the elevated blood urea returned to a normal level (Table I). 3. With one exception the kidneys of the operated animals were macroscopically normal. 4. No apparent interdependence was discerned between the level of blood urea and the state of severe anemia or between the former and body temperature or the development of obesity. The disturbance in the level of blood urea presumably is not associated with any discernible metabolic disorder.

The rise in blood urea may be due to an impairment of the ability of the kidney to clear urea at normal blood level; an increased blood urea may be a prerequisite for the adequate elimination of this substance when lesions are made in the anterior hypothalamic and preoptic regions.

13364 P

Precipitation of Active Influenza A Virus from Extra-Embryonic Fluids by Protamine.

LESLIE A. CHAMBERS* AND WERNER HENLE. (Introduced by D. W. Bronk.)

From the Department of Pediatrics, University of Pennsylvania, and the Children's Hospital, Philadelphia, Pa.

The allantoic and amniotic fluids of infected chick embryos have been found to contain large amounts of both active influenza virus and the complement fixation antigen[1, 2] in spite of their low total protein content. These extra-embryonic fluids, either fresh or lyophilized, constitute a very adequate antigen for the diagnosis of influenza by complement fixation test, and the suggestion has been made by us that they should be superior to the usual tissue emulsions as vaccines because of the relatively small amount of cellular debris.

We have carried out a series of experiments to determine the degree of protection afforded to mice by intraperitoneal vaccination with infected extra-embryonic fluids. Five-week-old Swiss mice were inoculated once with 0.05 cc of pooled dialyzed fluids. The fluids used were infectious for mice in dilutions of 10^{-8} to 10^{-10} and

* Fellow of the Nemours Foundation.

1 Henle, W., and Chambers, L. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 713.

2 Nigg, C., Crowley, J. H., and Wilson, D. E., *J. Immunol.*, 1941, **42**, 51.

contained about 0.20 mg of N per ml, dialysis having removed the large amount of uric acid nitrogen originally present.

Approximately 3 weeks subsequent to vaccination the mice were inoculated intranasally with 0.05 cc amounts of serial decimal dilutions of egg fluid infected with the homologous strain. In a series of experiments the vaccination resulted in protection of the mice against as much as 10^8 lethal doses of virus when test inocula of adequate strength were used.

While dialysis eliminates the uric acid, non-dialyzable substances other than virus remain in the fluids in relatively large amounts. The presence of egg proteins can be shown by precipitation with rabbit sera against ovalbumin or whole egg white.

We have attempted to separate the virus from these adventitious substances by various adsorptive and precipitative procedures which will be discussed elsewhere. One practicable procedure of immediate interest consists in precipitation of the active substance by the addition of protamine.

When about 1/5 of a volume of an aqueous solution (0.5 mg/cc) of a basic protein, such as spermine, is added to the infected egg fluid harvested from living embryos, a precipitate appears immediately. Precipitation can be effected either before or after dialysis of the fluid, but occurs only in the pH range 7.0-9.0. Fluids from normal eggs yield no precipitate or only a very slight amount.

The precipitate is sedimentable in the angle centrifuge at 5000 rpm. Since it is readily resuspended several washings may be carried out. Upon resuspension to the volume of the original fluid the precipitated material shows approximately the same infectivity as the original fluid although analysis shows that the suspension contains only about 0.4% of the original nitrogen. Some preparations have produced infection in mice in doses of 10^{-6} g (dry weight).

Immediately after precipitation the protamine-virus complex was resuspended to the original fluid volume and used as a vaccine for mice with the results indicated in Table I. Mouse protection appeared to be equal to that afforded by the whole extra-embryonic fluid despite the fact that only a small fraction of the total egg fluid protein was inoculated. Quantitative comparisons of the 2 vaccines are in progress.

The active agent in this form is distinctly different from preparations sedimented from infected lungs.[3] It is relatively simple in structure consisting largely of ribonucleoprotein. Analysis of the

[3] Chambers, L. A., and Henle, W., *Am. J. Path.*, 1941, **17**, 442.

TABLE I.

Protamine Vaccination. The effect of intranasal administration of 0.05 cc of the test virus solution to the vaccinated and unvaccinated groups of mice is indicated. D indicates death, the subscript showing the days of survival after infection. 4, 3, 2, and 1 indicate different degrees of lung consolidation on autopsy after 10 days.

Vaccinated with F-12-33 (6/3/41)	I.N. M.I.D.*	Tested with F-12-33 (6/23/41)	Results: Mouse No. 1	2	3	4	5	6	7	8	9	10
Original	10^{10}	undil.	3	2	1	±	±	0	0	0	0	0
		10^{-1}	±	±	0	0	0	0	0	0	0	0
Protamine Sediment	10^{9}	undil.	1	1	±	±	0	0	0	0	0	0
		10^{-1}	0	0	0	0	0	0	0	0	0	0
Not vaccinated		10^{-4}	D_6	D_6	D_7	D_9	D_{10}	D_{10}	D_{10}	4	3	3
		10^{-5}	D_4	D_7	D_9	D_9	D_9	D_{10}	3	3	2	2
		10^{-6}	4	4	4	3	3	3	3	2	1	1
		10^{-7}	3	3	3	3	2	2	1	1	0	0
		10^{-8}	2	1	1	0	0	0	0	0	0	0

*Intranasal maximal infectious dilution.

complex is being carried out. The dry weight figure of 10^{-16} g as an infectious dose limits the molecular weight of the complex to the order of 10^8 or less and a series of electron-micrographs,† to be described in a later publication, shows that the resuspended complex contains no particles of diameter greater than 15 mμ, but a considerable amount of aggregation was apparent.

In spite of the relatively high degree of homogeneity obtained by the procedure described, the material still contains complement fixation antigen.

It appears that protamine precipitation of the active agent of influenza from infected egg fluids constitutes a step toward isolation, and identification of the virus. The usefulness of the complex in either an active or inactivated form as a vaccine is indicated, and its efficacy in the protection of human subjects will be investigated.

† Taken by Dr. T. F. Anderson in the laboratories of the R. C. A. Manufacturing Company.