.52. In the tagged cell experiment, alpha dose to blood per μc Pb^{212} is .18 rad, and fraction of Pb^{212} atoms which decay in blood is .78.

At 30 hours Pb^{212} in excreta is about 6% of the total Pb^{212} at that time. Most of the excretion takes place 1 to 3 half periods after injection, so that the actual amount of Pb^{212} which decays outside the dog (*i.e.*, in excreta) is only 1 to 2% of the amount originally injected. Thus our error will be small if we use the simplifying assumption that all Pb^{212} decays inside the dog. Hence, in summary, when blood cells are tagged with Pb^{212} and injected into the dog, about 4 5 of the alpha energy is delivered to blood and 1 5 to the remainder of the dog. When Pb^{212} is injected intravenously, half decays in blood and half in the rest of the dog. It follows then, that when thoron decays in blood, half the resulting Pb^{212} can be expected to decay in blood and half elsewhere in the dog. Therefore, with information gained from these experiments, we will be able to calculate the amount of thoron which decays in blood if we know Pb^{212} concentration in blood and total blood volume.

Summary. Beagle metabolism of short-lived Pb^{212} has been studied following intravenous injection, and after transfusion of blood cells tagged with Pb^{212} *in vitro*. The latter proved to be a satisfactory method to determine blood volume. When Pb^{212} was given intravenously, *in vivo* tagging of blood cells occurred. A maximum of 65% of the activity in cells was reached at 2 to 3 hours after injection, then Pb^{212} in blood cells decreased with a biological $t_{1/2}$ = 37 hours and an effective $t_{1/2}$ = 8.2 hours. The same decrease occurred after injection of *in vitro* tagged cells. In both experiments essentially all Pb^{212} decayed inside the dog. Half decayed in blood in the *in vivo* tagging experiment; 4 5 decayed in blood when tagging was done *in vitro*.

The author acknowledges assistance of D. R. Atherton, N. Keller, and R. L. Floyd.

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Received October 31, 1958. P.S.E.B.M., 1959, v100.

Isolation of Asian Virus from Extrapulmonary Tissues in Fatal Human Influenza.*† (24597)

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Thirty-three influenza-associated deaths were observed in greater Cleveland during the 1957 pandemic of Asian influenza. Asian virus was isolated from lung or trachea in 25 of the 33, and from extrapulmonary tissues in 3 of the 25 respiratory tract-positive cases. Al-

* This investigation was conducted under sponsorship of Comm. on Acute Respiratory Diseases, Armed Forces Epidemiological Board, supported in part by Office of Surgeon General, Dept. of Army, and the Brush Fn., Robert Hamilton Bishop, Jr. Endowment Fund, and Republic Steel Corp.

† The authors are indebted to Dr. L. Adelson of Dept. of Pathology, WRU and Cuyahoga County Coroner's Office, for study material and generous use of facilities, and to Dr. F. W. Denny, Jr. and D. A. Stevens for their assistance.

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though influenza virus has been recovered from blood and extrapulmonary organs in experimental infection in animals, occurrence of viremia has not been shown in natural influenza infection in man(1). This report deals with viral studies on pulmonary and extrapulmonary tisues from fatal human cases. Recovery of virus from liver, spleen, kidney, heart, tonsil and lymph node supports the concept that viremia does occur during overwhelming human infection. Detailed analyses of clinical, bacteriologic and anatomic findings of these studies will be reported elsewhere(2).

Methods. Source of material. Tissues were obtained from 33 influenza-associated deaths, 25 of which met the following criteria: 1) history of influenza-like illness; 2) characteristic anatomic findings (acute hemorrhagic and necrotic tracheobronchitis; pulmonary edema and congestion; and intra-alveolar hemorrhage with or without pneumonitis); and 3) isolation of influenza virus from lung or trachea. Extrapulmonary tissues were available in 15 of 25 respiratory tract-positive cases. Tracheobronchial lymph nodes and tonsils were not collected regularly and therefore were tested in only 2 instances. Necropsies were performed within 12 hours after death. *Viral studies.* Organ samples were collected as soon as body cavities were opened and before systematic examination of viscera was carried out. Using washed, flamed instruments, tissues were removed, placed in sterile lusteroid containers, and either tested immediately to establish presence of virus or stored at −70°C until processing. Lung and trachea were examined initially and, when positive, the extrapulmonary tissues were tested. Other organs from respiratory tract-negative cases were not studied. Tracheal mucous membrane was stripped from underlying structures, ground with alundum®, and 10% suspension in veal infusion broth approximated. Twenty % suspensions by weight of lung and extrapulmonary tissues were prepared in similar manner. To each ml of suspension were added 200 units of penicillin and 0.2 mg streptomycin. One-tenth ml was inoculated into the amniotic cavity of each of six 11-day-old embryonated hens' eggs. Amniotic fluid was harvested after incubation at 37°C for 3 days and tested for hemagglutination with chicken red cells at room temperature. Each isolate was identified as Asian type by specific antisera. A negative result with lung or trachea was defined as failure to demonstrate hemagglutination after the original and 1 blind passage on 2 consecutive attempts from original material, and with extrapulmonary tissues after the original and 2 blind passages. Each positive isolate obtained from extrapulmonary tissue suspensions was confirmed 3 times by repeating the procedure from the original tissue. Tissue suspensions were assayed for virus content employing 10-fold dilutions (1:10 to 1:10,000,000) of lung and trachea, and 1:5, 1:25, 1:50 and 1:500 dilutions of extrapulmonary organ suspensions. One-tenth ml of each dilution was inoculated into amniotic cavities of six 11-day-old embryonated hens' eggs. Amniotic fluid was harvested after 3 days' incubation at 37°C and tested with chicken red cells at room temperature. When virus was not found on first passage of a given dilution of organ suspensions, 2 blind passages were made in eggs from pooled amniotic fluids of the first passage. The concentration of virus in lung and trachea is expressed as the reciprocal of highest dilution of suspension from which virus was isolated after first amniotic passage, and in extrapulmonary tissues, after first and subsequent passages.

Results. Asian strain influenza virus was isolated from extrapulmonary organs in 3 of 15 cases: in one it was present only in the tonsil; in the second, in spleen and lymph node and in the third, in liver, spleen, kidney and heart. The clinical, anatomic and bacteriologic findings in the 3 cases are shown in Table I. The 2 children were previously healthy, but the adult had serious pre-existing liver and kidney disease.

The histologic appearance of virus-positive extrapulmonary tissues (Table II) revealed acute nonspecific inflammatory changes in addition to pre-existing chronic disease. Although virus was isolated from heart muscle (Case 19) showing acute arteriolar lesions, virus could not be demonstrated in myocardial suspensions from 13 respiratory tract-positive cases, 3 of which exhibited acute myo-

TABLE I. Clinical, Anatomic and Bacteriologic Findings.

Case No.	Age (yr)	Sex	Race	Duration illness (days)	Clinical features	Postmortem findings	Bacteriology Lung	Bacteriology Trachea
6	10	♂	Non-white	2	Sudden onset of influenza-like illness; 7 siblings simultaneously ill; developed bilateral pneumonia	Pulmonary edema, congestion and hemorrhage; Focal pneumonitis; Acute necrotizing tracheobronchitis; Acute nonspecific changes in splenic and lymphoid follicles; Cerebral edema and congestion; Pseudotubular change in adrenals; No pre-existing disease	Hem. staph.	Hem. staph.
19	59	♀	White	4	Sudden onset of influenza-like illness; found dead at home; chronic alcoholic with liver cirrhosis	Pulmonary edema, congestion and hemorrhage; Acute necrotizing tracheobronchitis; Fibrinoid degeneration of myocardial arterioles; Portal cirrhosis; Nephrosclerosis; Chronic pyelonephritis	Sterile	Sterile
28	4	♀	"	4	Coryza treated by parent with penicillin; found dead at home	Pulmonary edema, congestion and hemorrhage; Focal pneumonitis; Acute necrotizing tracheobronchitis; Acute myocarditis; Acute follicular tonsillitis; Necrosis of splenic and lymphoid follicles; No pre-existing disease	"	Hem. staph.

carditis histologically, nor in brain suspensions from 13 cases, 2 of which were characterized clinically by widespread involvement of the central nervous system. Virus titrations were performed on tissues from both extrapulmonary-positive group and 3 other cases from which virus had been recovered only from the respiratory tract (Table III). Concentration of virus, expressed as reciprocal of highest dilution of suspension from which virus was isolated, ranged in pulmonary tissues from 100 to 100,000 and in extrapulmonary tissues from 25 to 500 after the first amniotic passage. After one or 2 blind passages of those dilutions of organ suspensions negative on first passage, virus was detected in the 1:5 dilution of spleen in Case 19, the 1:500 dilution of spleen in Case 6, and the 1:1000 dilution of tonsil in Case 28.

TABLE II. Histologic Appearance of Virus-Positive Tissues.

Case No.	Organ	Histologic lesion
6	Spleen	Nonspecific inflammatory reaction
	Lymph node	*Idem*
19	Liver	Portal cirrhosis
	Spleen	Nonspecific inflammatory reaction
	Kidney	Nephrosclerosis; chronic pyelonephritis
	Heart	Fibrinoid degeneration of arterioles with focal fibrin thrombi
28	Tonsil	Acute follicular tonsillitis; necrosis of follicles

Discussion. Hamre and her co-workers(1) demonstrated virus in blood and extrapulmonary tissues in experimental influenza infection in animals, and suggested that viremia might also occur in natural influenza in man. Attempts to isolate virus from blood were not carried out in our study, but virus was obtained from extrapulmonary tissues. Localization of virus in distant organs lends strong support to the presence of viremia during overwhelming human infection.

Flewett and Hoult(3) have recently de-

TABLE III. Concentration of Virus in Tissues from Cases of Human Influenza.*

Organ	No. positive/No. tested†	Case No. 6	19	28	4	16	29
Lung	19/32	1,000	1,000	0	100	1,000	100,000
Trachea	22/29	100	100,000	100,000	10,000	100	NT
Liver	1/14	0	50	0	0	0	"
Spleen	2/14	25 (500)‡	0 (5)§	0	0	0	"
Kidney	1/13	0	25	0	0	0	"
Heart	1/14	0	50	0	0	0	"
Lymph node	1/ 2	25	NT	NT	NT	NT	"
Tonsil	1/ 2	NT	"	500 (1,000)§	"	"	"

* Conc. = reciprocal of highest dilution of suspension from which virus was isolated after first amniotic passage.

† One or more extrapulmonary tissues were available for testing from 15 cases.

‡ Reciprocal of highest dilution after third passage.

§ *Idem* second .

0 = Negative. NT = Not tested.

scribed fatal cases of Asian strain influenza associated with encephalopathy at height of acute attack. Virus was isolated from both brain suspension and a pool of lung and trachea in 1 case. A 1:10 dilution of brain suspension failed to yield virus, although virus grew well in eggs inoculated with a 1:1000 dilution of lung and trachea. The authors suggest their findings may have been due to postmortem room viral contamination of brain samples, or to viremia.

Despite the relatively low concentration of virus in extrapulmonary tissues in our study, it is unlikely that the findings are the result of contamination either in autopsy room or laboratory. Collection of specimens was carried out before the pathologist conducted routine dissection. Instruments were washed and flamed immediately prior to use. Samples of tissue were placed in sterile lusteroid containers as soon as they were removed from body. Positive isolation of virus from extrapulmonary tissues was repeated at least 3 times for each sample tested. Finally, systemic manifestations, both clinical and anatomic, suggest the likelihood of blood-stream dissemination of virus in fatal influenza in man.

Summary. Asian strain virus was demonstrated in extrapulmonary tissues from 3 influenza-associated deaths. This finding suggests the occurrence of viremia in overwhelming influenza in man.

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Received November 3, 1958. P.S.E.B.M., 1959, v100.