TABLE II. Intraperitoneal Mouse Pathogenicity with Gastric Mucin of Penicillin-Sensitive Strains of *Staphylococcus aureus* before and after Artificial Induction of Resistance to Penicillin.

Strain	Mouse mortality/10		
	Original isolate	Passage control on antibiotic-free medium	After induction of penicillin resistance
25	2	1	0
88	2	4	1
89	4	4	9
90	7	4	0

were subjected to statistical analysis by means of the chi square test.* a chi square of 5.44 and a P value of 0.02 was obtained thereby indicating that the observed differences were definitely significant. Thus based upon the above findings with the series of staphylococci investigated it may be concluded that although no direct relationship is evident between penicillin susceptibility and virulence, penicillin-resistant strains are more apt to be virulent than are those sensitive to the antibiotic.

Mouse pathogenicity of 4 penicillin-sensitive strains before and after induction of resistance as well as the mortality produced after passage on antibiotic-free agar is recorded in Table II.

After serial transfer on graded concentrations of antibiotic, strains #25, 88, 89, and 90 were able to grow on agar plates containing 80, 100, 1,000, and 400 units of penicillin per ml whereas their original isolates as well as their subcultures on antibiotic-free media were inhibited by 0.05, 0.1, 0.5, and 0.05 unit of penicillin per ml respectively. No consistent pattern with respect to change in virulence following induction resistance is discernible. Thus, strains #25 and 88 exhibited a slight decrease, strain #89 a marked increase, and strain #90 a marked decrease in virulence after being made resistant.

Summary. No distinct correlation was established between penicillin susceptibility and virulence of 80 coagulase positive, hemolytic, mannitol fermenting strains of *Staphylococcus aureus*. Both penicillin-resistant and penicillin-sensitive strains vary considerably in their mouse pathogenicity but a significantly higher proportion of the former proved to be virulent as compared to the latter which were fairly evenly divided between virulent and avirulent strains. No definite pattern with respect to change in virulence was observed with 4 originally penicillin-sensitive strains following artificial induction of resistance to the antibiotic.

* We are greatly indebted to Mrs. Constance Percy for the statistical analysis.

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Establishment of Human Adult Tonsil Cells in Continuous Culture and Their Virus Susceptibilities.* (23599)

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This paper describes the development in tissue culture of a continual line of epithelial-like cells from adult human tonsil tissue and compares the susceptibility of these cells to a number of viruses with that of a human carcinoma cell line (strain HeLa). The tonsil line has been subcultured serially over 40 times in the course of a year and has been maintained from the start in a medium free from human serum.

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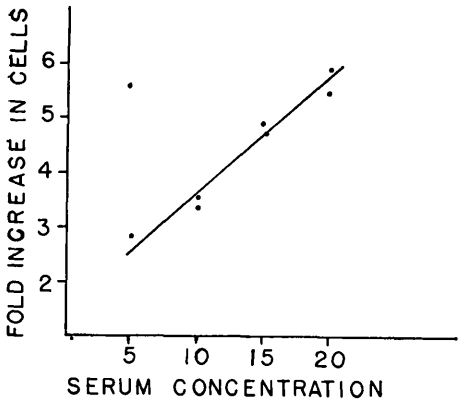
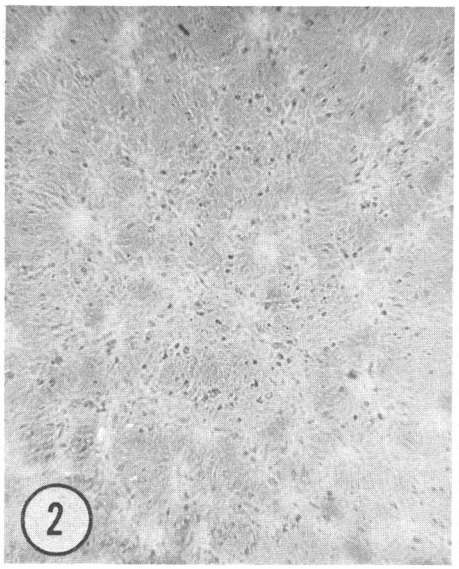


FIG. 1. Effect of varying % of serum concentration on fold increase in tonsil cells after 4 days. Medium 199 was used throughout.

Materials and methods. Tissue culture technics were similar to those generally employed(1). Mixture 199(2) was used for growth of cells with 10% calf serum (Cappel). For maintenance of cells, Scherer's solution(3) with 5% calf serum was satisfactory. In the initial growth of tissue fragments, minced pieces were cultivated using a plasma clot technic. In each ml of media 100 units of penicillin, 100 μg of streptomycin and 50 units of nystatin were routinely incorporated. The viruses employed included adenovirus types 1-7, some obtained through Dr. Robert Huebner, poliomyelitis virus types I-III representing standard prototypes from Dr. Gilbert Chang, mumps virus (Enders' strain), Coxsackie virus strains from Dr. Lois Kitze, hemagglutinating virus of Japan (Sendai strain) from Dr. D. L. Walker, Newcastle disease virus of the Victorian and G.B. types from Dr. R. L. Hanson, influenza strains from the Naval Medical Research Unit at Great Lakes, vaccinia virus from Lederle calf vaccine, and an intranuclear inclusion-producing virus resembling measles isolated in this laboratory from monkey kidney cells. Titrations were made by inoculation of half log dilutions into 4 tubes for each dilution and are expressed as log TC 50/ml of inoculum. The final reading was made on different days depending on the virus tested.

Results. Clinical data: Tonsil designated as T16 was obtained from a 24-year-old female student nurse who had had enlarged tonsils for 15 months and repeated sore throats. Surgery was performed on January 31, 1956 under local anesthesia.

Establishment of cell line: Tubes of tonsil tissue were prepared by a plasma clot technic and some were maintained in the original tubes for 9 months. No cytopathogenic effect was noted and no virus could be demonstrated in the supernatant fluid tested over 11 times from the 11th to 190th day of culture using



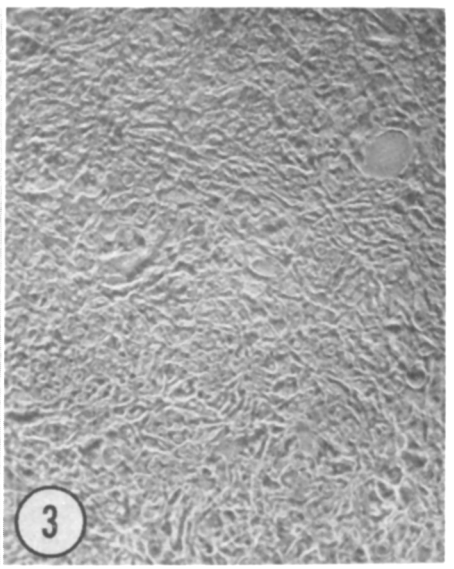


FIG. 2. Unstained preparation of the tonsil line showing cell sheet. × 87.
FIG. 3. Phase microscope appearance of heavy sheet of tonsil cells. × 87.

HeLa, human intestine, human amnion, human tonsil, and monkey kidney cells. No complement fixing activity for adenovirus was found in the supernatant fluid. Repeated histological sections stained with hematoxylin and eosin failed to reveal foamy changes, intranuclear inclusions, or other evidence suggesting a virus infection. Initial growth was both epithelial and fibroblast in appearance. After 2 months cultivation, epithelial-like material from 2 of the original tubes was scraped off, pooled, and implanted in a serum

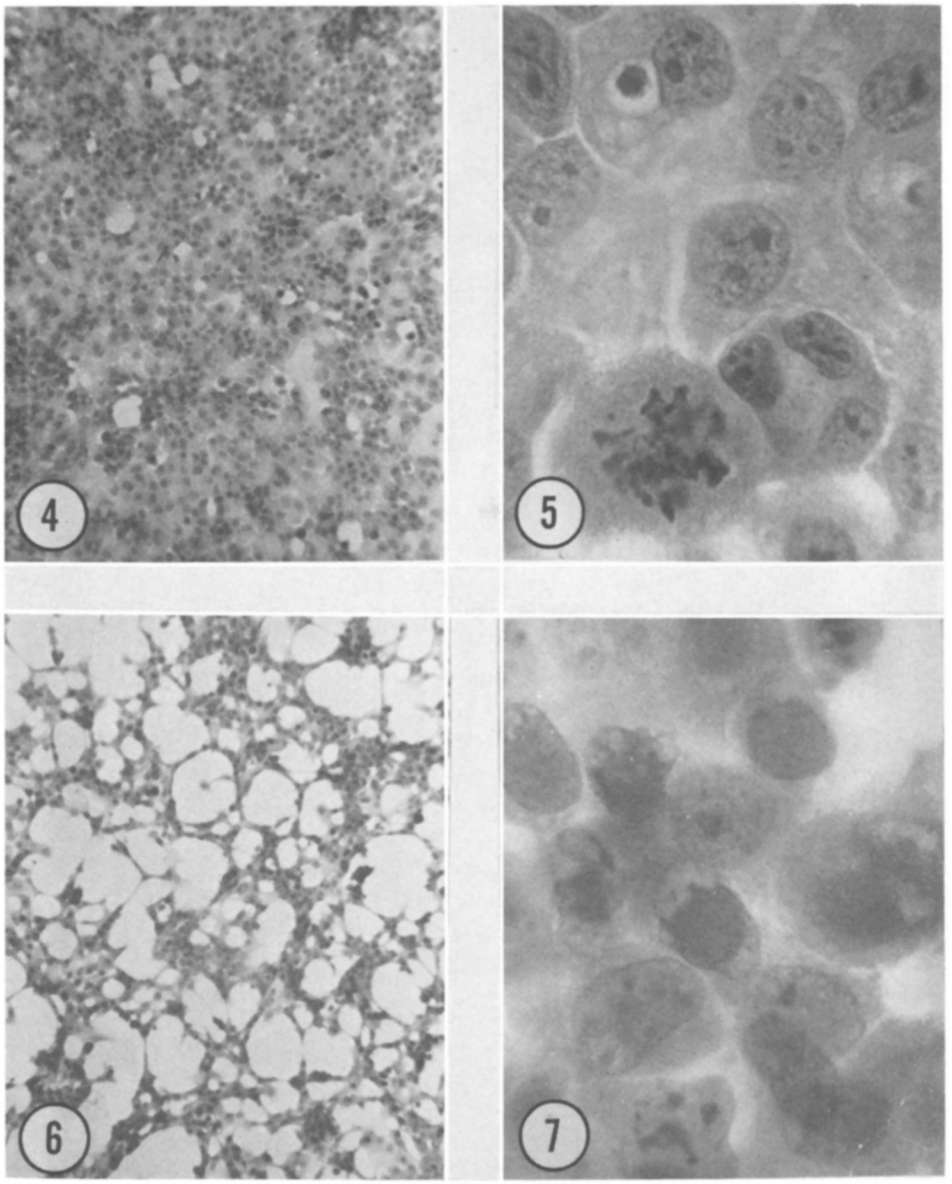


FIG. 4. Collodion preparation of tonsil cells in 19th subculture. Hematoxylin and eosin stain. × 87.

FIG. 5. Same at higher magnification showing mitotic figure, small aggregate of nuclei, and variation in nucleoli. × 667.

FIG. 6. Stained preparation of tonsil cells 8 days after inoculation with adenovirus type 7 showing disruption of cell sheet. Hematoxylin and eosin stain. × 87.

FIG. 7. Same under higher power showing nuclear changes. × 667.

sampling bottle. After 3 such passages 0.5% trypsin was used for 5-10 minutes at room temperature to disperse the cells. Over 40 subcultures have been made over a period of a year each in the complete absence of human serum.

Growth characteristics: Total cell counts on tonsil cells exposed to trypsin were made at different intervals to determine the best growth medium. At a 10% serum concentration Mixture 199(2), the maintenance-tryptose phosphate combination described by Ginsberg(4), and the basal medium of Eagle's for HeLa cells(5) all resulted in about a 4-fold increase in cells over a week's period. Scherer's maintenance medium(3) resulted in no appreciable multiplication under similar circumstances. *Serum concentration* influenced the growth of cells. This is shown in Fig. 1 which records the results of duplicate counts made on cells grown in bottles for a 4 day period. An almost linear increase in cell population was found as serum concentration increased. *Scherer's maintenance* with 5% calf or horse serum maintained cells well for 2 and sometimes 3 weeks with changes at 3-4 day intervals. A maintenance medium of Hanks balanced salt solution (94.5 parts), lactalbumin hydrolystate (0.5 parts) and a calf serum (5 parts) could be used for short term experiments of less than a week's duration. The cells could be stored without degeneration in a 30°C incubator for 2-3 weeks prior to inoculation.

Morphology: In unstained preparations the tonsil line was epithelial-like in appearance and yielded a solid sheet of cells when grown on glass with appearance of a few rounded cells after maintenance of a week or more (Fig. 2-3). They could not be differentiated readily from other cell lines originating from normal human tissues (intestine, liver, kidney, conjunctiva) or malignant human tissues (KB, Hep-2, HeLa) maintained in this laboratory. Stained preparations showed epithelial-like cells and occasionally darker stained and somewhat smaller cells resembling lymphocytes (Fig. 4-5). Alterations in both the stained and unstained cell preparations were seen after exposure to different viruses. The late effects of adenovirus (type 7) are illustrated in Fig. 6-7. Nuclear changes in stained cells were also observed with poliomyelitis virus and with an agent resembling measles which produced a syncytial pattern, multinucleated giant cells, and eosinophilic intranuclear inclusion bodies.

Virus effects: A number of viruses have been qualitatively tested for their capacity to produce cytopathogenic changes in tonsil epithelium (T16) with simultaneous testing in a strain of HeLa cells adapted to growth in horse serum. The results are presented in Table I. The tonsil line is susceptible to a wide range of viruses similar to the HeLa line. In addition, the tonsil line has been successfully employed in the original isolation of adenoviruses, herpes simplex virus, and mumps virus. It has been used as a source of adenovirus antigen for complement fixation tests and in neutralization tests for adenovirus, herpes simplex, and poliomyelitis. Hydrogen ion changes permit color tests with adenovirus.

Virus titrations: Susceptibility of the tonsil cell line was compared to that of the HeLa line using several viruses. The growth, maintenance media, and inocula were the same for both cell lines and the titrations were carried out simultaneously. The results are presented in Table II. High titers in both HeLa and tonsil cells (T16) were found for adenovirus, Coxsackie, and poliomyelitis viruses with cytopathogenic changes that were early in appearance (except for adenovirus 4) and clear cut in effect. No real difference was found between the 2 cell lines.

Discussion. The feasibility of establishing a line of cells in continuous culture from normal adult human tonsil tissue has been demonstrated. The line described here is a vigorous growing, relatively hardy one carried from the start in the absence of human serum. It has a wide range of virus susceptibilities similar qualitatively and quantitatively to the HeLa cell lines. While this line has been free from latent virus infection, it should be recalled that adenovirus and salivary gland virus may appear in some "normal" tonsil or adenoid tissue after prolonged cultivation (6-7) in a combined frequency in a younger

TABLE I. Viral Susceptibilities of Tonsil Cells (T16) and HeLa Cells.

Virus group	Strain or type	Virus source	Single dilution tested*	Cytopathogenicity Tonsil (T16)	Cytopathogenicity HeLa
Adenovirus	1	HeLa culture	10^{-4}	+	+
	2	"	"	+	+
	3	"	"	+	+
	4	"	"	+	+
	5	"	"	+	+
	6	"	"	+	+
	7	"	"	+	+
	Bertha	"	10^{-1}	+	+
Coxsackie	A2 (Dalldorf 2)	Suckling mouse	10^{-3}	0	0
	3 (" 3)	"	"	0	0
	B1 (Conn 5)	"	"	+	+
	2 (Nancy)	"	"	+	+
	2 (Ohio)	"	"	+	N.D.
	E4	"	"	0	0
	High Point	"	"	0	0
	Wiederhold "C"	"	"	0	0
Hemagg. virus of Japan	Sendai	Allantoic fluid	"	0	0
Herpes simplex	H.F.	HeLa culture	"	+	+
Influenza	A_1 (PR^8)	Allantoic fluid	10^{-4}	0	0
	A (Great Lakes)	"	"	0	0
	B (Lee)	"	"	0	0
Measles	Wisconsin	"	Undil.	0	0
Mumps	Enders'	Amniotic fluid	10^{-2}	+	+
Newcastle	Victorian	Allantoic fluid	10^{-4}	+	+
	G.B.	"	"	+	+
Poliomyelitis	I (Mahoney)	HeLa culture	"	+	+
	II (Lansing)	"	"	+	+
	III (Leon)	"	"	+	+
Vaccinia	Lederle	Calf vaccine	"	+	+

* Indicates dilution of original material inoculated into tissue culture tubes. Only one dilution for each virus was tested and 0.1 ml inoculum was used.
N.D. = Not done.

age group approximating 55%. This is of course a great disadvantage in establishing continuous cultures from such cells. There should be an observation and testing period of about 2 months to eliminate this possibility.

Two other similar epithelial lines of tonsil cells have been established in this laboratory. One was deliberately discontinued after several trypsinized subcultures and the other is in the 9th passage. Fibroblast-like lines have also been started with both human tonsil and human lung but subcultured only with difficulty.

TABLE II. Comparative Titrations of Certain Viruses in Tonsil (T16) and HeLa Cells.

	Type	Day of reading	Virus titer* Tonsil	Virus titer* HeLa
Adenovirus	2	6	5.25	5.25
	3	6	4.75	4.75
	4	7	5.75	5.75
Coxsackie	B1 (Conn 5)	3	7.25+	7.25+
	B2 (Nancy)	3	7.25+	7.00+
Polio	I (Mahoney)	6	7.75	7.75
	II (Lansing)	6	9.50+	8.75+
	III (Leon)	6	9.50+	9.50+

* Log TC 50/ml of inoculum.

Summary. 1) A line of epithelial-like cells derived from tonsil tissue surgically removed from a normal adult female has been established in serial culture in the absence of human serum and maintained for over 40 passages over the course of a year. 2) Cytopathogenic effects in this line of tonsil cells were produced by 7 types of adenoviruses, 2 Coxsackie virus strains, 3 poliomyelitis prototype viruses, 2 Newcastle Disease virus strains, herpes simplex virus, mumps virus, vaccinia virus, and after adaptation, an intra-

nuclear inclusion-producing agent resembling measles virus. No clear cut effect was seen with 6 other Coxsackie strains, 3 influenza prototype viruses, or hemagglutinating virus of Japan. 3) Simultaneous titration of several adenovirus, poliomyelitis, and Coxsackie virus strains in tonsil and HeLa cells gave high and comparable titers.

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On Inhibition of Vit. K Activity by Sulfaquinoxaline in Chicks.*† (23600)

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Supplementation of poultry rations with sulfaquinoxaline is known to increase the requirement of chicks for vit. K(1,2). Mushett and Seeler(3) have shown that the action of this sulfonamide is not likely to be related to curtailment of the biosynthesis of vit. K in the intestinal tract, or to histological changes in the liver. A quantitative relationship between sulfaquinoxaline and vit. K has been indicated by preliminary work done at the Ill. Agric. Exp. Station. It is the purpose of the present report to investigate some aspects of this relationship.

Methods. Female crossbred chicks were kept on a purified diet deficient in vit. K to the age of 2 weeks, when the chicks were distributed into groups of 10, and graded levels of sulfaquinoxaline (0.025—0.4%) added to their diet. The basal diet is given in Table I. After 6 days graded levels of menadione sodium bisulfite complex (MSBC) dissolved in water, and containing 33% 2-methyl-1,4-naphthoquinone, were force-fed to the chicks. As the feed, and therefore the intake of sulfaquinoxaline, were influenced by the size of the chick, the doses of MSBC were adjusted to the body weight of each chick and were expressed in μg/100 g of body weight. Twenty-four hours later blood was drawn by heart puncture and plasma prothrombin times determined by a modification of the onestage method of Quick(4), using sodium citrate as an anticoagulant and acetone-dehydrated chick brain as a source of thromboplastin.§ Due to considerable

TABLE I. Composition of Basal Diet.

Ingredient	%
Drackett protein	30
Cerelose	30
Starch	29.36
Salt mixture*	5.34
Solka floc†	3
Refined corn oil	1
Glycine	.50
DL-methionine	.40
Choline-Cl	.20
Total‡	100

* Griminger *et al.*(6).

† Non-nutritive fiber, containing 99.5% cellulose.

‡ Plus the following vitamins (mg/kg diet): Thiamine HCl 25; riboflavin 16; niacin 100; calcium pantothenate 20; pyridoxine HCl 6; folic acid 4; biotin 0.6; cyanocobalamine 0.02; ascorbic acid 200; alpha-tocopherol acetate 20; 10,000 I.U. vit. A and 600 I.U. vit. D_3.

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§ An outline of the method employed will be sent on request.