inhibit the intestinal absorption by rats of glucose, galactose, and possibly of mannose and sorbose, but not of fructose. This may indicate the presence in the intestinal mucosa of a specific enzyme for fructose phosphorylation which is not inhibited by phlorhizin.

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Observations on the Toxin of Influenza Virus.* (18107)

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Henle and Henle(1) reported the demonstration of a toxin associated with influenza virus and later studied this factor more extensively(2-4). During an investigation in our laboratory of further toxic manifestations of the virus on the circulatory system of the host(5,6) difficulties in maintaining the toxin-producing properties of the virus were encountered; therefore, it was felt desirable to repeat and extend some of the experiments of Henle and Henle. Only the PR8 strain† of influenza A virus was used. With careful attention to conditions of storage of the virus, consistent production of toxin was attained. In general, our observations of the lesions produced by the toxin, the relation of toxin to other established virus properties, its production and stability and host factors affecting toxicity have confirmed and amplified the findings of Henle and Henle, with a few differences. In addition, the susceptibility of the ferret to influenza virus toxin was demonstrated.

Methods. Toxin of the influenza virus was produced by inoculating the allantoic sac of 10 to 11 day chick embryos with 0.15 ml of a 10^{-6} dilution of infected allantoic fluid in brain-heart infusion broth. The eggs were then incubated at 37°C for 48 hours, after which they were chilled before harvesting the allantoic fluids. The fluids were tested for bacterial contamination before pooling. The allantoic fluid was tested for toxicity by injecting 1.0 ml amounts intravenously into the tail vein of 12 to 15 g CFW mice. Dilutions for intravenous injection were made in normal allantoic fluid. When virus was purified and concentrated it was done by adsorption and elution from red blood cells followed by high speed centrifugation(5). Virus was tested for infectivity by injecting 0.15 ml of 10-fold dilutions of viral suspensions into 10 to 11 day embryonated eggs. The presence of virus in fluids harvested from these eggs was determined by the hemagglutination technic using the method of Salk(7) with the exception that the concentration of the chick red cell suspension used was 0.5 instead of 0.25%.

Experimental. As the Henles have shown, the intravenous injection of allantoic fluid containing PR8 virus produced toxic manifestations consisting of gross lesions of the liver, spleen, frequently the intestines, the kidney, and engorgement of pulmonary vessels without parenchymal consolidation. In our experiments, alterations in some or all of

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† This toxin-producing strain was kindly sent to us by Dr. Werner Henle.

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these organs were observed in 92% of 352 mice. Microscopically, representative sections of these lesions showed hemorrhagic necrosis.‡ The relationship between the amount of virus injected and the time of death after injection indicated that our toxin was less potent than that prepared by Henle and Henle(3), since the maximum number of deaths did not occur until 48 hours after injection of the toxin, rather than within 24 hours. Further, their toxin in a dilution of 1:4 regularly killed mice on intravenous injection while less than 5% of 251 mice died following the injection of a 1:4 dilution of various preparations of our toxin. The toxic activity was neutralized by specific antiserum. The Henles noted in a few instances that moribund mice suspended by their tails reacted with convulsions, and a few animals were observed in spontaneous convulsions; these findings were also observed in our mice.

In their observations on the lungs Henle and Henle reported only engorgement of the pulmonary vessels; the concentration of virus in the lung tended to remain constant in contrast to a marked fall in virus concentration in the liver and peritoneal fluid after intraabdominal injection. In our experiments pulmonary consolidation occurred even in mice which died within a period in which the deaths might have been due, at least in part, to the action of the toxin. It seems unlikely that virus could have invaded the lung and multiplied so rapidly that the early consolidation was due to infectivity alone. Of 75 mice which died within 24 hours, 8% had at least 50% pulmonary consolidation; of 134 mice which died between 24 and 48 hours, 20% had consolidation. Combining these data, 15% of the mice which died within 48 hours after intravenous injection had pulmonary consolidation. Of 33 mice which died between 5 and 10 days after injection, when infection by the virus had taken place, 79% had pulmonary consolidation. On investigating the relation of other properties of the virus to the toxic agent, Henle and Henle observed that, as in the production of infective virus and the hemagglutination factor, a dilute inoculum produced preparations of greater toxicity than more concentrated seed. They found that there was a correlation between infectivity titer and toxicity. This correlation was not absolute, however, as differences in time of appearance and duration of maximum titers indicated.

‡ We are indebted to Dr. Cecil Krakower for making the microscopic examinations of the tissues studied.

TABLE I. Relationship of Virus Inoculum to Infectivity and Mouse Toxicity of Harvested Virus

Dil. of inoculum	Titration of infectivity in embryonated eggs				Intrav. toxicity for mice	
	10^{-7}	10^{-8}	10^{-9}	10^{-10}	Undil.	1:4
10^{-1}	3/5*	0/5	1/5	0/2	0.8	0/3
10^{-3}	5/5	5/5	2/5	0/2	7/8	0/6
10^{-4}	5/5	3/5	1/5	0/2	8/8	0/7
10^{-5}	6/6	6/6	3/6	1/3	5/8	0/7
10^{-6}	6/6	4/6	1/6	0/3	8/8	3/7
10^{-7}	6/6	6/6	2/6	0/3	8/8	5/7
10^{-8}	6/6	5/6	0/6	0/3	1/8	0/3
10^{-9}	0/3	0/3	0/3	0/3	1/4	—

* Numerator = No. of mice dead.
Denominator = No. of mice inoculated.

In Table I are recorded the results of an experiment in which groups of 6 to 8 eggs were inoculated with dilutions of virus ranging from 10^{-1} to 10^{-9}. After 48 hours, the allantoic fluids of each group were pooled and tested for chick embryo infectivity and for toxicity. The harvest from the initial inoculum of 10^{-1} presumably demonstrated the interference phenomenon both for infectivity and toxicity. When the initial inoculum ranged from 10^{-3} to 10^{-8}, the infectivity titer of the fluids, all harvested at 48 hours, was 10^{-8} or slightly higher in each case; the toxicity titer, however, was greatest when the initial inoculum was 10^{-6} or 10^{-7}. Injection of toxin harvested from eggs which received an initial inoculum of 10^{-8} produced only one death. The reason that optimum toxin production occurred with a highly dilute inoculum is unknown at present. Although attempts to separate the toxin from the infective portion of the virus molecule have been unsuccessful(3), the findings of Henle and Henle and those here reported indicate that there may be different fractions of the virus, or that qualitatively different virus may exist. An alternative explanation would depend on

TABLE II. Relation of Toxin to Hemagglutination Titer of Virus.

Hemagglutination titer of virus	No. of mice injected	% mortality
1:6400	4	100
1:3200	102	87
1:1600	122	80
1:800	53	72
1:400	8	13

the demonstration of an inhibitor of toxin.

In Table II it may be seen that there is a correlation between the hemagglutination titer of the virus and the toxic property. Virus with a titer of 1:400 was of low toxicity; as the hemagglutination titer increased the toxicity also increased, although not proportionately. The Henles' findings also suggested a rough correlation with hemagglutination titer.

Since there is some indication that the toxic fraction may be a separate entity of the virus molecule, a study was made to determine whether there was an optimal incubation temperature of the inoculated eggs which would result in maximal toxin production. Between 33 and 37°C, the toxin production and infectivity were relatively constant. Outside this range (31 to 39°C), the toxicity was greatly decreased but the hemagglutination titer was also very low so that there was no indication of the toxic factor's being produced under temperatures different from those which were optimum for the virus as a whole.

Genetic variation of the mouse host might also be expected to be of importance in the manifestation of toxicity. Testing 4 strains of mice, the Henles(4) found that the LD_{50} varied with the strain, although the data indicate that the differences were small (LD_{50} 1:3.7 and 1:5.7). In our experiments, 10 strains of mice were tested; all were males weighing from 10 to 15 g. These included CFW, Harlan, Rockland, DBA, C-57, A-K Leukemia, C_3H, fostered C_3H, CFl, and stock white mice. As seen in Table III no significant difference in susceptibility was noted among these strains in the dilutions tested.

The Henles concluded that younger mice were more susceptible to the toxic activity of the virus. They injected 1.0 ml amounts of the undiluted or diluted toxin preparations. In our tests, the amount of inoculum was 1.0 ml per 15 g of mouse body weight. Under these conditions, the 3- to 4-week-old mice were somewhat more susceptible than 6- to 12-week-old mice. On testing the virus in the ferret *(Putorius putorius)* it appeared that the virus was also toxic to this species. In Table IV it will be observed that a virus preparation containing 154 hemagglutinating units per g of body weight§ was required to kill 6 of 8 animals within 18 hours, while 46.5 units per g were on the borderline of toxicity. Material with 10.1 units per g of body weight produced no deaths and no gross or microscopic abnormalities with the exception of an enlarged liver and spleen in one ferret. Animals receiving the larger amounts of virus (Groups 2 and 3) showed enlargement of the spleen and occasionally of the liver if they survived for 24 hours; ferrets dying in less than 24 hours showed no gross changes, but microscopically congestion and infiltration with polymorpho-

TABLE III. Susceptibility of Various Strains of Mice to Toxin.

Strain	Intrav. inj. of virus undiluted	1:4
CFW	10/10	2/10
Harlan	9/10	1/10
Rockland	8/10	0/10
DBA	8/10	0/10
C-57	10/10	0/10
A-K leukemia	9/11	0/11
C_3H	11/11	0/11
C_3H fostered	11/11	1/11
CF1	4/4	0/4
Stock white mice	6/11	0/11

TABLE IV. Relationship Between Hemagglutination Titer of Influenza Virus and Its Toxicity for Ferrets.

Group No.	Hemagglutination units per g of body wt	Mortality ratio
1	10.1	0/5*
2	46.5	1/4
3	154.0	6/8

* Numerator = No. of ferrets which died.
Denominator = No. of ferrets which were injected.

§ Hemagglutinating units per g =

$$\frac{\text{Hemagglutination titer} \times \text{ml injected}}{\text{g of body wt}}$$

nuclear cells were observed in the liver, spleen and adrenal glands. These changes were not considered sufficient to cause death and it was believed that the toxin in high concentration was capable of acting on vital centers before significant demonstrable lesions had occurred. On comparing the toxicity of the virus for mice and ferrets, it may be observed that virus with a hemagglutination concentration of 154 units per g of body weight was required to kill 75% of the ferrets, whereas 53 units per g of body weight were required to kill approximately 75% of the mice. Thus it appeared that mice were more susceptible to the toxin than ferrets.

A knowledge of the stability of the virus is of obvious practical importance. The Henles found that toxicity was unaltered for 1 to 3 months at 4°C, decreasing slowly thereafter. In our experiments, the toxicity was very slight after periods of longer than one week at 4°C; this was true whether the virus was preserved unpurified and unconcentrated in allantoic fluid or in the purified and concentrated state in 0.1 M phosphate buffer solution at pH 7.0. However, there was no decrease in toxicity in material stored in allantoic fluid for 3 months at –30°C or at –60°C. On two occasions our stock virus lost its capacity to stimulate production of the toxic factor on inoculation into eggs while it retained its ability to produce the hemagglutination and infectivity factors when it had been stored for periods of 6 months at –60°C. This loss of toxin production was also observed on one occasion on serial passage in the embryonated egg. These findings lend further evidence to the hypothesis that the toxic factor is different from the infective moiety.

Summary. The mouse toxic factor of influenza virus, PR_8 strain, originally reported by the Henles was reinvestigated, and similarities and differences between their observations and ours are reported and discussed. The toxin also was shown to affect ferrets.

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Excretion of Phenylalanine and Derivatives in Phenylpyruvic Oligophrenia. (18108)

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It is established that patients affected with phenylpyruvic oligophrenia excrete in the urine phenylalanine(1,2), phenylpyruvic acid (2-8), and phenyllactic acid(1,2,6). Quantitative determinations of phenylpyruvic acid in a few patients have been published(2,4,5), but only in one patient were data reported on the excretion of these 3 phenyl compounds (2). It is the purpose of this note to present quantitative data on the urinary excretion of phenylalanine, phenylpyruvic and phenyllactic acid in 20 patients, under varying dietary conditions.

Materials and methods. Twenty individuals affected with phenylpyruvic oligophrenia ranging in chronological age from 2 to 42 years and in the intelligence quotient from 5 to 47, were used as experimental subjects. For the determination of free phenylalanine

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