

jection, and repeated by intramuscular injection. The LD₅₀ of mercuric cyanide in dogs without treatment is 2.71 ± 0.17 mg per kg; and that with nitrite-thiosulfate therapy and BAL injections, 8.66 ± 0.66 mg per kg.

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Trachoma Viruses Isolated in United States.*† VII. Relative Toxicity of Different Strains for White Mice. (29251)

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Since 1959 several strains of virus have been isolated in this laboratory from cases of trachoma and inclusion conjunctivitis(1). These strains were adapted to growth in the yolk sac of embryonated hens' eggs by serial passage. When high, constant egg-lethal titers of between 10^{-6} and 10^{-7} ELD 50/g of yolk sac were reached, usually after 4-5 passages, 50% yolk-sac pools were stored in stoppered glass vials at -50°C in a mechanical freezer. It was of interest to determine if these suspensions were lethal for mice following intravenous inoculation, to compare toxicity with egg lethality and with the total numbers of virus particles present, and to determine if strains with different biological properties differed in relative toxicity(2,3,4). The results of such studies are presented here.

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Materials and methods. Viruses.§ Isolates from the United States were employed at the following yolk-sac passage levels: Trachoma isolates BOUR YS 14 and 16, ASGH YS 14, AP 2 YS 10 and inclusion conjunctivitis isolates IC Cal 3 YS 10 and 13, IC Cal 4 YS 11, IC Cal 5 YS 12.

In addition, one of the strains originally isolated in Peking(6), and passed for many years in mouse brain and in eggs, was supplied to us by Dr. J. Snyder. It was TE55 and used as our YS6. Stock virus suspensions were made from pools of infected yolk sacs harvested from live eggs and stored at -50°C for 1-9 months as 50% suspensions in broth-saline. They were titrated for egg lethality (ELD 50/g of yolk sac) as previously described(2). Bacteriologically sterile pools of high egg-lethal titer were rapidly thawed, serial 2-fold dilutions made in chilled broth saline, and 0.5 ml amounts inoculated intravenously into weanling (3 week old, 12-15 g) Swiss white mice obtained from a commercial,

§ The nomenclature of these isolates by the proposed system(5) is as follows: BOUR = TRIC/ /USA-Cal/Cal-1/OT; ASGH = TRIC/ /USA-Cal/Cal-2/OT; AP 2 = TRIC/ /USA-Cal/Cal-4/OT; IC Cal 3 = TRIC/ /USA-Cal/Cal-9/ON; IC Cal 4 = TRIC/ /USA-Cal/Cal-10/ON; IC Cal 5 = TRIC/ /USA-Cal/Cal-11/ON.

TABLE I. Mouse Toxicity (MLD 50/g of Yolk Sac), Egg Lethality (ELD 50/g of Yolk Sac) and Total Particles/g of Yolk Sac of TRIC Virus Suspensions.

Suspension, strain and yolk sac passage No.	MLD 50/g	ELD 50/g	Total particles/g	ELD 50 per MLD 50	Particles per MLD 50
BOUR YS 14	24	2.0×10^7	6.4×10^9	8.4×10^5	2.7×10^8
"	28	"	7.4×10^9	7.2×10^5	2.6×10^8
BOUR YS 16 (examined twice)	32	3.6×10^7	5.0×10^9	1.1×10^6	1.6×10^8
ASGH YS 14	28	5.0×10^7	1.0×10^{10}	1.8×10^6	3.5×10^8
AP 2 YS 10	12	1.0×10^7	5.4×10^9	8.4×10^5	4.5×10^8
IC/Cal 3 YS 10	48	9.0×10^6	4.0×10^9	1.9×10^5	8.4×10^7
IC/Cal 3 YS 13	16	2.5×10^7	5.2×10^9	1.6×10^6	3.5×10^8
IC/Cal 4 YS 11	24	8.0×10^6	5.5×10^9	3.3×10^5	2.3×10^7
IC/Cal 5 YS 12	"	9.0×10^5	1.2×10^9	3.7×10^5	6.0×10^7
TE 55 YS 6	20	3.4×10^8	1.8×10^9	1.7×10^6	9.0×10^7
"	12	1.0×10^8	5.0×10^9	8.4×10^5	4.2×10^8
"	24	2.0×10^8	5.2×10^9	"	2.2×10^8

heterozygous stock. Six or 8 mice were inoculated with each virus dilution. Mouse deaths which occurred within one hour were regarded as due to trauma, those within the next 48 hours as specific. Gross examination of the latter revealed hemorrhagic lesions of the small intestine, congestion of the lungs, and, in addition, hemorrhagic lesions at the bases of the claws. Fifty percent end points were estimated and expressed as the mouse-lethal dose per g of yolk sac (MLD 50/g).

The elementary bodies in smears made from the suspensions after treatment with trypsin, centrifugation, and resuspension were stained with Giemsa and dark-field illumination(7).

Results. All the strains tested were toxic for mice, but only at low dilutions. Injection of 0.5 ml of a 1:32 dilution was never lethal. Uninfected yolk-sac suspensions were never lethal for mice, even when tested as 50% broth-saline suspensions. The strains showed little difference in amount of toxin present, from 12-48 MLD 50/g of yolk sac, and required between 3.3×10^5 and 8.4×10^6 ELD 50 per MLD 50, as shown in Table I. Particle counts of suspensions revealed similar numbers of elementary bodies, usually about 6.0×10^9 /g of yolk sac; thus, the amount of mouse toxin per particle was constant for all the strains, one MLD 50 containing about 2.2×10^8 particles. Strain TE 55 at its present passage levels grows to higher titers in embryonated eggs than recent U.S. isolates, up to a titer of $10^{-8.2}$ ELD 50/g of yolk sac. This was about 10-fold higher than the other strains tested, but this

strain was no more toxic for mice than the others tested. Although the number of egg-lethal doses per mouse-lethal dose was greater with strain TE 55, the total number of particles per mouse-lethal dose was the same as for all the other strains.

Discussion. Most viruses of the TRIC-PLT group cultured in the yolk sac of embryonated hens' eggs and grown to high titers are capable of killing mice when injected intravenously, and both Murray *et al*(8) and Chang *et al*(9) have demonstrated the toxicity of several strains of trachoma and inclusion conjunctivitis viruses for mice. The strains tested here were no exception since all were toxic for mice, albeit at comparatively high concentrations. Wang and Grayston investigated the minimal toxic dose for mice (TD 100) with egg infectivity of the same suspensions for several strains of trachoma and inclusion conjunctivitis(10). They concluded that about 10^6 EID 50 were required to kill mice, but they did not compare the toxicity of different strains. In the present study, using stored pools, without any special preparation to obtain highly toxic material, the mouse-lethal dose was found to contain about 10^6 ELD 50, which is similar to the findings of Wang and Grayston.

The strains tested differ in certain biological properties; thus, strain BOUR induces a markedly more severe infection when instilled into the eyes of primates than does strain ASGH(3), and strain TE 55 is apparently unable to induce any disease in primates(11). Conversely, TE 55 is the most virulent TRIC strain when grown in embryonated eggs(4),

killing chick embryos more rapidly and reaching higher titers than any of the other strains tested; and strain ASGH consistently kills chick embryos one day earlier than strain BOUR for all doses inoculated(2). Reeve and Taverne(4) suggested that the greater virulence of some TRIC strains for the chick embryo could be due to the greater toxicity of these strains: The results presented here suggest that all strains were equally lethal for mice, and thus toxicity tested in mice cannot explain these differences in virulence. Since all strains, including TE 55, appeared equally toxic, the ability to produce disease in primates may not depend on this factor; with strains pathogenic for the conjunctiva toxicity appears unrelated to the severity of disease induced in experimentally inoculated primates.

Summary. Yolk-sac suspensions of 3 strains of trachoma and 3 strains of inclusion conjunctivitis virus isolated and grown in this laboratory, and the TE 55 strain of trachoma, were tested for toxicity for mice following intravenous injection. All suspensions were equally toxic for mice, requiring between 3.3×10^5 and 8.4×10^6 ELD 50 per MLD 50, and all contained similar numbers of virus particles, an average of 6×10^9 /g of yolk sac. The egg-lethal titers of TE 55 virus

suspensions were 10-fold higher than those of the other strains tested, but this strain was no more toxic for mice and possessed the same total number of particles per g of yolk sac. Biological differences of the strains, such as virulence for chick embryos and pathogenicity for the conjunctiva, do not appear to depend on the relative mouse toxicity of the strains.

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Distribution of Cesium¹³⁷ After Chronic Exposure in Dogs and Mice.* (29252)

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The distribution of cesium in mammalian tissues resembles that of potassium, in that the higher concentrations are found in muscle and other soft tissues(1-3). Reports from Japan(4) and from the United States(5) indicate that the concentration of fallout Cs¹³⁷ is at least as high in bone as in muscle. Exposure to fallout products usually is a chronic exposure to a varying daily dose, and equi-

librium conditions (output equals intake) can only be temporary or fortuitous. Body burdens vary with time and intake(6,7) and can be distributed among tissues in different proportions from those that follow acute exposure. The distribution of Cs¹³⁷ in 3 dogs and 6 mice, after more than 400 days of continuous exposure to a constant daily dose, is reported here.

Methods. The three 7-year-old male beagles weighed between 14.4 and 15.1 kg each at the beginning of exposure. Each dog was

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