fore significant amounts of radiostrontium were absorbed, and the whole process was slower. 3. It was possible to measure radiostrontium absorption on two different occasions in the same animal, first with Sr^{89} and then with Sr^{85}. This should prove of value in studying factors affecting strontium absorption.

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Evaluation of Laboratory Diagnostic Procedures with A/Asian Influenza. (24629)

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The recent influenza epidemic stimulated many virus diagnostic laboratories to determine whether recommended laboratory procedures were adequate and to evaluate alternative diagnostic procedures(1). Throat washings and serum specimens we received were processed by standard procedures, and results analyzed to investigate the following: 1) comparison of complement fixation and hemagglutination-inhibition tests for routine diagnosis, 2) relationship between time of appearance of antibodies titrated by above methods, 3) correlation between increased antibody titers and virus isolation, and 4) comparison of several isolation methods.

Materials and methods. The majority of acute and convalescent serums were obtained from individuals 10 to 65 years of age. Samples were submitted from many areas of the United States. Selection of patients and collection of throat washings were made by numerous physicians and health officers with varying degrees of experience. Due to this fact, specimens were often subjected to suboptimal conditions for storage and transportation. Infected allantoic fluids served as specific antigens in both serologic tests. These were produced by injection of approximately 10,000 EID_{50} by the allantoic route in 11-day embryonate eggs. Following 48 hours incubation at 37°C, the eggs were chilled several hours, fluids harvested, clarified by low speed centrifugation and stored at 4°C. Virus strains used were A/Asian/Japan/305/57, A/Denver/1/57, and B/GL/1739/54. *Serologic procedures.* Complement fixation (CF) test employed was the standard technic in the Virus and Rickettsia Section. With this method, 0.1 ml of serum and antigen dilutions are mixed with 0.2 ml of complement and later sensitized sheep erythrocytes to give a final total volume of 0.6 ml. Optimal dilutions of antigen as determined in box-type titrations were used. Complement titers were measured in presence of each antigen, and dilutions contained 2 optimal units. The test was incubated overnight at 4°C followed by 20 minutes at room temperature, at which time sensitized cells were added. The test was read after incubation at 37°C for one-half hour. The tube containing greatest dilution of serum in which less than 50% hemolysis (estimated) occurred was considered as the positive end point. Hemagglutination-inhibition (HI) tests were performed as previously described

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(2,3) with the modification that serums were heated at 56°C for 30 minutes and then treated with M/90 solution of potassium periodate to destroy nonspecific inhibitors. One volume was mixed with 2 volumes of periodate and held overnight at 4°C. Two volumes of 1% glycerol saline were then added to stop the reaction of the periodate(4). *Isolation procedures.* Penicillin and streptomycin, approximately 200 units and 100 mg/ml respectively, were added to aliquots of each throat washing before inoculation. All throat washings were inoculated into the amniotic cavity of 11-day-old embryonate hens' eggs. A number of suspected throat washings were inoculated into 3 tissue culture tubes of monkey kidney cells and a few were tested in HeLa and human amnion cells. Each test system received 0.1 ml inoculum. At least 2 transfers were completed before a specimen was considered negative.

Results. To compare efficacy of 2 recommended serologic methods, results were analyzed from 422 cases in which diagnosis could be made on the basis of a 4-fold or greater increase in antibody measured by one or both test methods. Analysis of positive results is presented in Table I. Only a little over half of the cases were positive by both tests (56%). While an additional 33% were positive by the CF test alone, only an additional 11% were positive by HI test alone. The question arose as to whether these differences might relate to the convalescent period when serum was collected. The data were examined

TABLE I. Relationship between Time of Collection of Serum and Serologic Results.

Days after onset*	Total positive cases†	CF & HI No.	CF & HI %	CF only No.	CF only %	HI only No.	HI only %
7- 9	23	7	30	12	52	4	17
10-12	57	22	39	34	60	1	2
13-15	165	89	54	58	35	18	11
16-18	63	38	60	16	25	9	15
19+	114	79	69	19	17	16	14
Total	422	235		139		48	
	100%	56%		33%		11%	

* Acute phase specimens were drawn during first week of illness. Days after onset indicate interval before collection of convalescent serum.

† In all cases 4-fold or greater increase in antibody titers were demonstrated by one or both methods.

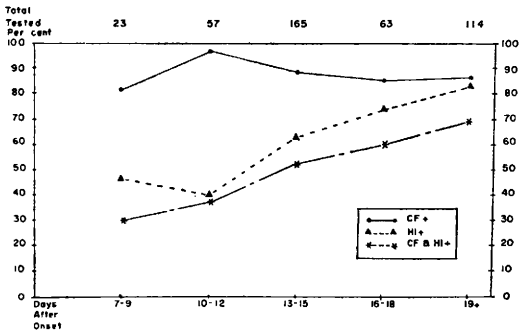

FIG. 1. Influence of interval between onset and convalescent serum specimens on rates of diagnosis by CF and HI methods in 422 serologically proven cases of influenza infections.

with this in view, and the results listed in Table I.

When specimens were collected 10 to 12 days after onset of illness, approximately 60% of the cases diagnosed by CF were missed by the HI test. In contrast, the marked disparity between results with the 2 serologic procedures was not evident when convalescent serums were collected 19 days or longer after onset, at which time approximately 70% were diagnosed by both methods. It is evident, however, even after the longer interval, that some cases were detected by one method and not the other. It is desired to point out that the total cases diagnosed by the CF test at a particular time interval varied between 82 and 99%, whereas total cases diagnosed by the HI test varied between 41 and 83%. Those cases where diagnosis was made by HI test and not by CF test may have been because the acute phase serum was obtained when CF antibody level was elevated and did not rise further. Relative differences in sensitivity between CF and HI procedures are contrasted in Fig. 1. With an increasing number of days after onset, results obtained by both tests become nearly parallel. The obvious conclusion is that the CF test would be preferred when convalescent serum was collected early after onset, whereas if the HI test alone is to be employed the convalescent serum specimen should be collected 3 weeks after onset. It must be concluded that the greatest efficiency of diagnosis results when both methods are employed. From a practical standpoint the CF test would be used for

routine work and when CF test results are negative, use of the HI test is indicated.

Isolation attempts. A total of 530 throat washings and specimens of lung from autopsies were tested and from these 102 isolates (19.2%) were obtained by usual chick embryo technics. In one limited study of 43 successful isolations, 19 were positive and typed from the first egg inoculation, whereas 24 required a transfer in additional eggs. In 29 positive cases, it was found that in only 2 cases were allantoic fluids harvested with hemagglutinin titers suitable for typing when 0.1 ml of throat washing was inoculated into both amniotic and allantoic cavities. Negative allantoic fluid required one or 2 additional transfers before all could be typed.

With 102 specimens, a comparison was made of relative sensitivities between standard chick embryo amniotic isolation technics and hemadsorption procedure developed by Vogel and Shelokov(5). These specimens were tested simultaneously, and 35 were positive by one or the other procedure. Only 3 specimens yielded virus by monkey kidney tissue culture hemadsorption method, so that this method was definitely less sensitive in our laboratories. On the other hand, 2 of the 3 positive by tissue culture method were missed by egg inoculation procedure.

In addition to inoculation of above described tissues, other cell lines (HeLa and human amnion) and mice were also inoculated. No isolations were successful in any of these systems. Several blind passages were made in each case.

Attempts to correlate virus isolations with positive serologic diagnosis were seriously hampered by the fact that both kinds of specimens were not usually obtained from the patient. In only 67 cases were throat washings submitted with acute and convalescent serums. There were 42 cases diagnosed by either method and 16 cases demonstrated the presence of virus in their throat washings. From 16 cases where influenza virus was isolated, 15 were positive by the complement fixation test, while 8 were positive by the HI test. In 26 cases from which no virus was isolated but positive serologic diagnosis was made, rates of diagnosis by CF or HI tests were similar to those found with positive virus isolations. These results indicate greater difficulty in isolation of virus from specimens than might be expected if patients were carefully selected, and if throat washings were properly collected and transported. Under these circumstances, however, positive serologic support of a diagnosis is much more reliable than virus isolation attempts.

Discussion. As part of an extended evaluation of laboratory diagnostic procedures currently in use for recognition of influenza, the following conclusions seem warranted. The CF test appears preferable to the HI method for routine diagnosis because antibodies measured by CF test often appeared in elevated concentrations approximately 10 days before HI antibody levels were increased. Furthermore, the difficulties encountered with inhibitor sensitive strains of influenza rule out the HI test for many inexperienced laboratories. In addition, a strain specific CF test(6) may yield information similar to that obtained by the HI test without the disadvantage of having to utilize serum treatment procedures for removal of nonspecific inhibitors. The more rapid rise in CF antibody titer apparently results from an anamnestic response to antigens which are not concerned in the virus neutralizing or hemagglutinin inhibiting reaction. The slower response as measured by the HI test must be that resulting from the first experience with a new set of antigens. If this is correct, then a more parallel response could be expected with CF and HI tests in epidemics which occur when closely related viruses had been prevalent for several years. It is possible, therefore, that the increased advantage of the CF test would be evidenced only during epidemics such as the one just experienced. In spite of the need for rapid diagnosis, our data made it clear that the embryonate egg remained the most sensitive host for attempts at isolation of influenza viruses. More recent modifications reported by Shelokov *et al.*(7), may result in increased efficiency of the new method.

Summary. For routine serological diagnosis of influenza, the CF test is preferable to HI test. A/Asian influenza virus was more

readily isolated in the embryonate egg than in monkey kidney cell tissue culture.

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Factors Related to Psittacosis Virus (Strain 6BC) Growth. V. Folic Acid-like Factor in Infected Cells.* (24630)

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Inhibition of growth of the 6BC strain of psittacosis virus by sulfonamides and reversion of this action of sulfonamides in a competitive manner by p-aminobenzoic acid and pteroic acid and in noncompetitive manner by folic acid or citrovorum factor led Morgan to suggest(1) that the cell infected with psittacosis virus might be capable of synthesis of folic acid from precursors. If this is true, one would expect that folic acid content of viral-infected cells would be higher than that of noninfected cells, and studies were initiated to determine if it was possible to detect such net increase in folic or folic acid-like factors in viral-infected cells. L cells were chosen since these cells require folic acid(2) and they support growth of psittacosis virus when cultivated in a semi-synthetic medium(3). These experiments indicate that L cells infected with psittacosis virus contain increased amounts of folic acid or a folic acid-like factor.

Materials and methods. The mouse fibroblast cell line, strain L, obtained from Dr. Wilton R. Earle, Nat. Cancer Inst., Bethesda, was maintained in T flasks‡ in growth medium consisting of Earle's balanced salt solution; 0.06% yeast extract§; 0.18% lactalbumin hydrolysate‖; and 10% horse serum. Semi-synthetic medium (SSM) was a modification of medium described by Bader and Morgan(3) in which folic acid was omitted, concentration of p-aminobenzoic acid (PABA) was doubled, and 1% dialyzed horse serum was added. The egg-adapted strain of psittacosis virus (6BC) was used, as described previously(1,4). To minimize folic acid content of virus inocula, virus stock was prepared from virus grown in L cells which were maintained in SSM for 8 days before infection. After infection, daily microscopic observations of cells was carried out until a definite cytopathic effect of the virus was noted; then the flasks were frozen and thawed slowly to break up the cells and the entire culture harvested and stored in a dry-ice chest for use as stock virus which was diluted in SSM as needed. Sulfadiazine (SDZ) was used as a sterile solution of sodium salt. P-aminobenzoic acid (PABA) was dissolved in distilled water as the sodium salt and sterilized by filtration. Folic acid determinations were carried out according to the method adopted by Assn. of Official Agricultural Chemists(5), using *Streptococcus faecalis* as test organism (ATCC #8043). Chicken pancreas extract was used as source of glutamic acid conjugase. Bacterial growth was measured turbidimetrically with Bausch

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‡ Obtained from Kontes Glass Co., Vineland, N. J.

§ Obtained from Difco Labs., Detroit, Mich.

‖ Obtained from Nutritional Biochemicals Corp., Cleveland, O.