short-lived cultures from parenchymal fragments of mouse mammary gland released by exposure to collagenase. The present studies employed glucose-potassium chloride-sodium chloride solution as diluent for collagenase rather than Hanks' or other such phosphate-buffered solutions, because the enzyme has been reported unstable in phosphate buffer (11). Present experience indicates that the commercial enzyme in stock solution is stored better at 5°C than frozen, and that best results are obtained when enzyme solutions in GKN are prepared shortly before use. Although prior digestion of lung tissue with trypsin often was advantageous to reduce erythrocyte contamination, well dispersed cell suspensions were prepared with collagenase alone. The purified bacterial enzyme as commercially prepared may include sufficient proteolytic enzyme, in addition to collagenase, to separate cells released from collagen-containing stroma. Use of collagenase(12) to release cells in quantity from tissue parenchyma extensively interlaced with connective tissue can be expected to make available new cell types for diagnosis of viral infection, for wider study of virus-host cell relationships, and for new sources of virus vaccine. Use of the enzyme makes available a convenient system for study of swine influenza virus, in particular, since porcine fetal lung together with a supply of serum can be obtained from local abattoirs.

Summary. Primary monolayer cultures of human, rabbit and porcine fetal and adult lung were prepared conveniently by dispersion of tissue with commercially available collagenase in phosphate-free salt solution.

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Mammalian Cell Cultures for Study of Influenza Virus. II. Virus Propagation.* (24819)

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Cells easily dispersed from continuous cultures or from particular tissue sources such as kidney and amnion are the mainstay of cell culture procedures for detection and assay of viruses and antibodies. For specific study of host-cell relationships, however, it is desirable to work with primary and/or continuous cultures of cell types known to be affected *in vivo*. This paper describes susceptibility to human and swine influenza viruses of primary cultures of cells released from lung parenchyma by treatment with collagenase.

Materials and methods. Cultures of human, rabbit and porcine lung, and whole por-

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cine kidney for comparison, were prepared as previously described (paper 1). *Virus sources* included 14th egg-passage Asian A influenza virus obtained from Dr. Marvin Field, Pfizer Laboratories, Terre Haute, Ind.; the PR-8 strain of influenza A (T. Francis, Jr., passaged in ferrets 34x, mice 593x, eggs 165x), and Lee strain of influenza B, (both by purchase from the American Type Culture Collection, Washington, D.C.) passed 6 times in eggs before use; and swine influenza virus (#1976) kindly supplied by Dr. Morris Schaeffer, Communicable Disease Center, Montgomery, Ala. Virus stocks represented 1:10 dilutions of lyophilized material (stored at −70°C) in Hanks' solution supplemented with 5% horse serum. *Virus assays* with cell cultures were performed by inoculation of 5 culture tubes per 10-fold serial dilution and calculation of cytopathogenic 50% endpoints by the method of Reed and Muench(1). For similar calculations of egg infectivity titers (EID_{50}), allantoic sacs of 10-day embryonated eggs were inoculated with 0.1 ml doses of virus dilutions; eggs were incubated at 35°C until infectivity was apparent or for a period not exceeding 96 hours. Hemagglutinating potency of virus material was determined by standard procedure(2). Propagated influenza viruses were identified by use of hyperimmune ferret serum (kindly supplied by Dr. Morris Schaeffer) in the Salk(3) hemagglutination-inhibition pattern test.

Results. To test susceptibility of primary human, rabbit and porcine lung cultures, confluent monolayers were washed free of nutrient medium with Hanks' solution, and re-covered with 0.9 ml of maintenance solution(4,5) containing 5% inactivated serum (homologous for culture species and free of influenza virus antibody) and penicillin and streptomycin to final concentrations of 200 μg per ml. Prepared tube cultures were inoculated with 0.1 ml of virus of known titer and incubated slanted and stationary at 37°C. For serial virus passage, inoculated culture tubes were frozen and thawed consecutively 3 times by immersion in alcohol-dry ice mixture, and inoculated into new cultures. Inoculated cultures were passed when cytopathogenic damage affected 70-80% of each monolayer, or after 6-7 days of incubation if cell destruction was minor. Human lung cultures unequivocally propagated A and B human influenza viruses and virus hemagglutinins despite cumulative dilution of original inoculum to insignificance (Table I). All 3 human influenza viruses retained egg infectivity after 15 passages in cell cultures. Contrariwise, infectious and hemagglutinating activity of swine influenza virus was lost rapidly with control serial passage. Cytopathogenic effect of the human influenza viruses on the human lung cells was minimal: peripheral rounding of monolayer cells, usually first observed 48-72 hours after virus inoculation, progressed for an additional 48-72 hours, but no further (Fig. 1). Identity of propagated human viruses was confirmed by hemagglutination-inhibition titers with specific antiserum (Table I).

Porcine adult and fetal lung cultures propagated swine influenza virus and hemagglutinin to high titer through 20 serial passages (Table I) despite theoretical extinction of original inoculum. Although only low cytopathogenic titers and no hemagglutinins were recorded for A strains of human influenza virus serially passed in porcine lung cultures, evidence of propagation was substantiated by final harvest of low-titer egg infectious virus. Results with influenza B virus were equivocal: minimal cytopathogenic effect persisted despite high cumulative dilution of original inoculum, but hemagglutinating activity was not found after 15 passages and egg infectious virus was not detectable. In contrast to behavior of viruses in human lung cultures, cytopathogenic effect of swine virus in swine lung cultures was marked after 48 hours incubation at 37°C (Fig. 2), and almost complete destruction was seen after 72-96 hours.

Rabbit lung and porcine kidney cultures were uniformly unresponsive to blind passage of human influenza viruses. Porcine kidney cultures, prepared from mixed cortical and medullary tissue, propagated swine virus hemagglutinin and yielded egg infectious virus after 15 passages, although cytopathogenic effects were not seen.

Discussion. For both epidemiologic and

TABLE 1. Propagation of Influenza Viruses in Monolayer Cultures of Mammalian Lung Dispersed with Collagenase.

Cell culture source	Influenza virus	Passage No.	Total days in culture	Cumulative dilution of original inoculum (log)	Cytopathogenic titer (log $TCID_{50}$)	Supernate hemagglutination titer, 1:	Hemagglutination inhibition titer as reciprocal of initial serum dil.	Egg infect. titer (–log EID_{50})
Human lung	A (Asian)	0					NT	>5
		1	4	2	1.0	40	"	
		5	24	7	3.2	80	"	
		10	54	12	2.8	80	"	
		15	79	17	2.9	80	1280	5.2
	A (PR-8)	0					NT	>6
		1	4	2	3.3	20	"	
		5	24	7	1.4	30	"	
		10	54	12	2.0	40	"	
		15	79	17	2.4	80	512	3.8
	B (Lee)	0					NT	>4
		1	5	2	2.4	80	"	
		5	35	7	1.9	20	"	
		10	65	12	3.0	80	"	
		15	95	17	2.8	160	256	5.6
	Swine virus results were negative.							
Rabbit lung	A (Asian), A (PR-8), B (Lee) and swine virus results were negative.							
Porcine lung	A (Asian)	0					NT	>5
		1	4	2	neg.	10	"	
		5	24	7	1.2	neg.	"	
		10	54	12	2.0	"	"	
		15	79	17	1.8	"	"	1.0
	A (PR-8)	0					NT	>6
		1	4	2	neg.	neg.	"	
		5	24	7	"	"	"	
		10	54	12	1.3	"	"	
		15	79	17	1.0	"	"	1.6
	B (Lee)	0					NT	>4
		1	4	2	2.3	20	"	
		5	24	7	1.5	10	"	
		10	54	12	1.3	10	512	
		15	79	17	1.0	neg.	NT	neg.
	Swine	0					NT	>5
		1	4	4	6.5	160	"	
		5	19	12		640	"	
		10	34	22	7.9	640	"	
		15	49	32		320	"	
		20	65	42	8.0	640	512	8.4
Porcine kidney (cortex & medulla)	A (Asian), A (PR-8) and B (Lee) virus results were negative.							
	Swine	0					NT	>5
		1	4	2	neg.	40	"	
		5	27	9	"	20	"	
		10	51	14	"	20	"	
		15	80	20	"	20	"	2.0

NT signifies not tested.

fundamental studies of host-cell virus relationships, cultures of cells normally the target of *in vivo* infection are desirable. Although poliovirus notably has been studied successfully with cells not susceptible *in vivo*(6), other viruses including influenza viruses have not been so easily propagable in cultures of heterologous relationship(7,8,9). In these studies, human and swine influenza viruses clearly multiplied in and were usefully cytopathogenic for primary cultures of human and porcine lung, respectively. These findings emphasized the value of collagenase treatment for dispersal of tissues held together by

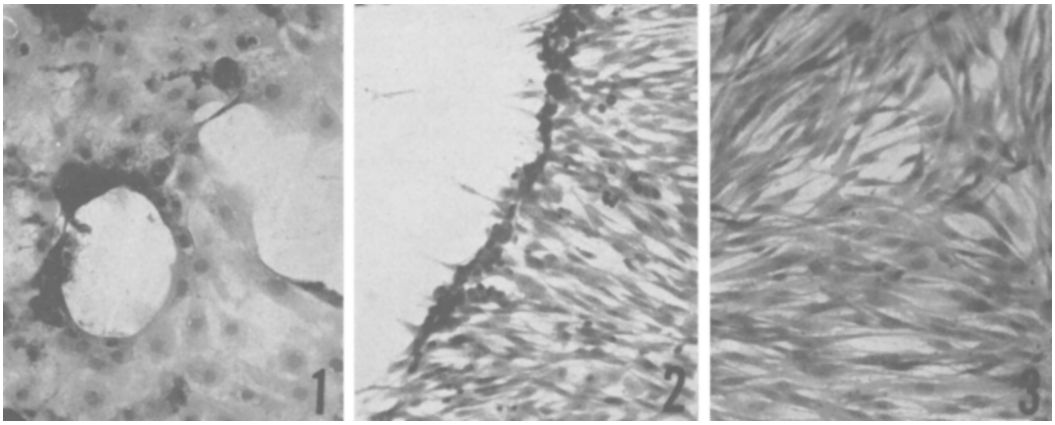

FIG. 1. Porcine lung cells of primary culture of collagenase-dispersed tissue, 48 hr after inoculation of swine influenza virus. ×200.

FIG. 2. Human lung cells of primary culture of collagenase-dispersed tissue, 96 hr after inoculation of human influenza virus. Peripheral cells are typically rounded. ×120.

FIG. 3. Rabbit lung cells of primary culture of collagenase-dispersed tissue, 6 days after inoculation of swine influenza virus. ×120.

fibrous stroma. Interestingly, specific host-cell virus relationships were disclosed. Swine influenza virus, for example, was propagated readily in swine lung cells but not swine kidney or human lung cells. The human Asian, PR-8 and Lee viruses multiplied in human lung cultures, and appeared to multiply also in porcine lung cultures, although cytopathogenic titers did not increase with serial passage and hemagglutinins were lost finally. Although propagation of human viruses in porcine lung cultures was of lesser magnitude (or equivocal in the case of Lee virus), it was sufficient to merit further investigation. If human strains could be adapted to better growth in swine cells without loss of antigenic character, a non-primate cell culture source of virus for human vaccine might be made available. Initial results with swine virus and porcine lung cultures are adequate to indicate that adult or fetal collagenase-dispersed lung should be useful for detection and propagation of this virus. The system appeared susceptible to quantitation because plaque formation was seen in infected monolayers, even without agar overlay. Although cytopathogenic effect of human influenza viruses on human lung cells was much less severe than that of swine virus on swine lung cells, the former as well as the later was neutralized specifically by diagnostic hemagglutination-inhibiting antiserum. The relative susceptibility to influenza viruses of human and porcine lung cultures, and presence of morphologically recognizable epithelial elements, suggest that collagenase-dispersal released parenchymal cells in large numbers. Although the same result might be expected for rabbit lung, it is not apparent whether the complete insusceptibility of collagenase-dispersed rabbit lung cultures to influenza viruses (Fig. 3) should be attributed to species or cell type.

Summary. Collagenase-dispersed human and porcine lung cells in primary culture unequivocally propagated human strains A and B, and swine influenza viruses, respectively. Swine lung cells also propagated human influenza A viruses to some extent. Swine virus infection destroyed, and human virus infection less severely damaged porcine and human lung cells respectively. Rabbit lung cells were insusceptible to human and swine influenza viruses. Swine kidney cells propagated egg-infectious swine influenza virus and hemagglutinin without cytopathogenic effect.

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Metabolism of Acid Mucopolysaccharides of Rabbits with Serum Sickness.* (24820)

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The clinical description of serum sickness by Von Pirquet and Schick(1), aroused considerable interest. More recently, Gregory and Rich(2) demonstrated that serum sickness can be produced readily in rabbits and can serve as a useful experimental model to study phenomena of hypersensitivity. In studies with rabbits, lesions of connective tissue structures are observed, similar to those associated with the group of diffuse diseases of human connective tissue, as lesions associated with polyarteritis nodosa. One factor that seems common to the human diseases, experimental serum sickness, and related hypersensitivity states is the similar pathologic involvement of matrix or ground substance of connective tissue. This similarity is the basis for considering an etiologic relation between hypersensitivity and diseases of connective tissue. Although such relation remains to be established, the inflammatory response of components of connective tissue to disease due to hypersensitivity is provocative. For purposes of studying this inflammatory response, biochemical technics were used to investigate components of the matrix of connective tissue. The effect of serum sickness on acid mucopolysaccharides (MPS) of skin and cartilage of rabbits was observed.

Methods and materials. Six groups of 4 to 6 rabbits, each of approximately 2.6 kg weight, were studied. The rabbits were offered water and laboratory chow freely. Serum sickness was induced in one-half or more of each group by 2 intravenous injections of horse serum (10 ml/kg) given 2 weeks apart (3). From each group 2 rabbits were selected: one demonstrating most clinical evidence of serum sickness, judged by sedimentation rates and changes in weight, was matched approximately by weight with a normal rabbit. Rabbits were sacrificed 6 days after second injection of horse serum, when maximal lesions from serum sickness are expected(3). Examination of gross and histologic sections† confirmed the presence of pathologic lesions of serum sickness. During period of observation after second injection of horse serum to sick animals, the pair selected from each group (one animal with serum sickness and its control) received subcutaneous injections (50 μc) of C^{14} carboxyl-labeled sodium acetate‡ in 0.15 *M* solution 3 times a day. One series of 3 pairs received the isotope for 3 days (450 μc) and another 3 pairs for 5 days (750 μc) before sacrifice. Timing of administration of labeled acetate was chosen for comparison with other observations in normal animals(4). MPS, hyaluronic acid and chondroitin sulfate, were isolated from skins of rabbits by methods previously described(5), and chondroitin sulfate was isolated from cartilage(6) obtained from various sites of body, such as ears, trachea and sternal cartilages. The amount of MPS isolated was determined by weighing or estimated from solutions by uronic acid deter-

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