with little effect on the amount of alum required to precipitate the virus.

Effect of Inactivation with Formaldehyde. Inactivation of the virus with formaldehyde had little or no effect on the precipitation of virus with alum. The allantoic fluids were treated with 0.25% formaldehyde at a temperature of 5°C for 24 hours. In some instances the formaldehyde slightly reduced the titer of the fluids. The formalinized virus was then tested for infectivity in mice, precipitated, washed, and resuspended as previously described.

Immunization of Mice. Sets of 8 mice were inoculated intraperitoneally with 0.5 cc of graded five- or tenfold dilutions of the material to be tested. For controls, mice were inoculated with 0.5 cc of 10% horse serum broth.

[6] Bodily, H. L., and Eaton, M. D., *J. Immunol.*, 1942, **45**, 193.

[7] Eaton, M. D., *J. Immunol.*, 1940, **39**, 43.

TABLE II.
Results of Immunization of Mice with Alum Precipitated Influenza Virus.

Strain	Preparation used	Lesions in mice immunized with dilutions of* 1:10	1:50	1:250	1:1250	1:6250
PR8	Untreated allantoic fluid	7.5	0	15	27.5	47.5
"	Formalinized and precipitated with alum	—	15	25	95	100
Lee	Untreated allantoic fluid	0	0	0	6.6	25
"	Formalinized and precipitated with alum	0	2.5	0	25	33

*Results given as percentage of lung tissue showing consolidation in each group of mice. Controls: 90-100% consolidation.

Two weeks later all the mice were inoculated intranasally with 0.05 cc of a dilution of mouse-adapted virus containing from 10 to 100 m.l.d. Deaths were recorded during a 10-day period at which time the remaining mice were killed and the extent of lesions in the lungs recorded. The results of some of the immunization experiments are given in Table II. The inactivated alum-precipitated suspension concentrated 10 times appeared to be slightly less antigenic in mice than the untreated virus. Most of this reduction in the antigenicity was due to the fact that inactivation with formaldehyde impaired the immunizing power of the unconcentrated fluid.[7]

Stability of the Alum-precipitated Virus. Alum-precipitated suspensions were stored at 4°C and −70°C and tested at intervals during a period of 3 months. At both temperatures the suspensions when redissolved in sodium citrate retained practically the same agglutination titer as they possessed when prepared. One sample of alum precipitated virus retested in mice after an interval of about 3 months was found to have retained its immunizing power. Some of the preparations lost part of their agglutinative activity and pH measurements on these indicated that acidity was responsible for the reduction in titer.

Concentration of Virus with Chicken Cells. Recently Hirst[8] has observed that influenza virus can be eluted from agglutinated chicken cells. This observation has been applied by Francis and Salk[4] to the concentration of influenza virus. It was of interest to know if influenza virus concentrated in this manner could be precipitated with alum.

Allantoic fluid infected with strain PR8 was treated with sterile chicken cells to a final concentration of 1% and placed at 4°C for one hour. The suspension was then centrifuged at low speed, the cells resuspended in 1/5 of the original volume of allantoic fluid, and incubated at 37°C for 2 hours with intermittent shaking. At the end of the incubation period the suspension was again centrifuged and the supernatant carefully removed. By this method the virus was concentrated almost quantitatively.

Fluids infected with strain Lee gave essentially the same results. In this case, however, 2.5 to 3.0% cells were required to remove the virus from the fluid instead of 1% required by strain PR8.

Following concentration with chicken cells the fluids were treated with formaldehyde and precipitated with alum with no more difficulty than was experienced on untreated fluids. The few attempts to produce alum-precipitated virus by this method have given better yields than were obtained with alum precipitation alone.

Immunization of Human Beings. Six human volunteers were inoculated subcutaneously with 1.0 cc of a mixture of equal parts of alum precipitates of PR8 and Lee strains concentrated tenfold by the combined method. A comparable group was inoculated with type A and B vaccine received from Dr. Hirst and prepared by adsorption to the precipitate of allantoic fluid.[2] Blood specimens were collected before and 2 weeks after vaccination, and the antibody titers determined by the agglutination-inhibition technic with chicken erythrocytes. The results of these preliminary tests as presented in Table III indicate that the alum precipitates produce a very definite antibody response.

Discussion. The possible value of alum-precipitated influenza virus as a vaccine is suggested by these experiments. By this

[8] Hirst, G. K., *J. Exp. Med.*, 1942, **76**, 195.

TABLE III.
Antibody Response of Persons Receiving Concentrated Influenza Vaccines.

Vaccine	No. tested	Mean titers type A		Mean titers type B	
		before	after	before	after
Alum ppt.	6	28.5	406	16.0	362
Hirst method[2]	8	29.3	430	32.0	234

method the virus is separated from the formaldehyde used in inactivation, and from other extraneous materials. The continued action of formaldehyde apparently tends to diminish the antigenic potency of influenza vaccines. Formalin also produces some discomfort after subcutaneous inoculation. Suspensions of the alum precipitates are relatively stable at 4°C without the necessity of drying. By the combined method, using adsorption and elution from chicken cells and precipitation with alum, vaccines more concentrated than those now in use could be made.

Summary. The virus of influenza grown in allantoic fluid of chick embryos can be precipitated in a stable form with alum and the precipitate redissolved in sodium citrate. This procedure does not alter the infective, antigenic, or hemagglutinating properties of the virus. Adsorption on sterile chicken cells was also used to concentrate the virus before inactivation and precipitation with alum.

The immunizing power of formalinized and alum-precipitated virus in mice was slightly less than that of active fluid. Inactive concentrated alum precipitates inoculated subcutaneously produced a satisfactory antibody response in human beings. The suspensions of alum precipitates freed of formaldehyde and acid retain their antigenicity when stored at 4°C for at least three months.

14073

Method of Producing Open Skin Wounds Uniformly Infected with Staphylococci, Suitable for Local Chemotherapy Studies.*

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While engaged in the evaluation of various methods of local sulfonamide chemotherapy of infected wounds, the problem arose of producing controlled, equally involved, infected skin wounds, before different methods of local chemotherapy could be compared.

In evaluating the effectiveness of local chemotherapy of infected wounds, quantitative bacteriological studies were first considered. At first thought, it might be believed that the wounds could be excised completely, ground up in a tissue mill, and the degree of infection estimated by bacterial population of the macerates. This method is open to the criticism that in excising the wound, some normal tissue necessarily must be included, making it impossible to obtain quantitatively reproducible results. Moreover, it is extremely difficult to grind the skin of animals either by sterile mechanical mills, or by freezing-grinding methods. It was finally decided that for all practical purposes, epithelization rate, healing time, and qualitative bacteriological studies would be the best ways in which to evaluate the effectiveness of local chemotherapy of infected open wounds.

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