From histologic evidence alone, there does not appear to be significant difference in reaction of either the thyroid gland or the submaxillary gland to desiccated thyroid or to propylthiouracil between young weanling or older adult animals.

Conclusions. The effect of feeding either desiccated thyroid or propylthiouracil was studied to determine if the former drug resulted in an anticariogenic effect and the latter produced a cariogenic effect in adult rats to the same degree as in weanling animals. Propylthiouracil resulted in significantly more caries than were found in weanling animals, but desiccated thyroid did not reduce the caries experience. Similar histologic changes were found in both thyroid and salivary glands in the adult rats as were found previously in weanlings.

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Effects of High Pressures on Influenza Viruses.* (25236)

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Various viruses have been subjected to high compression pressures of 30,000 to 120,000 lb/in^2 (2000 to 8000 atmospheres) to test the effect of these pressures on infectivity, but the results were complicated by temperature increases accompanying the pressure rise. Since pressure was generated by a hydraulic press operated at room temperature, increase in heat of the virus suspension in the press was unavoidable. Using the hydraulic press apparatus Basset(1,2) studied a number of viruses including 2 plant agents (tobacco mosaic and tobacco mecrosis), 6 animal (vaccinia, herpes, rabies, yellow fever, Rous sarcoma, and equine encephalomyelitis) and staphylococcus bacteriophage. The most susceptible to inactivation was the bacteriophage which was inactivated at 30,000 lb/in^2 and the most resistant was tobacco mosaic inactivated at 12,000 lb/in^2. All of the above studies were reported prior to 1941 so that much modern data regarding measurements of infectivity were not available. An ingenious device recently developed by Arthur D. Little produces pressures of over 30,000 lb/in^2 at below freezing temperatures. The method depends on freezing and expansion of water in a steel cell. The sample to be tested is placed inside the cylinder which is then filled with water. The cell is sealed and placed in a deep freeze at various freezing temperatures. As the water freezes it expands against the contracting steel and internal compression pressures result which can be measured by a strain gauge. Using this device Curtis *et al.*(3) studied avian erythroblastosis and mouse hepatitis (Nelson) viruses and found inactivation complete at 30,000 and 25,000 lb/in^2 respectively. Experiments to determine minimum pressure were not reported. None of the previous studies of the effects of pressure on viruses have included influenza virus. Since this virus is represented by many diverse strains all of which have 2 distinct and easily

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measured biological properties, hemagglutinin and infectivity, it was of interest to investigate the effects of pressure developed at below freezing temperatures on these agents.

Materials and methods. Viruses. Virus strains used included SW, PR8, FM-1, Lee, IB BCal, and 1233 ("C") all obtained from the Army Medical School, Walter Reed Medical Center, Washington, D. C. The 1233 "C" strain was maintained by amniotic passage; all others were allantoic adopted strains. *Hemagglutinin and infectivity.* Hemagglutinin titrations were performed in the usual manner at temperature indicated using 0.5% chicken red blood cell suspension. Infectivity titrations were made by inoculation of full-log dilutions into groups of 4-6 eggs. The eggs were then tested for the presence of hemagglutinin and endpoints calculated by the Reed and Muench method. *Pressure studies.* Virus suspensions to be subjected to pressure were placed in a soft plastic tube 3-4″ long and sealed so that no air bubbles were present. Water to be used in filling the cell was first boiled to remove all possible absorbed air and then chilled to 7°C. Likewise the steel cell itself was chilled to 7° prior to use. Virus samples were then placed in the chamber and this filled with water which was then overlaid with hydraulic oil as a seal. The steel cap was screwed down and the pressure rise followed with the strain gauge. Any initial leaks were immediately detected by a drift in the strain gauge pressure. With the cap in place and a proper seal obtained initial pressure reading was 2400 lb/in^2. Experience has shown that for reproducible results, this exact initial pressure must be obtained. Virus control samples were placed in similar plastic tubes and attached to the exterior of the steel cell which was then placed at the appropriate freezing temperature. After the desired pressure was reached the cell was returned to room temperature and the presure fall followed until it reached 2400 lb/in^2 at which point the cell was opened and the samples removed. The strain gauge is very sensitive to slight fluctuations in pressure. Moreover, the cell pressure will vary with any changes in freezer temperature. Thus, opening and closing the freezer door while pressure is being developed will change the shape of the pressure curve. The pressure curve is directly related to temperature at which freezing occurs and Fig. 1 shows the characteristics of pressure curves at –12 and –22°C, the temperatures used in the present experiments. With this particular apparatus maximum attainable pressure is about 32,000 lb/in^2. After this pressure is reached a fall in pressure occurs if the cell is allowed to remain at the same temperature, generally to about 27,000 lb/in^2. Finally, under the present experimental conditions, it is not possible to hold a pressure between 2500 and 27,000 lb/in^2 once it is attained.

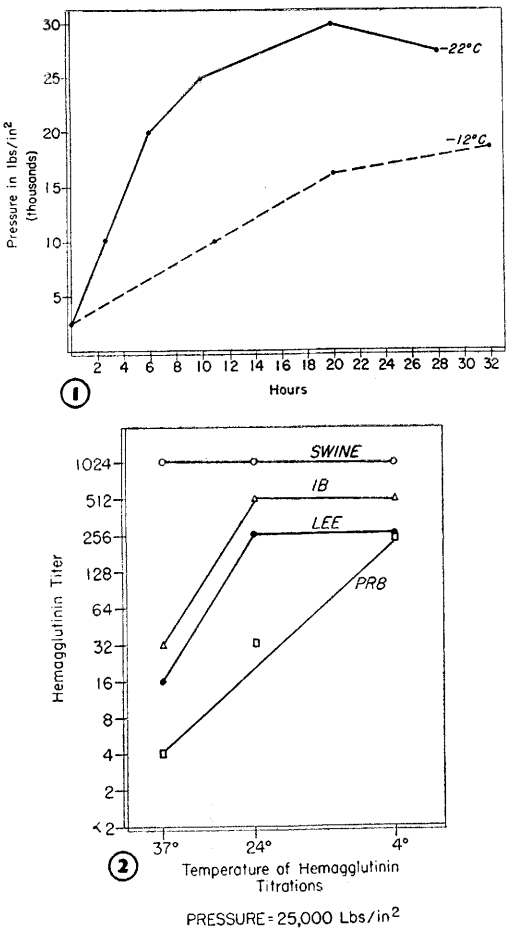

FIG. 1. Relation between pressure and temperature at which cell is filled with water and virus samples is placed.

FIG. 2. Relation of titer of virus subjected to pressure and temperature at which hemagglutinin test was performed.

Results. Initially, all pressure experiments

TABLE I. Effects of Pressure Developed at –22°C and –12°C on Influenza Virus Hemagglutinin.

Influenza virus strains	Virus hemagglutinin titers at 24°C: Lb/in² pressure developed at –22°C					Lb/in² pressure developed at –12°C	
	15,000*	17,000	20,000	24,000	30,000	15,000	17,000
SW	512	512	512	512	512	512	512
PR_8	"	"	"	64	<2	"	"
FM-1	"	256	128	128	256	"	128
Lee	"	"	"	"	"	"	64
IB	"	512	512	512	512	"	128
1233 ("C")	1024	1024	1024	1024	1024	1024	1024

* Titers equivalent to control samples of virus not subjected to pressure.

were performed by placing the cell at –22°C. Allantoic fluids containing virus strains described were diluted 1:2 in normal saline and subjected to pressures ranging from 2500 lb/in² (the baseline pressure) to 30,000 lb/in². At end of run, control and pressurized fluids were removed and hemagglutinin titered at room temperature. The results are given in Table I. One of the most striking features has been the reproducibility of the data. Thus, in replicate experiments amount of decrease in hemagglutinin titers for any given strain at any given pressure was always the same. Considering individual results, there is a marked difference in effect on hemagglutinin from various influenza virus strains. Thus, although the SW and PR8, strains of type A influenza virus are related rather closely antigenically, there is a vast difference in susceptibility to inactivation by pressure of these hemagglutinins. In other experiments, SW virus was subjected to a peak pressure of 32,000 (later falling to 27,000) for 4 days without effect on the virus hemagglutinin. Likewise, no effect of pressure has been found with the 1233 strain of influenza "C" virus. The FM-1, Lee, IB and BCal strains were variably affected by pressures developed at –22°C. The slight (one tube) rise in hemagglutinin titer in FM-1 and Lee upon increasing pressure from 24,000 to 30,000 lb/in² probably is not significant. In addition to strains described, a few studies with mumps, Newcastle Disease virus and NWS showed no pressure inactivation of the hemagglutinin. Since addition of serum tends to protect virus from inactivation by various physical methods, serum was added to virus fluids to be tested in the pressure cell. No serum concentration tested (the highest was equal quantities of allantoic fluids and horse serum) showed any protective effect. Due to a mechanical failure of one freezer unit it became necessary to conduct the experiments with the cell at a much higher temperature, –12°C. This, of course, resulted in more prolonged rise in pressure. For example, at –22°C a pressure of about 16,000 lb/in² can be obtained in about 4 hours whereas at –12°C 16 or more hours are required to attain the same pressure level. Unfortunately, the highest pressure obtainable at this temperature is only about 19,000 lb/in². The results of pressure experiments obtained at this temperature also are presented in Table I. Surprisingly, the Lee, FM-1, and IB virus hemagglutinins were more affected under these conditions than at presures more rapidly attained in the previous experiments.

Effect of temperature of titration on hemagglutinin titers of virus subjected to pressure. Since hemagglutinin was affected by pressure it was of interest to determine the role of virus elution in this process. Accordingly, the virus strains subjected to pressures with the cell at –12 and –22°C were then titered at temperatures of 4, 24, and 37°C. The results of these experiments are presented in Table II and Fig. 2. In general, virus samples subjected to various pressures showed a decrease in titer as the titration temperature was raised. Moreover, in certain instances the higher temperatures demonstrated a pressure effect on the hemagglutinin not apparent with room temperature titration. Thus, IB virus compressed to 17,000 with the cell at –23°C had a titer equivalent to the control when titered at 24°C but showed a 4-fold decrease at 37°C. Again, the difference in effect on the Lee, IB and

TABLE II. Titer of Influenza Virus Hemagglutinin at 24°C and 37°C of Virus Subjected to Pressures Developed at −22°C and −12°C.

Influenza virus strains	Lb/in² pressure developed at −22°C				Lb/in² pressure developed at −12°C	
	17,000 lb/in²		24,000 ib/in²		17,000 lb/in²	
	Temperature of hemagglutinin titrations					
	24°	37°	24°	37°	24°	37°
SW	512	512	512	512	512	512
PR_8	"	"	64	16	"	"
FM-1	256	64	128	<2	128	<2
Lee	"	128	"	16	64	"
IB	512	"	512	32	128	64

FM-1 hemagglutinin when rate of increase of pressure was varied was apparent. Lee virus for example brought to 17,000 lb/in² pressure (−23°C) showed a 4-fold decrease at 37°C titration temperature yet if the same pressure were slowly attained and the sample then titered at 37°C the resultant decrease was more than 128-fold. To determine more precisely the relationship between titer and temperature of titration various virus strains were subjected to 25,000 lb/in² pressure and titered at 37°, 24°, and 4°C. The results of this experiment are shown in Fig. 2. The titer of the control samples in this experiment was 512 for IB, Lee and PR8 and 1024 for the SW strains. Obviously, there is a relationship between temperature of titration and titer of the IB, Lee and PR8 strains but this is not always proportional. Again, no effect on SW hemagglutin was found.

Infectivity and antigenicity inactivation of influenza virus by pressure. Since the effect of pressure on hemagglutinin varied with the strain of virus used it was of interest to determine whether such variation could be demonstrated with infectivity titration. The results of experiments on 3 strains are shown in Table III. Whereas no pressure affected the SW hemagglutinin, a pressure of 24,000 lb/in² (1600 atmospheres) almost completely inactivated the infectiousness of this strain. Moreover, this pressure resulted in loss of most of the infectivity of the other 2 strains of virus tested.

TABLE III. Effect of Pressure Developed at −22°C in Infectivity of Influenza Virus.

Influenza virus strains	Pressure, lb/in²			
	15,000		24,000	
	Control	Exp.	Control	Exp.
PR8	7.5*	7.5	7.5	2.5
SW	7.8	7.6	7.6	1.4
Lee	7.8	7.2	7.5	1.0

* Log ID_{50} in eggs.

The PR8 virus was used in immunization studies to determine whether infectivity and/or hemagglutinin could be destroyed by pressure without affecting the antigenicity. For this purpose virus was semipurified by differential centrifugation and the virus then divided into control and pressure-treated lots. After 25,000 lb/in² pressure the resultant virus suspensions showed a marked loss of antigenicity when tested in rabbits. Rabbits immunized with pressurized virus developed an antihemagglutinin titer of 1:80 compared with the control titer of 1:1280 in animals immunized with the same aliquot sample not subjected to pressure.

Electron microscope studies of PR8 virus particles subjected to maximal pressures have not shown detectible changes in particle morphology.

Discussion. The present experiments indicate a marked difference between strains of influenza virus in the effect of pressure on viral hemagglutinin. The variation in resistance to presure inactivation is particularly marked with the swine as compared to the PR8 strains of type A influenza virus. Thus, maximal pressures for periods as long as 4 days failed to affect SW hemagglutinin yet more than 90% of PR8 hemagglutinin was lost at lower pressures applied for much shorter intervals. Other physical agents such as heat(4) and ultraviolet irradiation(5) have also demonstrated some differences between influenza virus hemagglutinins of the strains tested. However, the differences were most apparent in comparison of type A and type B

viruses and, to our knowledge, no studies previously reported have shown such marked differences in influenza virus hemagglutinins closely related antigenically.

As might be anticipated from other experiments concerned with physical agents and influenza virus, inactivation of infectivity and hemagglutinin does not occur coincidentally. Part of the aim of the present experiments was to determine whether pressure inactivation might be useful as a technic to prepare killed-virus vaccines. Unfortunately, the results indicate that antigenicity is lost at pressures requisite for infectivity inactivation so that, at least with the influenza virus strains tested, pressure inactivation for vaccine preparation would not appear feasible.

Summary. High compression pressures attained at below freezing temperatures have been studied for their effects on influenza virus hemagglutinin, infectivity and antigenicity. Influenza virus hemagglutinin from the virus strains tested varies markedly in its resistance to pressure inactivation.

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Separation of Lymphocytes in Human Blood by Means of Glass Wool Column.* (25237)

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With the exception of the magnetic technic of Levine(1), previous procedures for separation of lymphocytes from the other white cells found in human blood(2,3) have been based on differences in density of the various formed elements. This report describes the successful application of the principle of differential adsorption to separation of human lymphocytes. In this technic, granulocytes are removed from whole blood by passage through a short column of siliconized glass wool, while the lymphocytes in good yield and apparently intact are recovered in the effluent.

Materials and methods. The column and attachments are shown in Fig. 1. All glass surfaces were siliconized with a mixture of monomethyltrichloro- and dimethyldichlorosilane.‡ The vapor of this mixture (obtained by forcing air through a bubbler) was passed through the glass wool for 1 minute and then quickly flushed out with air. The glass wool was washed 10 times with water, heated 30 minutes at 110°C, soaked in water 30 minutes, rinsed many times with distilled water, and finally air dried. The glass wool used was Pyrex fiber No. 3950.§ Two grams of glass wool were packed into the column and held under firm pressure overnight by a rubber stopper fitted with a glass rod. The column was first conditioned by passing through it about 10 ml of fresh serum under 5% carbon dioxide, 85% nitrogen, 10% oxygen. Preliminary experiments carried out since the series reported in this paper indicate that this conditioning may be unnecessary. A freshly drawn 30 ml sample of the same subject's blood was then obtained, heparinized with 3.0 mg of heparin, and added to the column. No attempt was made to control the gas phase over the whole blood. The flow rate was adjusted to about 0.8 ml/minute by turning the screw clamp on the vent tube. The first 4 ml of effluent blood were discarded, and the next

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