hours after the first injection. The ovaries were serially sectioned. The results are presented in Table I.

It appears that in some cases the immature rat ovary will respond to gonadotropic hormone with a follicle stimulating reaction as early as 26 hours after the first injection. This reaction becomes progressively more marked and reaches its maximum at approximately 64-72 hours after injections are begun. After 64 hours luteinization begins to occur and progresses steadily thereafter. In the majority of animals luteinization reaches its maximum at the end of 96 hours. In one instance (M31UA42) only follicle stimulation was present at the end of 96 hours and 120 hours. At 144 hours, however, extensive luteinization was found.‡

From these experiments it appears that the ovaries of normal immature animals respond to gonadotropic hormone extracts when given in sufficient dosage, first by follicle stimulation which is followed, after a variable period of time, by luteinization. From this and the preceding study it is evident that at least 3 factors influence the production of luteinization, *viz.*, the method of extraction, the quantity of extract administered, and the time.

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Resistance of the Viruses of Poliomyelitis, Human Influenza and Swine Influenza to Intense Vibration.*

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There is great need for a technique whereby the pathogenicity of viruses may be destroyed without altering the immunological properties of their antigenic constituents. The results of past work indicated that sonic vibration might be the agent by means of which this result could be accomplished. Thus, it was recently reported[1]

‡ From this it might appear that when a subthreshold dose of luteinizing factor is given, prolongation of the time beyond 96 hours may result in luteinization.

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1 Chambers, L. A., and Flosdorf, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 631.

that disintegration of *Eb. typhi* and *S. hemolyticus* by the action of sonic vibrations of audible frequency (9,000 c.p.s.) and high intensity resulted in the liberation, in an apparently unaltered state, of certain labile components of the antigenic complex of the organisms. Takahashi and Christensen[2] have reported that tobacco mosaic virus, in the form of the juice of infected leaves, was inactivated by exposure to sonic vibration of 450,000 c.p.s. frequency. Stanley[3] confirmed these results and observed in addition that a partially purified virus preparation was much less readily inactivated than was the crude leaf juice, and that in no case was inactivation possible when the irradiation was conducted *in vacuo* (absence of oxygen and cavitation). Hopwood,[4] on the other hand, found that vaccinia virus was not inactivated by exposure to supersonic vibration, but that there was indeed an increase in potency.

Accordingly, we have applied the sonic method to preparations of the viruses of poliomyelitis, human influenza and swine influenza, using the technique described for bacteria.[1] Under conditions of the experiments, the viruses proved to be completely resistant to the action of sonic vibration.

Poliomyelitis. The Philadelphia 1932 strain of the virus has been used in these experiments. Partially purified preparations for exposure to the sonic vibrations were made by submitting saline extracts of the spinal cords of monkeys *(Macaca mulatta)* which had succumbed to the experimental disease to a process of adsorption and elution similar to those described by Sabin[5] and Schaeffer and Brebner.[6] These methods and the properties of the products obtained by their use will be more fully described in another communication.[7] After the preparation had been exposed to sonic vibration, it was tested for the presence of active virus by intracerebral inoculation into normal monkeys, weighing not more than 5 pounds, under deep ether anesthesia. When the resulting infection was not in every respect clinically typical of poliomyelitis, the result was confirmed by histological study of the animal's spinal cord.†

So far as one is able to judge from the results of the inoculation

2 Takahashi, W. N., and Christensen, R. J., *Science*, 1934, **79**, 415.

3 Stanley, W. M., *Science*, 1934, **80**, 339.

4 Hopwood, F. L., *Nature*, 1931, **128**, 748.

5 Sabin, A. B., *J. Exp. Med.*, 1932, **56**, 307.

6 Schaeffer, M., and Brebner, W. B., *Arch. Path.*, 1933, **15**, 221.

7 Scherp, H. W., and Wolman, I. J., to be published.

† The authors are indebted to Dr. Irving J. Wolman for examining these specimens.

TABLE I.

Exposure of the Virus of Poliomyelitis to Sonic Vibration.

Oxygen was present unless otherwise noted. The temperature did not exceed 20° C at any time.

Exp. No.	Conc. virus exposed,* %	Length exposure, min.	Conc. tested in monkey, %	No. monkey	Incr. dose, cc.	Result of intracerebral test
1†	10	0	10	208	1	Complete paralysis, 10 days
		0	1	207	1.5	" " 6 "
		20	10	209	1.2	" " 12 "
		20	1	181	1	" " 10 "
2	10	0	10	220	1	" " 7 "
		5	10	218	1	" " 7 "
		15	10	219	1	" " 6 "
3	10	0	10	221	0.5	" " 8 "
		15	10	222	0.5	" " 7 "
4	10	0	10	249	1	" " 8 "
		60	10	248	1	" " 7 "
5	10	0	10	260	0.5	" " 6 "
				261	0.5	Part. paral., 9-10 d. Recovered
		60	10	258	0.5	Complete paralysis, 10 days
				259	0.5	" " 7 "
6	10	0	1.0	275	1	Temp. 105.4-106.4°F, 4-7 days‡
	crude	0	0.1	273	1	Complete paralysis, 10 days
	suspn.	30	1.0	274	1	" " 10 "
		30	0.1	272	1	Legs completely paralyzed, 12th day. Recovered partially

*Expressed in the same terms as the suspension from which the preparation was made. Thus, "10%" indicates that the eluate was equal in volume to the volume of the saline cord extract used in its preparation.

†Carried out in an atmosphere of hydrogen.

‡Histological examination of the spinal cord revealed no evidence of poliomyelitis.

test, which is inherently unsuitable for quantitative interpretation, the virus is entirely unaffected by even long exposure to intense sonic vibration. The results of several experiments are summarized in Table I. It is particularly noteworthy that, except for the first experiment which was conducted in an atmosphere of hydrogen, all tests were made in the presence of oxygen, under conditions favorable for the sonic activation of chemical systems.[8]

Buggs and Green have reported[9] that homogenization of herpetic rabbit brain in a colloid mill resulted in a suspension which produced herpes encephalitis in test rabbits with a much reduced incubation time and at much higher titer than that obtained by using material triturated in the customary manner in a mortar. Thus, using comparable doses, homogenized virus produced death in 12 to 24 hours, whereas triturated virus required 2 to 6 days. There was a direct relationship between the degree of dispersion of the infected tissue

[8] Flosdorf, E. W., Chambers, L. A., and Malisoff, W. M., *J. Am. Chem. Soc.*, 1936, **58**, 1069.

[9] Buggs, C. W., and Green, R. G., *J. Infect. Dis.*, 1936, **58**, 98.

and the activity of its virus content. The minimal fatal dose of homogenized virus was of the order of 0.0001 mg. of brain, or much less than is usually obtained with this virus. Unfortunately, the authors did not run a control series of their own with triturated material.

An experiment was carried out to determine if there was any evidence that a similar result could be obtained with poliomyelitis virus, using sonic vibration as the homogenizing agent. Accordingly, a spinal cord, freshly removed from a monkey completely paralyzed with poliomyelitis, was thoroughly ground in a mortar without abrasive, and made up to a 10% suspension in saline. The suspension was filtered through 2 layers of gauze to remove gross particles. Part of the filtrate was set aside for injection into monkeys without further treatment. The remainder was exposed to the action of the sonic apparatus for 30 minutes. The suspension was smoothly homogenized, and the insoluble matter settled out much more slowly than it did in the case of the untreated suspension. The results of the inoculation of these fractions into monkeys are shown as Experiment 6 in Table I. It was obvious that here was no result analogous to those obtained by Buggs and Green with herpes virus.

To check upon the relative efficiency of simple trituration and sonic homogenization in the extraction of soluble material from the nerve tissue, protein nitrogen and total Kjeldahl nitrogen determinations were carried out in duplicate upon the supernatant fluid ("saline extract") obtained when the suspensions were vigorously centrifuged. The sonic extract contained 0.51 mg. total nitrogen per cc. and 0.24 mg. protein nitrogen per cc. The corresponding figures for the extract prepared by simple trituration were 0.49 mg. and 0.29 mg. It would appear that sonic extraction liberated slightly more total solute but resulted in the denaturation of some fraction of the protein.[10]

Influenza. The strain of human influenza virus was the PR8; of swine influenza virus, the S15. These strains were originally obtained through the courtesy of Dr. Thomas Francis, Jr., and Dr. Richard E. Shope, of the Rockefeller Institute. Both strains were used for the experiments in the form of saline extracts of the lungs of mice that had succumbed to the experimental disease. The lungs were removed at autopsy with sterile precautions and stored in the frozen state at a temperature of −8 to −10°C. for not more than 2 weeks before use. In making the extract, the lungs were ground

[10] Chambers, L. A., and Flosdorf, E. W., *J. Biol. Chem.*, **1936, 114, 75.**

with powdered sterile pyrex glass in a mortar and made up to a suspension of the desired strength of 0.85% sodium chloride solution which was centrifuged at refrigerator temperature for 30 minutes at 2000 R.P.M. These extracts were cloudy with suspended material. Short exposure to sonic vibrations resulted in clarification but the cloudiness usually reappeared upon prolonged treatment. After exposure, the fractions were diluted in saline and portions of the dilutions were instilled intranasally into healthy albino mice 4 to 5 weeks old, under light ether anesthesia.‡ The mice were killed when moribund or at the end of the fifth to seventh day. Their lungs were then removed and examined for the lesions of influenzal pneumonia.

The results obtained with the 2 strains of influenza virus are

TABLE II.

Exposure of the Viruses of Human and Swine Influenza to Sonic Vibration. Oxygen was present in all cases. The temperature did not at any time exceed 20°C.

Exp. No.	Conc. virus exposed	Length exposure, min.	Mouse test dilutions.*			
			1:10	1:100	1:1,000	1:10,000
1	10%	0		A D5 D5	D5 2+ 2+	3+ 2+ 1+
	PR8	5		E D5 D5	4+ 2+ 1+	1+ 1+ 1+
		15		D3 D5 D5	D5 3+ 2+	2+ 1+ 1+
		30		E E D5	3+ 2+ 2+	2+ 1+ 1+
2	10%	0		D3 D3 2+	D5 D5 2+	A D5 3+
	PR8	5		D3 D4 D7	D5 3+ 2+	3+ 3+ 2+
		30		D4 D7	D5 3+	2+
3	10%	0		D6 D6 3+	2+ 2+ 2+	1+ 1+ 1+
	PR8	5		D6 D6 D6	2+ 2+ 1+	2+ 2+ OL
		30		D6 D6 3+	D6 2+ 1+	1+ 1+ 0
4	10%	0	2+ 3+ 3+	2+ 2+ 4+	1+ 1+ 0	A A 0
	S15 glycerolated	30	D5 D5 3+	1+ 1+ 2+	1+ 0 0	0 0 0
5	7.2% PR8	0	1:1,400 D1 3+ 3+	1:14,000 D1 2+ 2+		
	Tissue sediment from above extract	30	E 3+ 3+	1+ 1+ 0		

*Dilutions are expressed in parts, wet weight, of lung tissue to parts of saline by volume.

Notation:

"A" Mouse died with atypical lung involvement.

"D3," "D4," etc. Mouse died with typical lesions of influenzal pneumonia in 3 days, 4 days, etc.

"E" Mouse eaten.

"L" Large, older Mouse—not as susceptible to the virus.

"0" No detectable influenzal lung lesions at autopsy.

"1+" Lesion of influenzal pneumonia involved up to ¼ of lungs at autopsy.

"2+" Lesion of influenzal pneumonia involved ¼ to ½ of lungs at autopsy.

"3+" Lesion of influenzal pneumonia involved ½ to ¾ of lungs at autopsy.

"4+" Lesion of influenzal pneumonia involved ¾ to all of lungs at autopsy.

‡ The authors take pleasure in acknowledging their indebtedness to Mrs. Dorothy R. Shaw, who carried out these animal tests.

summarized in Table II. The findings were entirely comparable to those obtained with the poliomyelitis virus. Even prolonged exposure to sonic vibration failed to affect the titer of the viruses, with the possible exception of the experiment in which glycerolated lungs were used as the source of the virus. In that case, the presence of glycerol was probably a factor of importance, since it has been shown that glycerol is one of many organic substances which are activated by intense sonic vibration in the presence of oxygen.[11]

An homogenization experiment (No. 5 of Table II) was carried out with the PR8 virus. The lungs were ground in a mortar without abrasive and made up in saline to a 7.2% suspension, which was then centrifuged for 10 minutes at 2000 R.P.M. The supernatant saline extract was set aside as a control and the sedimented tissue was suspended in the original volume of saline and homogenized by 30 minutes exposure to the sonic vibrations. It will be seen from the data in Table II that the virus content of the homogenized lung tissue suspension and of the saline extract were nearly the same, showing that simple trituration of the lung tissue resulted in only partial extraction of the virus. However, there was clearly no evidence of enhanced activity in the homogenized material.

Summary. The pathogenicity of preparations of the viruses of poliomyelitis, human influenza and swine influenza was not affected by exposure to intense sonic vibrations (9,000 c.p.s.) under conditions which suffice to disintegrate such bacteria as *Eb. typhi* and *S. hemolyticus*. No evidence was obtained that homogenization of virus-containing tissue by exposure to intense sonic vibration resulted in increased activity of the virus content.

[11] Chambers, L. A., unpublished data.