sexually mature virgin rats. RNA/DNA ratio was reduced 31.9% one day after weaning and by the 12th day it was lower than that of glands from sexually mature virgin rats.

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Experimental Influenza in Sheep.* (28234)

JAMES L. MCQUEEN† AND FRED M. DAVENPORT
Department of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor

Investigators in Hungary recently reported the isolation of Asian (A_2) influenza virus from an adult sheep suffering with respiratory disease(1). They also described attempts to infect lambs by intratracheal inoculation of egg-adapted strains of A/Swine, A_2, and PR8 virus. The lambs reportedly manifested clinical, pathological, and immunological responses to the inoculation. However, no mention was made of attempts to recover virus from the inoculated animals. Since influenza had not previously been reported in sheep, a limited study was undertaken to determine their susceptibility to artificial infection with Type A influenza isolates.

Materials and methods. Virus. The virus strains A/PR8/34 ($F_{109}E_{4-7}$), A/Swine/Wis/3/57(E_{12})‡, and the A_2 strain isolated from sheep in Hungary, A/Bor/111/60(E_4)§, were used in this study.¶ Stock virus was prepared using standard egg inoculation techniques(2). Infected allantoic fluids no more than 72 hours old were diluted in broth for inoculation of lambs.

Experimental animals. Three male Merino-type and 6 male Suffolk-type lambs, ranging in age from 3 to 10 weeks, were obtained from 2 farms near Ann Arbor, Mich. The lambs were housed indoors and fed on alfalfa hay and antibiotic-free starter ration. All lambs were observed for at least one week prior to initiating the experiments.

Inoculation and observation of animals. Unanesthetized lambs were restrained in an upright position. The trachea was penetrated just below the larynx with an 18 gauge needle, and from 10 to 40 ml of virus-containing broth was given by syringe. Body temperatures were recorded twice daily for 7 days and once daily thereafter for a total of 14 days.

Reisolation attempts. Specimens for attempted isolation of virus were collected from 4 lambs. Nasal swabbings obtained from 2

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† Veterinary Officer, Dept. of HEW, USPHS, CDC, Atlanta, Ga., assigned to School of Public Health, Univ. of Michigan, Ann Arbor.

‡ A/Swine/Wis/3/57, obtained from R. Q. Robinson, Respiratory Disease Unit, CDC, Atlanta, Ga.

§ A/Borzsony/111/60, obtained from Dr. E. Farkas, State Inst. of Hygiene, Budapest, Hungary.

¶ The letters F and E and numerical subscripts denote the number of passages in ferrets and eggs.

lambs 48 and 72 hours after inoculation, and lung and tracheal tissues obtained from 2 others by autopsy at 72 hours were inoculated into eggs according to standard procedures (2). A single blind passage of amniotic fluids was made.

Serologic methods. Blood samples were drawn immediately prior to administration of virus and 14 days after each inoculation. A nonspecific inhibitor (heat stable at 56°C) was noted in most preinfection sera. This inhibitor was removed, with no loss of specific antibody titer, by heating the sera at 60°C for 30 minutes. Except for that minor variation in the method of inactivating non-specific inhibitor, the standard serological procedure for influenza testing was used for detecting hemagglutinating inhibiting (HI) antibody (2).

Results. Clinical response. Two 10 week old Merino-type lambs were given 900×10^6 EID_{50} of A/PR8 influenza virus. These animals manifested a febrile response beginning 24 and 50 hours after inoculation. Maximum temperatures of 105.4°F and 106.0°F were noted during the febrile period of 5 to 6 days. No symptoms of acute respiratory distress were observed in either animal. In attempts to repeat this experience, virus was administered to 5 Suffolk-type lambs. Three lambs, 10 to 12 weeks of age, were given from 348,000 to 4178×10^6 EID_{50} of A/PR8 influenza virus. Two 18 week old lambs were given 200×10^6 EID_{50} of A/Bor influenza virus. None of these animals manifested clinically detectable illness throughout the 14 day period of observation. Body temperatures after inoculation remained within normal limits(3).

Attempts to reisolate virus. Swabs of the posterior nares and turbinates, taken 48 and 72 hours after inoculation of the first 2 animals, were expressed in one ml of broth and inoculated into eggs. Virus was not recovered. Because the next 5 animals remained afebrile, attempts were not made to reisolate virus from them.

Two lambs, a 10 week old Suffolk-type and a 4 week old Merino-type, were given respectively 4178×10^6 EID_{50} of A/PR8 and 100×10^6 EID_{50} of A/Bor influenza virus.

TABLE I. Homologous Antibody Response of Lambs to Influenza Virus.

Virus given	HI titer of sera		
	Pre-inoculation	1st Post-inoculation	2nd Post-inoculation
PR 8	<8	64	256
PR 8	<8	64	1024
PR 8	<8	12	2048
A/Bor	<8	8	32
A/Bor	<8	8	128

These animals were afebrile when sacrificed 72 hours following administration of virus. Six shallow, subpleural lesions, 2 to 4 cm in diameter were present in the lungs of one. The lungs and trachea of the other showed no gross pathological evidence of infection. Sections of lung and trachea were removed and blended in broth to yield 10% suspensions. Two-tenths ml aliquots of the supernatant fluids were inoculated into 11 day old embryonated eggs at log dilutions of 10^{-1} through 10^{-6}. No virus growth was detected in the eggs after 72 hours. In addition, 20 ml of lung supernate was passaged intratracheally in another 10 week old Suffolk-type lamb, but the recipient did not develop any signs or symptoms of illness, nor did it respond immunologically. Subsequently, this animal did respond with a rise in HI titer when given infective allantoic fluids.

Serologic results. A primary postinoculation antibody response was detected in the sera of all animals, except those sacrificed at 72 hours. Serologic responses of 5 of these animals are shown in Table I. A second inoculation of virus, given 6 to 7 weeks after the first, elicited a considerable booster response (Table I). In this limited series, no relation was detected between dose of virus given and level of homologous antibody titer reached.

In 3 animals tested, third and fourth inoculations of PR8 virus did not significantly increase the maximum homologous HI titers reached after the second inoculation. However, multiple inoculations did broaden the antibody response of these animals as measured with swine strain (Table II).

Heterologous antigenic stimulation was observed in a single lamb inoculated first with PR8 virus and later with A/Swine/Wis strain.

TABLE II. Heterologous Antibody Response of Lambs to Multiple Inoculations of PR8 Influenza Virus.

Viral test antigen	HI titer of sera			
	Pre-inoculation	1st Post inoculation	2nd Post inoculation	3rd Post-inoculation
A/Sw/Wis	<8	<8	<8	64
"	<8	<8	96	64
"	<8	<8	<8	12

The PR8 titer rose from less than 8 to 64 after the first inoculation. After the second inoculation, the A/Swine/Wis titer rose from less than 8 to 512 and, as a result of this heterologous antigenic stimulation, the PR8 titer rose from 64 to 1024.

Summary and conclusions. Of 9 lambs given viable Type A influenza virus *via* the intratracheal route, only the first 2 inoculated suffered clinical illness. Their illness was not respiratory in nature. Infectious virus was not recovered from 4 animals using nasal swab materials or lung and tracheal tissues. All lambs responded immunologically to primary intratracheal inoculation of virus, and second inoculations resulted in significant rises in homologous HI antibody titer. Further inoculations of virus served to broaden the antibody response without increasing the peak homologous antibody titer. The antibody titers reached suggest the feasibility of using lambs for large-volume production of anti-influenza typing sera.

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Post-Heparin Plasma Lipoprotein Lipase Levels in Cirrhosis of the Liver.* (28235)

D. V. DATTA (Introduced by Sheila Sherlock)

Department of Medicine, Royal Free Hospital, University of London, London, England

Hahn(1) observed that injection of heparin into dogs showing alimentary lipemia resulted in the disappearance of plasma turbidity. This was due to release into the circulation of a substance called clearing factor(2) and subsequently shown to be a lipoprotein lipase(3).

The precise role of lipoprotein lipase in the metabolism of triglycerides is not certain although it may permit the clearing of chylomicron triglycerides from the circulation(4). Circulating lipoprotein lipase is removed by the isolated rat liver(5,6) and also by the human liver(7), while high levels of post-heparin lipoprotein lipase activity have been reported in rats and humans with liver damage (8,9,10). It has therefore been inferred that the normal liver removes lipoprotein lipase from the circulation while the diseased liver fails to do so. Most of the results leading to this conclusion have been obtained by measuring lipoprotein lipase activity turbidometrically or under suboptimum conditions.

The present work describes an improved method of estimation of lipoprotein lipase activity measuring initial velocity of the reaction permitting zero order kinetics. Results are given for post-heparin plasma lipoprotein lipase activity in control and cirrhotic subjects.

Materials and methods. The 31 patients comprised 10 with postnecrotic cirrhosis, 10 with primary biliary cirrhosis, 9 with juvenile

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