endotoxin was reduced from 100% to 40-60% by antibiotic therapy given prior to or at the time of injecting the endotoxin. This was true even if the antibiotic was given orally and was completely non-absorbable. Therefore, endotoxins from intraintestinal bacteria are involved in the death from an intravenous MLD/100 of endotoxin. Data are given to show that antibiotics act solely as antibacterial agents, and not as anti-endotoxic agents. 2. A sublethal intravenous dose of endotoxin promptly induced in rabbits an increased sensitivity to a supplementary fractional dose of endotoxin so that a second dose given one hour later increased the mortality from zero to 67%, even though the total of both doses, if given as a single initial dose, was not lethal. Recovery of nearly normal resistance to the second dose of endotoxin required about 4 hours. Antibiotics given with the initial dose rendered the second dose harmless; but the same antibiotics given with the second dose did not prevent death. Therefore, it is inferred that death from the second dose involved the production of additional endotoxin. 3. Reasons are given for inferring that similar phenomena operate in hemorrhagic shock and account for the development of irreversibility to transfusion.

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Serial Propagation of Adenoviruses (APC) in Monkey Kidney Tissue Cultures. (22577)

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Production of adenovirus (APC) vaccines suitable for general usage, is dependent on the serial propagation of virus in cultures of acceptable non-malignant tissue. Since monkey kidney tissue cultures demonstrated cytopathogenic effects and supported limited growth of adenoviruses(1), it seemed possible that with further adaptation, these agents might be induced to grow satisfactorily in such cultures. This report describes the procedures used in establishing monkey kidney passage lines of 7 serotypes (1 through 7) of

adenoviruses. None of the strains was grown in HeLa cell or other cancer cell cultures at any point in its passage history.

Methods. Rhesus monkey kidney tissue cultures* prepared by the Youngner procedure(2) in stationary tubes and 32 oz. bottles were grown in 2.0% calf serum, 0.5% lactalbumin hydrolysate, and 97.5% Hanks' solution(3), and maintained in 1.0 and 40.0 ml respectively of Mixture 199(4). Bottle cultures were washed 3 times with 20.0 ml of Hanks'-Simms' solution prior to inoculation. Nutrient fluids were changed routinely twice a week for tube cultures and once a week for bottle cultures. Occasionally, only one-half of the bottle culture fluid was changed, and in advanced passages bottles were maintained without fluid change (usually 7 to 10 days).

Monkey kidney passage lines of types 1, 2, 5, and 6 were initiated with material from early passages in human embryo tissue of fluids from spontaneously degenerating adenoid or tonsil tissue cultures(5). Strains of types 3 and 7 were obtained by inoculating a number of virus-containing throat washings into monkey kidney cultures. Virus strains recovered from several such specimens were carried for one or two additional passages and the most rapidly cytopathogenic strain of each type selected for further passage. Attempts were made to initiate passage lines of type 4 by direct isolation in monkey kidney culture, but of 8 known-positive nasal washings tested, only one produced cytopathogenic changes, and this strain could not be passaged further. Therefore, this strain was reisolated in human embryo trachea tissue culture and then passaged to monkey kidney cultures. Initiation of passage lines and routine serial passages were carried out by the inoculation of 0.1 ml of sample into monkey kidney tubes containing 0.9 ml of Mixture 199; supernatant fluids harvested at the time of maximal tissue involvement were usually used for passages. If cytopathogenic effects were delayed in passage attempts, cultures were reinfected at the time of the first fluid change with pools of tissue scrapings and several fluid harvests of the preceding passage, or passages were repeated with 0.2 to 0.4 ml inoculum per tube.

Attempts at establishing the viruses in bottle cultures were made by inoculating 0.5 to 2.5 ml of supernatant fluids from infected tube cultures; the bottles were frequently reinfected with similar amounts of fluid if cytopathogenic effects were delayed or progressed slowly. The final fluid harvests and frequently intermediate fluids from bottle cultures were titrated as antigen in the complement-fixation test using a standard pool of human convalescent serums(1). Since in early passages replicate bottles occasionally varied as much as 16-fold in antigen yield, the fluids showing the highest CF antigen titers were selected for passage. Fluids of early bottle passages were centrifuged for 4 to 4½ hours at 30,000 rpm in a Spinco Model L centrifuge; the pellets were resuspended in the lower one-fifth of the supernates and 3.5 to 5.0 ml quantities used as inocula for subsequent passage. Serial passages were made with ultracentrifugal concentrates until harvests with high virus and CF antigen titers were regularly obtained.

Results. Serial passages of all 7 serotypes were established initially in tube cultures by selection of strains giving the shortest incubation periods and by the use of large inocula and reinfection of cultures. In the first kidney passage, cytopathogenic changes developed in 1 to 5 days, but in subsequent passages the incubation periods were usually markedly prolonged, and the virus and CF antigen yields were very poor. One or 2 bottle passages using concentrated inocula resulted in adaptation of types 1, 2, 5 and 6 to give satisfactory virus and CF antigen titers in further passages. On the other hand, 3 to 5 such passages of types 3, 4 and 7 were necessary before satisfactory harvests were obtained, and occasional subsequent passages with concentrated virus were necessary to maintain yields of virus having high titers (Table I). All 7 serotypes were carried through 10 or more serial passages in monkey kidney cultures. Infectivity titers for HeLa cells(1) at 4th to 9th passage levels of types 1, 2, 3, 5, 6, and 7 were $10^{4.5}$ to 10^{6} TC50

* Cultures were obtained from Microbiological Associates, Bethesda, Md.

TABLE I. Representative Serial Passage Lines of Adenoviruses in Monkey Kidney Tissue Cultures.

Type	Passage	Type of culture	Vol of inoculum per culture, ml	Days to 1st cytopathogenic effect	CF titer of harvest (highest level)
3	MK* 1	Tube	.1‡	5	
	2	"	.1	6	
	3	"	.1	2	
	4	Bottle	1.0	4	1:1
	5	"	5.0 5× concentrate	5	1:1
	6	"	3.5 "	3	1:16
	7	"	1.0	5	1:16
	8	"	2.0	3	1:4
	9	"	3.5 "	<6	1:16
	10	"	2.0	2	1:16
4	HE Tr† 1	Tube	.1§	6	
	MK 1	"	.1	1	
	2	Bottle	2.0	10	1:4
	3	"	5.0	5	1:1
	4	"	4.0 5× concentrate	5	1:4
	5	"	4.0 "	4	1:4
	6	"	4.0 "	2	1:8
	7	"	4.0 "	1	1:32
	8	"	1.0	2	1:32
	9	Tube	.01	1	
	10	"	.1	1	

*Monkey kidney. † Human embryo trachea. ‡ Throat wash J.F. § Nasal wash R.N.

per 0.1 ml, representing virus activity at theoretical cumulative dilutions of the original inocula of 10^{-15} to 10^{-19}. After 8 passages, type 4 titrated $10^{3.5}$ or higher, when the cumulative dilution of inoculum was 10^{-23}. After 6 to 10 passages, all types produced CF antigen titers of 1:16 or greater in 2 or more bottle harvests. The identities of the passage viruses were confirmed in neutralization tests performed in monkey kidney cells; all strains were completely neutralized by 8 units of homologous rabbit antiserum. Since the rabbit antiserums had been prepared with viruses grown in HeLa cells or human embryo tissue and with no history of passage in monkey kidney cells, these tests also indicated that the passage lines were not contaminated with monkey renal viruses(6). Formalinized and heat-inactivated vaccines prepared with the monkey kidney passage line of type 3 stimulated neutralizing antibody responses and prevented an experimentally induced illness in volunteers(7). Similarly, a formalinized polyvalent vaccine containing the monkey kidney passage strains of types 3, 4, and 7 induced neutralizing antibody responses to all 3 serotypes in rabbits and humans, and reduced febrile respiratory disease rates in Navy recruits(8).

Summary. Strains of adenovirus types 1 through 7 were carried through at least 10 serial passages in monkey kidney tissue cultures, yielding harvests of virus with titers of $>10^{3.5}$ to 10^{6} per 0.1 ml, and frequently CF antigen titers of 1:16 or higher.

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