assimilation of food was incomplete due to a deficiency in the external secretion of the pancreas and that fatal hypoglycemia developed more rapidly for this reason. There was no diarrhea in these experiments, the feces were well formed and firm but were larger than normal in the depancreatized rats. The completeness of intestinal absorption was not studied. Similarly, it is possible that the depancreatized rats were debilitated by operation and by the period of uncontrolled glycosuria so that their resistance to insulin shock was lower than normal. Although the depancreatized animals appeared vigorous and healthy during the period of insulin administration, it would be unsafe to conclude that their lower than normal resistance to insulin was due solely to the absence of the pancreatic islets.

Summary. Eighteen extensively depancreatized male rats and 20 normal rats were forcefed a medium carbohydrate diet. Following periods of adaptation and control, all of the animals were tested for their resistance to twice daily injections of regular insulin. The initial dose was 5 units and was increased in increments of 5 units per dose every second day until the animal died. The average lethal dose for the depancreatized rats was 50.5 units per 24 hours as compared to an average of 68.5 units for the normal animals. This difference in averages was calculated to be statistically significant.

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17177. Isolation of Three Strains of Type B Influenza Virus Incompletely Related to Lee.

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During December 1948, reports of widespread distribution of influenza began to appear in the Seattle area. The majority of illnesses were of relatively short duration and were accompanied in many cases by gastrointestinal disturbances. Proper specimens of throat washings and serum were not obtainable until the return of students to the University of Washington campus after the New Year holidays.

During the first 9 weeks of 1949, there were 116 admissions for upper respiratory infection to the University of Washington Health Center. It was apparent, through random questioning of the campus population, that many others were ill at home. Since approximately 60% of the 16,000 University students live at home in or close to the City of Seattle, and there were a number of students known to be ill in living quarters on and off the campus, it seems obvious that the outbreak was much more widespread than the 116 admissions to the Health Center would indicate. A conservative estimate of 500 cases, of varying severity, during the period from the end of December to the first of March does not seem unreasonable. Observations of classroom attendance supported this estimate.

The majority of illnesses were mild, with the average period of hospitalization being between 3 and 4 days. The mildness of the disease further supports the idea that the cases seen in the campus infirmary represented only a relatively small proportion of the total number occurring in the Seattle area.

Materials. Blood specimens and throat washings were obtained during the acute stage from patients in the infirmary whenever possible. Serum was separated promptly and stored at −2°C until used. Throat washings were obtained by having the subjects gargle 15 ml of nutrient broth (Bacto) to which had been added penicillin G in an approximate concentration of 1000 units per ml. The garglings were collected in clean beakers, trans-

ferred to sterile screw cap bottles, and stored in a CO_2 ice box at approximately –76°C until used.

Convalescent blood specimens were obtained whenever possible. The interval between the acute and convalescent specimens ranged from 10 to 25 days, with a few exceptions. Twenty-eight convalescent sera were obtained from suspected influenza cases, and were stored with the acute specimens at –2°C.

Methods. Throat washings were inoculated to the allantoic cavity of 8- to 10-day chick embryos, using 0.25 ml per egg and a minimum of 5 eggs per washing. Inoculated eggs were incubated at 36°C and were examined daily by candling. Eggs showing dead embryos in 24 hours were discarded. A small portion of allantoic fluid was aspirated daily from surviving eggs from the second to fifth days after inoculation, and was tested in a 1 to 10 dilution for ability to agglutinate washed human cells of group O. Fluids showing typical agglutination were inoculated to a new set of eggs and were further tested against standard antisera in an attempt to determine the type of virus most prevalent in the outbreak.

The technic for examination of paired sera was that recommended by the Influenza Information Center of the U. S. Public Health Service[1] for laboratories participating in its program. The original method,[2] as modified by the Army Medical Center,[3] involves the addition of 4 hemagglutinating units of the standard virus antigens (PR8, FM1 and Lee) to serial two-fold dilutions of inactivated serum, followed by addition of washed group O human red cells. Incubation was for one hour at room temperature.

Results. Three strains of influenza virus were isolated during the early part of the outbreak, and gave typical agglutination results up to 1 in 320, in the first passage. Subsequent allantoic passages yielded fluids with titers ranging up to 1 in 2560. The strains were designated as Seattle 1-49, 2-49, and 3-49, and were forwarded to the Influenza Strain Study Center.[4]

The 3 virus strains were separately tested by the standard agglutination-inhibition technic against high-titer monovalent rooster sera.* These sera were able to inhibit red cell agglutination by PR8, FM1 or Lee antigens in titers of 1 in 1600 or 1 in 800, with cross-inhibition only in the first tube between the A and A prime viruses. However, when the 3 newly isolated strains of influenza virus were set up against the standard sera, no significant evidence of inhibition of hemagglutination could be obtained. Additional egg passages up to a total of 10 did not modify these results. The specific rooster sera were not able to supply the typing information desired, and because of doubt regarding the nature of the prevalent virus, a general program of vaccination was not undertaken.

Twenty-eight paired sera were investigated for evidence of rise in antibody titer against types A, A prime and B viruses, and also against the Seattle strains, using the standard agglutination-inhibition technic. No pairs showed any evidence of significant increase against type A or A prime viruses and these data are therefore omitted. The results of the significant titrations are shown in Tables I and II.

Table I gives the results of titrations on 28 paired sera. Six patients showed no increase of antibodies able to inhibit hemagglutination by known virus antigens and were not considered as serologically proved cases. The titrations of the 22 sera showing a significant increase of such antibodies gave the results indicated. It will be noted that the evidence indicates a higher antibody rise against Lee virus than against the Seattle strains.

The difference in antigenicity between the Lee and Seattle viruses is demonstrated further in Table II. It is seen that of the 22 serum pairs showing a 4-fold or greater increase in titer against Lee virus, only 18 also showed

[1] Culbertson, James T., *Am. J. Pub. Hlth.*, 1949, **39**, 37.

[2] Anonymous, *Bull. U. S. Army Med. Dept.*, 1946, **6**, 777.

[3] Medical Division, Army Medical Center, letter of Sept. 7, 1948.

[4] Anonymous, *Science*, 1947, **106**, 542.

* Obtained through the kindness of the Department of Virus and Rickettsial Diseases, Army Medical Center, Washington, D.C.

TABLE I.
Titrations of Paired Sera.

Increase	4-fold	8-fold	16-fold or more	Total
vs. Lee	6	7	9	22
vs. Seattle strains	9	8	1	18
No significant increase in titer				6

TABLE II.
Increase in Titer of Paired Sera.

Significant increase	
vs. both Lee and Seattle strains	18
vs. Lee only	4
vs. Seattle strains only	0
No increase	6
Total	28

a significant rise against the Seattle strains. Four patients showing a rise in titer of antibodies able to inhibit hemagglutination by Lee virus and not by the Seattle strains, may represent cases due to viruses other than the more prevalent strains.

On the basis of titrations of paired sera only, the outbreak in Seattle would be considered as due to a virus antigenically related to Lee virus, since all the positive sera showed a significant increase in antibodies against Lee when compared with the acute phase sera. On the other hand, hemagglutination by the Seattle viruses was not inhibited by typing sera prepared in roosters. This fact emphasizes two points: first, an antigenic difference between the newly isolated strains and Lee virus, and second, the inadequacy of highly specific antisera for the typing of influenza virus having minor antigenic differences from the standard strains.

Because of the ability of Seattle viruses to produce antibodies against Lee virus, and the failure of specific Lee antiserum to inhibit hemagglutination by the new strains, it is apparent that the Seattle strains are not antigenically identical with Lee virus. This opinion has been confirmed by the virus laboratory of the California State Department of Health[5] and by the Influenza Strain Study Center.[6]

The evidence in the case of the Seattle strains seems to indicate that these viruses are not as highly antigenic as Lee virus, as demonstrated by the lesser rise in titer in paired sera, but that the new strains may be antigenically broader in pattern, as shown by the ability of all second sera to inhibit hemagglutination by Lee significantly, whereas specific Lee serum did not inhibit hemagglutination by the new strains.

Strain differences in influenza B virus have been reported previously by Eaton and Beck,[7] Gordon,[8] Burnet *et al.*,[9] and others. However, it is considered of value to record the continued antigenic heterogeneity of this virus, particularly in view of the problem of choice of strains for inclusion in influenza vaccines.

Summarizing, there is reported an outbreak of influenza on the University of Washington campus, from which 3 strains of influenza virus B were isolated. Paired sera from 22 patients showed a significant rise in hemagglutination-inhibition titer against Lee virus in all cases and against the new strains in 18 sera. Because of the inability of rooster antiserum prepared against Lee virus to inhibit hemagglutination by the Seattle strains, it appears that these new viruses are incompletely related to the standard type B virus.

[5] Meiklejohn, G., personal communication, Feb. 11, 1949.

[6] Magill, T. P., personal communication, March 18, 1949.

[7] Eaton, M. D., and Beck, M. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 177.

[8] Gordon, I., *J. Immun.*, 1942, **44**, 231.

[9] Burnet, F. M., Beveridge, W. I. B., and Bull, D. R., *Aust. J. Exp. Biol. and Med. Sci.*, 1944, **22**, 9.

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