Table 2. Comparison of human tissue culture and chick embryo adapted GL1134 influenza-A virus with other influenza-A and -B viruses by hemagglutination-inhibition.

Antiserum	Hemagglutination-inhibition titer, expressed as the reciprocal of the initial serum dilution					
	PR8	FM1	FW 1-50	Lee	GL1134 TC	GL1134 E5
PR8	3200				< 50	< 50
FM1		800			< 50	< 50
FW-1-50			800		200	200
Lee				800	< 50	< 50
GL1134 Acute	400	50	50	50	50	50
GL1134 Convalescent	400	200	200	50	400	100

in fresh bovine amniotic fluid increased eightfold after one passage through the amniotic sac of the chick embryo. Therefore the virus grown in human embryonic renal tissue was in the O phase (*4*).

Virus propagated in tissue culture was compared with the same virus that had been through five chick-embryo passages from the original nasal washing. The results of hemagglutination-inhibition with known influenza-A and -B antiserums are shown in Table 2, and it can be seen that this strain (GL1134A-53) is related to FW-1-50 influenza-A virus. Although convalescent serum inhibited hemagglutination by virus grown in tissue culture to a greater degree than that grown in the chick embryo, no assumptions should be made on the basis of this single serum, which was obtained 2 mo after the onset of illness.

Further studies on the growth of influenza viruses in cultures of human tissues, the characteristics of the virus, and the effects of multiplication on human cells are in progress.

Addendum. Since submission of this paper, additional strains of influenza-A and -B viruses have been isolated and propagated directly from nasal washings in both human embryonic lung and kidney tissue cultures.

References and Notes

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1. T. H. Weller *et al. J. Immunol.* **69,** 645 (1952)
2. G. L. Morann and J. L. Melnick, *Proc. Soc. Exptl. Biol. Med.* **84,** 558 (1953).
3. J. F. Enders, *ibid.,* **82,** 100 (1953).
4. F. M. Burnet and D. R. Bull, *Australian J. Exptl. Biol.* **21,** 55 (1943).

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Communications

Lipogenesis by Cells of the Cornea

The capacity for neutral fat formation by nonadipose tissue is of possible relevance to the problems of fatty degeneration, atherosclerosis, and certain lipid histiocytoses. Simms *et al.* (*1–4*) have studied fat formation in chicken fibrocytes and concluded that serum contains lipoid soluble substances (called lipfanogens) that can be converted into fat.

The cornea is especially suitable for the study of such fat formation, since it is a relatively simple structure, is avascular, and contains no stainable fat. In a series of observations (*5*) on the cornea of the living rabbit and the incubated cornea, we have found that oleic acid and sodium oleate induce sudanophilic fat formation in all the cells of the cornea (epithelium, stroma, and endothelium) so long as serum is present. The experiments *in vivo* were made by injecting approximately 0.1 ml of oleic acid suspension or 10 percent sodium oleate solution into the corneal stroma. The experiments *in vitro* were made, for the most part, by incubating corneal buttons at 37°C in 2 ml of rabbit serum to which 2 drops of 10 percent sodium oleate were added. This technique was varied for specific purposes as will be evident in the conclusions.

Sudanophilic globules began to appear in the cytoplasm of the cells in approximately 6 hr and continued to increase in amount for at least 2 wk. This process appeared to be a true lipogenesis and was exclusively intracellular. Immersion of the corneal explants in 1 percent sodium fluoride or 1 percent potassium cyanide, or exposure of the buttons to 60°C for 1 hr prior to the incubation, completely blocked the lipogenesis. No effect was found, however, with similar heating of the serum prior to incubation. Variation of *p*H showed fat formation only in the range of 5.6–8.2 with maximal fat formation in 6.0–7.8. Serum was essential and could not be replaced by glucose-saline or glycerol-saline mediums.

The oleic acid or oleate salt appeared to be a specific and essential substrate: Of the other aliphatic acids tried unsuccessfully were elaidic acid, undecylenic acid, palmitic acid, arachidic acid, pelargonic acid, n-caproic acid, butyric acid, and acetic acid. Stearic acid resulted in a minimal fat formation (possibly attributable to contamination by oleic acid).

The lipogenesis did not result from contact with necrotizing agents other than oleic acid and sodium oleate. It did occur, however, abundantly with hydrolyzed rabbit fat and hydrolyzed olive oil.

Similar results were obtained with rabbit, beef, cat, and human corneas and with rabbit, horse, or human serum.

The fat formation occurred exclusively in viable cells. Those close to the site of oleic acid injection showed pycknotic degeneration but no sudanophilia. Although lipogenesis occurred equally in all the native cells of the cornea, none was found in the invading polymorphonuclear cells and only moderate amounts in the macrophages. Comparative studies on nonocular tissue are now under investigation.

DAVID G. COGAN
TOICHIRO KUWABARA
Howe Laboratory of Ophthalmology,
Harvard Medical School,
Massachusetts Eye and Ear Infirmary, Boston

References

1. H. S. Simms and N. P. Stillman, *Arch. Pathol.* **23**, 316 (1937).
2. H. S. Simms and M. Sanders, *ibid.* **33**, 619 (1942).
3. H. S. Simms, M. S. Parshley, and R. B. Pitt, *J. Gerontol.* **2**, 205 (1947).
4. H. S. Simms, *ibid.* **6**, 159 (1951).
5. Work supported by funds from the American Heart Association and the Massachusetts Heart Association.

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Toxicity of Sarin in Bullfrogs

The Chemical Corps has a continuing and necessary interest in studies concerned with the mechanism of action of "nerve gases," compounds that have a powerful anticholinesterase activity. A major part of the fund of knowledge concerning the fundamental physiology of nervous activity has been obtained through studies on poikilotherms, especially the frog. Consequently, data concerning the effect of anticholinesterase drugs on cold-blooded animals are directly related to Chemical Corps research.

Sarin (isopropyl methyl phosphonofluoridate) is a powerful anticholinesterase drug (*1*). Details of its pharmacology and toxicology in the mouse, rat, guinea pig, rabbit, cat, dog, sheep, goat, and monkey have been published by British investigators (*2*). This communication summarizes the toxicity of this compound for the bullfrog, *Rana catesbeiana.*

Frogs, weighing 400 to 500 g each, were injected with the drug, dissolved in amphibian saline, into the dorsal lymph sac. Controls received similar injections of plain amphibian saline. The frogs were then put into glass aquariums kept at 22°C and observed until death or for 3 days after injection. Table 1 gives the results. If the logarithm of the dose is plotted as a function of percentage dead for that dose, the LD_{50} is seen to be about 6 mg/kg body weight.

Table 1. Illustrating the toxicity of sarin to bullfrogs.

No. of frogs	Dose (mg/frog)	Results
6	0.060	No effect
6	.080	No effect
6	.100	No effect
5	.500	All alive and active 3 days later
17	1.000	1 dead
11	2.000	1 dead
6	4.000	4 dead
6	8.000	4 dead

It has recently been reported that sarin is the most toxic of three known compounds of the nerve-gas type (*3*). The toxic dose for man is estimated to be 0.7 to 7.00 mg (*3*). For a 70-kg man, this value would amount to 10 to 100 μg/kg to kill. Rabbits given 40 μg/kg of sarin intravenously stop breathing in about 10 sec (*2*). The blood pressure of anesthetized cats, given 200 μg/kg of sarin intravenously, is rapidly depressed to about 30 mm Hg. At such pressure the heart continues to beat effectively for several minutes after respiration ceases (*2*).

It is evident that the bullfrog is resistant to relatively large amounts of sarin given by injection. The only signs of poisoning noticed were observed in frogs given doses exceeding 1 mg. Such animals were partially anesthetized. They showed no spontaneous movements and responded sluggishly to tactile stimuli. The righting reflexes were present. After 24 hr these signs disappeared, and the frogs seemed to be normal. No blood or tissue cholinesterase values were estimated.

The explanation of the great resistance to, and surprising recovery from, sarin poisoning in bullfrogs is not certain. In mammals two key effects of such poisoning are paralysis of external respiration and depression of circulation. In frogs such effects merely anesthetize the animals until detoxification takes place. In addition, the functional integrity of frog nerves persists even at remarkably low cholinesterase levels (*4*).

CHARLES G. WILBER
Chemical Corps Medical Laboratories,
Army Chemical Center, Maryland

References

1. B. Holmstedt, *Acta Physiol. Scand.* **25**, suppl., 90 (1951).
2. C. A. DeCandolle *et al.*, *Brit. J. Pharmacol.* **8**, 466 (1953).
3. Anon., *Chem. Eng. News*, **32**, 2006 (1954.)
4. F. Crescitelli, G. B. Koelle, and A. Gilman, *J. Neurophysiol.* **9**, 241 (1946).

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Morphologic Variation and Mode of Growth of Devonian Trepostomatous Bryozoa

Ramose fossil Bryozoa rarely are so situated in enclosing sediments that colonies can be assembled and studied in detail. The Hamilton group of Middle Devonian age in New York State contains colonies of at least three genera. Several have been restored and thin-sections made throughout their length.

The cortical, thick-walled portion of a colony (Fig. 1) contains diaphragms and other structures used in the classification of the order. The stage of development of the cortical region at any one level within the colony can be expected to control structural variations, at least of a quantitative nature. In the axial region of the colony the zooecia are thin-walled and diaphragms and other structures are rare.

Data commonly considered to be of value in dis-