3 hours of homogenization, while the temperature of an identical sample in a container without cooling coil increased from 0.5°C to 90°C after only 1 hour.

Internal cooling coils did not interfere with effective homogenization, and proved to be more efficient in controlling temperatures than water jackets on the glass containers. Jackets for these containers constructed from large polyethylene bottles and cemented in place with epoxy resin were only moderately successful because of poor heat transfer through the thick glass walls. When the cooling coil is used during homogenization in the presence of abrasive materials (such as small glass beads) there is an etching effect on the lowermost helix, and this section should be protected by a sleeve of plastic tubing. Cooling coils of ⅜-inch tubing were found to be more satisfactory than those of smaller size because of the faster flow rate of cooling water which they permit. We have used both aluminum and copper tubing; stainless steel may be employed where conditions require it.

Summary. Effective cooling during prolonged homogenization at 15,000 r.p.m. in the Waring Blendor was obtained by simple, inexpensive modifications to standard Waring Blendor containers. Homogenates in glass containers were cooled by insertion of coils made of metal tubing through which ice water was pumped; metal semi-micro containers were modified by installation of water jackets for circulation of a coolant.

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Viral Sensitivity of Cell Cultures Maintained with Skim Milk Medium. (25202)

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Development of a serum-free medium containing skim milk, which effectively maintains a number of cell cultures(1), prompted this study. Previous data showed that heat sterilization of milk in preparation for this medium completely destroyed any antibodies that might be present(1). The viral sensitivity of cell cultures in presence of skim milk maintenance medium was compared to sensitivity of the same cell lines on an established medium.

Material and methods. Virus. Virus strains and host cell lines are listed in Table I. *Cell cultures* employed were prepared in the cell culture laboratory, Division of Biologics Standards, Nat. Inst. of Health by Mr. George Gardner. Primary monkey kidney cells(2) were propagated in medium 199(3) containing 2% calf serum* and antibiotics† and maintained in medium 199 containing 20% skim milk at 30°C until inoculated with virus. All other cell lines were held at 36°C. HeLa cells(4) were propagated and maintained in medium 199 containing 10% calf serum. Primary rabbit kidney cells(5) were propagated in lactalbumin hydrolysate medium(6) containing 10% calf serum and maintained in basal medium Eagle (BME) (7) containing 10% calf serum. HEP II cells(8) were propagated and maintained in BME containing 20% calf serum. *Viral titrations* were performed in duplicate in each cell line. Cell cultures of 1 titration were fed medium 199 containing 20% skim milk. Cell cultures of second titration were fed as follows: monkey and rabbit kidney cells—medium 199; HeLa cells—tryptose phosphate broth medium (TPB)(9) consisting of 70% medium 199, 25% tryptose phosphate broth and 5% chicken serum; HEP II cells—BME

* All serum used for cell culture medium was inactivated at 56° for 30 min.

† The following concentration of antibiotics was used: 100 units of penicillin/ml, 100 μg of streptomycin/ml, 100 μg of mycostatin/ml (not used in maintenance medium).

TABLE I. Viral Infectivity Titers (Log_{10}) in Presence of Skim Milk Medium and Established Maintenance Medium.

Virus	Cell line	Mean titers $TCID_{50}$/0.2 ml: Established medium	Skim milk medium
Polio I (Mahoney)	P. monk. kid.*	7.6 (199)	7.6
II (MEF)	*Idem*	7.4 "	7.4
III (Saukett)	"	6.9 "	6.9
Echo 1	"	7.3 "	7.7
10	"	7.3 "	7.3
19	"	7.2 "	7.5
Coxsackie A7	"	6.8 "	6.5
A9	"	7.5 "	7.8
B1	"	5.7 "	6.3
B2	"	5.7 "	5.9
B3	"	6.5 "	6.5
B4	"	5.7 "	6.2
B5	"	6.8 "	7.3
Vaccinia	"	5.4 "	5.4
Influenza A (Asian)	"	4.8 "	4.8
A′ (Denver)	"	3.6 "	3.3
Coxsackie A-11	HeLa	2.5 (TPB)	4.5
Adeno 3	"	7.8 "	6.8
4	"	6.9 "	5.7
7	"	7.3 "	6.3
Herpes simplex	P. rab. kid.†	7.0 (199, 5% c.s.‡)	7.0
Measles	H. Ep. II	2.8 (BME, 5% ch.s.§)	2.8

* Primary monkey kidney.
† Primary rabbit kidney.
‡ c.s. = calf serum.
§ ch.s. = chicken serum.

containing 5% chicken serum. All cell cultures were washed 3 times with balanced salt solution before addition of assay medium. Serial 10 fold dilutions of virus were made in balanced salt solution and 3 to 8 cell culture tubes/dilution were inoculated with 0.2 ml of virus. All titrations were held at 36°C for 7 days except adenovirus and measles which were refed at 7 days and held at 36°C for 14 days. If 50% or more of the cells had undergone cytopathogenic change, the cell culture was considered infected. The ID_{50} end point was calculated by the method of Reed and Muench(10).

Results. Titrations showed no significant decrease in virus titer with use of skim milk medium as compared with the established medium for 19 of 22 viruses tested. Adenovirus 3, 4 and 7 showed a 1 log_{10} lower titer in skim milk than in TPB medium. Coxsackie A-11 titers were 2 log_{10} higher in skim milk than in TPB medium. Table I shows these results.

To determine whether skim milk acts directly to repress adenovirus multiplication or lacks the cell-mediated enhancing factor of tryptose phosphate broth(9), a number of experiments were performed: 1. Different combinations of constituents of TPB medium and skim milk medium were used for maintenance of cell cultures during titrations. 2. Sufficient skim milk to provide a 20% concentration was added to adenovirus titrations incubated 24 hours at 36°C in medium 199. 3. A 1:10 dilution of adenovirus made in 100% skim milk and a 1:10 virus dilution made in TPB medium were incubated at 36°C for 2 hours. Ten-fold dilutions of these incubated samples were made in TPB medium and inoculated into cell cultures maintained in TPB medium.

The following comparisons may be made from results shown in Table II. 1. In confirmation of results obtained by Ginsberg, *et al.*(9), tryptose phosphate broth enhanced the virus titer (col. 1 & 2); and use of TPB medium consistently resulted in higher titer of adenovirus than the other mixtures. 2. Addition of tryptose phosphate broth or chicken serum to skim milk medium elevated the titer somewhat above that obtained with skim milk medium (col. 3, 4 and 5). However, these elevated titers did not equal those recorded with use of TPB medium, indicating that the lower titers with skim milk were not entirely due to lack of tryptose phosphate broth. 3. Skim milk medium containing both tryptose phosphate broth and 5% chicken serum resulted in no higher titers than skim milk with either of the components (col. 4, 5 and 6). This medium may also be regarded as TPB medium containing 20% skim milk in which the virus titer was decreased by addition of milk. In this case, the action may be due to presence of a viral inhibitor in the skim milk or failure of skim milk medium to maintain the cells in optimal condition for virus multiplication. 4. Virus incubated 24 hours in cell cultures with medium 199 before skim milk was added, did not attain the titer reached by virus in TPB medium (col. 1 and 7). 5. No significant difference in titer was noted with virus incubated in 100% skim milk and

TABLE II. Effect of TPB Medium, Skim Milk Medium and Various Combinations of Their Components on Adenovirus Titers (Log_{10}) per 0.2 ml.

		$TCID_{50}$ in presence of								
		1	2	3	4	5	6	7	8	9
Virus	Cell culture	TPB*	199, 5% ch.s.	SM†	SM, 5% ch.s.	SM, 25% tpb‡	SM, 5% ch.s., tpb	199 (sm§) added after 24 hr to make SM	TPB (virus incub. 2 hr in SM before dilution)	TPB (virus incub. 2 hr in TPB before dilution)
Adeno 3	HeLa	7.8		6.3						
		8.2		7.2						
		7.5		7.0						
Adeno 4		7.0		5.8	6.5	6.3	6.3			
		7.3		6.5	"	6.8	6.8			
		6.8		5.6				5.9	7.0	7.0
	H.Ep. II	"	6.3	5.3	5.3	6.0	6.0	5.3	6.5	6.5
		"	6.0	5.5	6.0	6.3		6.0	7.0	6.8
Adeno 7	HeLa	7.3	5.8							
		"	6.5							
		"	"							

* TPB = Tryptose phosphate broth medium. † SM = Skim milk medium - 199, 20% skim milk. ‡ tpb = Tryptose phosphate broth.
§ sm = 100% skim milk.

diluted in TPB medium from that which was incubated and diluted in TPB medium (col. 8 and 9). These last 2 results indicate no evidence for the usual viral inhibitors(11,12).

Discussion. These comparative studies showed that skim milk maintenance medium afforded full sensitivity to the tested strains of polio, echo, coxsackie, influenza, herpes, measles and vaccinia viruses. Parallel titrations of coxsackie A-11 employing skim milk medium and TPB medium with 3 different lots of chicken serum demonstrated higher titers in presence of skim milk. Titer differences noted for adenovirus were not great enough to permit conclusions from 1 experiment; however, consistent differences in 14 experiments became meaningful. Results of further experiments with adenovirus and numerous maintenance media (Table II) revealed that the inhibitory action of the skim milk was probably an effect on tissue culture cells rather than antiviral. Similar cell-mediated effects have been observed by others (9 & 13).

Summary. (1) Comparative titrations of 22 virus strains were performed to determine sensitivity of cell cultures maintained with skim milk medium. Infectivity titers of tested strains of polio, echo, coxsackie, influenza, herpes, measles and vaccinia viruses in presence of skim milk medium were equal to or greater than those employing the established maintenance medium. Lower titers obtained with adenovirus in skim milk medium were attributed to a cell-mediated effect. (2) The results indicate that skim milk medium is free of inhibitor for infectivity and permits full sensitivity to infection by most of the viruses tested.

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Persistence of Antibodies to ECHO-16 Viruses Following Boston Exanthem Disease.* (25203)

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Of the diseases now considered to be of ECHO virus etiology, Boston Exanthem disease(1,2) was the first to be recognized as a clinical and epidemiologic entity and associated with its causative agent. Immunological relationship of Boston Exanthem viruses to ECHO-16 virus has since been demonstrated, although their antigenic patterns are not identical(3,4). Reports on the ECHO viruses have dealt with clinical associations, epidemiological studies, or antigenic relationships. Information on the immunological status of man several years after infection with ECHO viruses has not been available. Serum samples were obtained† from patients of the 1951 Boston Exanthem disease epidemic, and also from cases of the Pittsburgh outbreak of 1954. This report provides results of studies on complement fixing and neutralizing antibody levels 3 to 6 years after original infection.

Materials and methods. Neutralizing Antibody (NA) Tests. Explant type roller cultures of embryonic human skin-muscle tissue‡, grown with medium containing bovine embryonic fluids as described elsewhere(4), were used. Prior to inoculation the nutrient was changed to 90% bovine embryonic fluids and the Hanks component omitted. Seven days after inoculation one additional fluid change of the same medium was made. Pools of virus in the 4th to 7th *in vitro* passage were used—2 of the *Floyd* strain which originated from a Boston patient, and 2 of the Pittsburgh *McGrew* strain. Serum samples were stored at −15°C, thawed on the day of use, and inactivated at 56°C for 30 minutes. Except as noted in Table I, all serum specimens from a patient were tested simultaneously. Two- or 4-fold serum dilutions were mixed with virus, held at 5°C for 1½ hours, and 3 cultures each were then inoculated with 0.1 ml of the mixture. In each test 3 or more control cultures were inoculated with an equivalent virus dose. Serum NA titers were determined 3 days after the virus controls showed unequivocal and generalized cellular degeneration. Titration of the virus inoculum was performed concurrently with each test. NA titers are expressed as that dilution of serum, before addition of virus, which effected neutralization of 2 or more of the 3 cultures inoculated. If all 3 tubes at one dilution were neutralized and 2 or all 3 tubes of the next highest dilution failed to be neutralized, an arbitrary intermediate dilution was taken as the titer. *Complement-fixation (CF) tests.* Methods of antigen preparation and the micro-method CF test procedure are reported elsewhere(5). The antigens were concentrated from infected culture fluids and heated at 56°C before use. Three antigens, from the same strain (McGrew) of exanthem virus, were used; a few

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