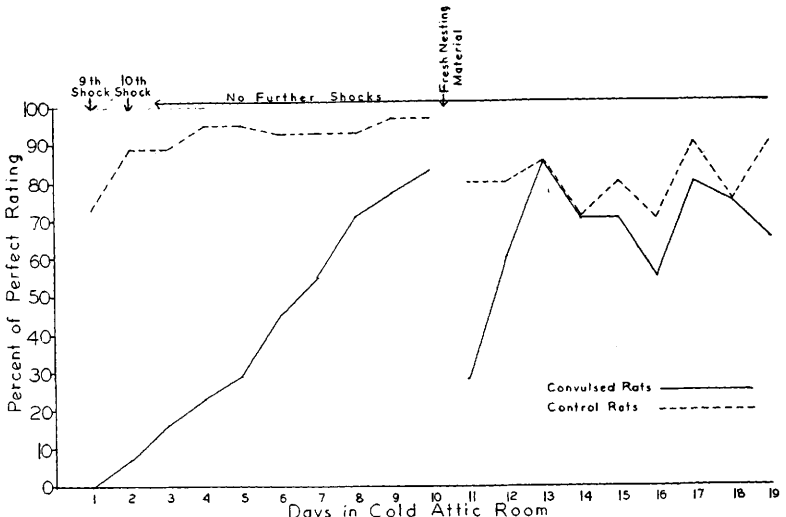

FIG. 1.

Beach[3] has stated that adequate functioning of innately organized reproductive behavior depends on the organism being under the influence of certain hormones at critical periods. Unless the hormones are present in sufficient strength at the right time, stimuli specific to an innately organized pattern of behavior have no effect. Rosvold[4] suggested that the inability of the parturient mother to respond to the paper and shavings as nesting materials may have been due to the fact that the innate organization for these responses had not been sensitized by the appropriate hormones. He implied that the hormones affected were related to the pregnancy-lactation cycle. Such an hypothesis, without qualification, is no longer tenable.

Summary. Twelve adult male albino rats were shocked once a day for 9 days after which they were placed in a relatively cold attic room and supplied with nest building materials. Nine, not shocked but otherwise treated like the others, served as controls. In the early post shock period the convulsed animals made no attempt at nest building whereas their controls built excellent nests. There was gradual recovery so that by the tenth day post shock, the convulsed animals built nests equal to those of the control rats.

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Hypotensive Action of Influenza A Virus on Rats.* (17404)

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The frequent clinical observations of cardiovascular disturbances complicating influenza infection[1,2] prompted us to undertake an experimental investigation of the toxic effect

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[1] Findland, M., *et al., Am. J. Med. Sc.,* 1945, **209**, 455.

of the virus on the cardio-vascular system of the host. During a study on the cardiac output of ferrets infected by the PR8 strain of influenza virus[3] arterial hypotension was occasionally noticed. Further observations disclosed that this phenomenon was invariably produced when albino rats were given intravenous injections of the virus. A series of experiments were then carried out to determine the nature of this factor in our preparation.

Preparation of the virus. A PR8 strain of influenza virus which was lethal for mice on intravenous injection[4] was originally obtained from Dr. W. Henle and maintained by passages in embryonated eggs by the usual procedures of allantoic sac inoculation, incubation and harvest.[5] The pooled, bacteria-free allantoic fluid was centrifuged at 1050 g to remove the debris and then purified by adsorption and elution from chick red blood cells.[6] The eluate was then centrifuged at 20,000 g for one hour and the precipitate was suspended in 0.1 M phosphate buffer solution of pH 7.0. A final centrifugation at 1050 g removed larger particles. In two experiments, the material was further purified by methanol precipitation according to the method of Cox *et al.*[7] The final product has an average hemagglutination titer[8] of 64,000 per mg of nitrogen, and its infectivity titer for chick embryo was $10^{-13.4}$ g. The extent to which our material was free from non-viral proteins was about the same as the preparations of other authors working on the purification of influenza virus.[9,10]

Preparation of the animals. 250-300 g male albino rats (Harlan strain) were anesthetized with sodium nembutal and the trachea was cannulated. Arterial blood pressure was obtained by direct cannulation of the common carotid artery and registered by mercury manometer writing on a kymographic drum. One ml of the virus suspension per 100 g of body weight was injected into the jugular vein at the rate of one ml per minute. The blood pressure was then observed for the next 30 minutes.

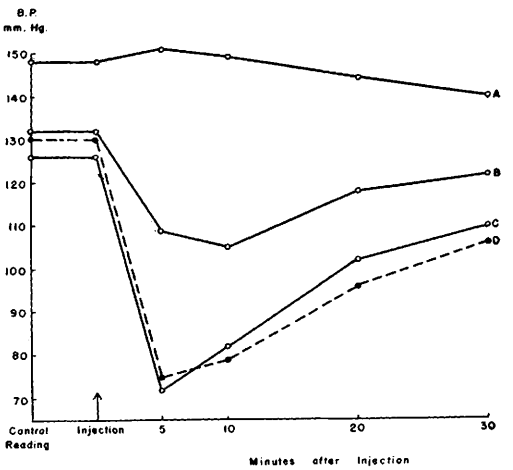

FIG. 1.

Hypotensive action of virus prepared by different purification procedures and that of normal allantoic fluid.

A—Inj. of conc. normal allantoic fluid.

B—Inj. of virus conc. by adsorption and elution from chick rbc.

C—Inj. of virus conc. by adsorption and elution from chick rbc. plus high speed centrifugation.

D—Inj. of virus conc. by adsorption and elution from chick rbc. plus high speed centrifugation and methanol precipitation.

Results. The data of the experiments can be summarized in the following categories:

1. Allantoic fluid of normal 11-day-old chick embryos was harvested, purified and concentrated by the same procedures used for the virus. Injection of this control material did not alter the blood pressure (Fig. 1, A).

2. The hypotensive factor seemed to be associated with the virus particles since it was similarly adsorbed and eluted from chicken red blood cells sedimented by high speed centrifugation and precipitated by methanol (Fig. 1, B, C, D). When the infectious allantoic fluid was centrifuged at 20,000 g, the precipitate resuspended in buffer solution gave

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TABLE I.
Effect of Heat on Hemagglutination, Chick Embryo Infectivity, Mouse Toxicity and Hypotensive Factor of the Concentrated Virus Preparation.

Treatment of the purified virus	Hemagglutination titer	Infectivity titer	Mouse toxicity*	% of drop in B.P. after inj. into rats†
Unheated	1:16000	10^{-10}	1:16	42.9
Heated at 56°C for 4 hr	1:8000	10^{-1}	1:4	28.2
8 hr	1:200	0	1:2	33.6
12 hr	0	0	0	18.2
16 hr	0	0	0	5.4‡

* Kills 4 out of 6 in 48 hr.

† % of drop in B.P. $= \frac{\text{Max. drop of B.P. in mm Hg}}{\text{Control B.P. reading before inj.}} \times 100.$

‡ This figure is equivalent to spontaneous variation in B.P. during the period of observation on control animals.

TABLE II.
Effect of Specific Anti-Serum on Neutralization of Hypotensive Factor of the Virus Preparation.

Preparations injected into rats	No. of rats used	Max. drop in B.P. during first 30 min. after inj.*	% drop in B.P. after inj.
A. Infectious allantoic fluid neutralized first and then concentrated	6	0 mm Hg	0
B. Purified virus plus specific immune ferret serum	5	17	11.2
C. Purified virus plus normal ferret serum	6	49	32.9†

* Initial B. P. for all these animals during control period before injection (average) = 150 ± 2 mm Hg.

† Difference between values for B and C is highly significant statistically. (P value less than 0.01).

a maximal decline of 40 per cent in blood pressure while the supernatant of the same preparation was entirely without effect.

3. The hypotensive factor was found to be relatively heat resistant being destroyed only after heating at 56° C for 16 hours (Table I). The hemagglutinins, infectivity for chick embryos and toxicity for mice were all inactivated by heating at the same temperature for shorter periods of time respectively.

4. Ferret immune serum titering 1:12500 by the hemagglutination inhibition test was produced by injection of infected mouse or ferret lung tissue suspension. Addition of this serum to pooled allantoic fluid of infected chick embryos resulted in complete neutralization of the virus as indicated by the hemagglutination and chick embryo neutralization tests. This neutralized mixture was then treated by adsorption and elution from chick red blood cells plus high speed centrifugation. Injection of the resulting preparation showed

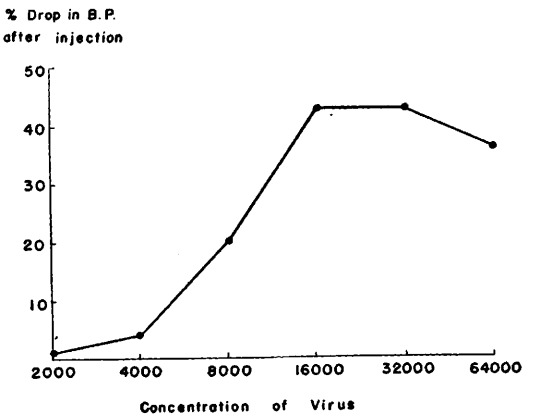

FIG. 2.
Relationship between concentration of the virus (shown by hemagglutination titer) and hypotensive response.

the loss of the hypotensive factor indicating specific neutralization of the factor (Table II, A).

To prove further that the hypotensive factor was a fraction of the virus particles, the concentrated and purified preparation was mixed directly with the immune serum. Injection of the mixture into rats showed a significant reduction of the hypotensive effect in comparison with the control material to which normal ferret serum was added (Table II, B, C).

6. To elicit the hypotensive response a threshold concentration of virus with hemagglutination titer of 1:8000 was required. Concentrations of virus with titer between 1:16,000 and 1:64,000 elicited about the same response (Fig. 2).

Conclusion. These observations indicate that a fraction of the PR8 strain of influenza virus possesses the toxic property of lowering arterial blood pressure in rats.

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Effect of Congo Red on the "MM" Virus. (17405)

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Conflicting reports on the effect of trypan red on the "MM"[1] neurotropic virus have appeared in the literature. Wood and Rusoff[2] reported this compound along with two other acid dyes, Congo red and brilliant vital red, to be definitely effective against the virus. Using the intraperitoneal injection route followed by intraperitoneal inoculation of the virus, these workers found an apparent protection which was overcome by inoculation of the virus in too high concentration.

Hammon *et al.*[3] have reported the failure of trypan red to protect against the "MM" virus when the dye was injected subcutaneously and the virus inoculated by intraperitoneal route. These authors interpreted the results of Wood and Rusoff as being due to a non specific protection such as afforded by various inert substances when injected previous to the inoculation of infectious agents by the same route.

Since the effect of Congo red had been reported for only one concentration of "MM" virus it was thought desirable to determine the activity over a range of dilutions.

In one series of experiments Congo red was injected intraperitoneally into 12 g mice and in a second series the dye was administered by stomach tube. As a matter of interest trypan red was included in 2 of the experiments. All virus inoculations were by the intraperitoneal route, serial dilutions being made from frozen stock material. A range (10-fold) of infecting dilutions was employed so as to permit calculation of LD50 values by the method of Reed and Muench.[4] Unless otherwise specified, dilutions of 10^{-6} through 10^{-9} were used for controls and 10^{-5} through 10^{-8} for test groups, 6 mice being used per dilution.

In all experiments calling for the injection of dye the schedule employed was that used by Wood and Rusoff. A 1% solution was injected in 0.1 cc amounts on each of 3 days and the virus was administered one day after the last injection.

For the experiments in which the dyes were given by stomach tube the schedule called for the feeding of 0.5 ml of a 1% solution twice daily for 9 feedings with the virus inoculation following the fourth feed-

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