act mechanism by which endotoxin induces radio-protection is unknown and this is being investigated.

Summary. When conventional and germ-free mice are administered 10 μg of *E. coli* endotoxin intraperitoneally 24 hours prior to whole-body X-irradiation, the $LD_{50(30)}$ X-ray dose is raised to about the same degree for both groups: from 739 r to 829 r for conventional mice and 834 r to 942 r for germ-free mice. For both groups the X-ray dose-reduction factors lie in the narrow range of 1.13 to 1.15.

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Plaque Studies with Certain Group B Arboviruses. III. St. Louis Virus on Chick Embryo Tissue Culture.* (30047)

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Although several group B arboviruses have produced plaques or shown distinct cytopathic effects (CPE) in chick embryo tissue culture (CETC), most reports indicate failure with St. Louis encephalitis (SLE) virus (1,2,3). In studies reported here several strains of SLE virus produced clear cut CPE in CETC monolayers and some strains produced excellent plaques under agar. Using 2 very similar strains recently isolated in Florida, 2 distinct plaque variants were noted in each, one much larger than the other, each producing what appear to be genotypic progeny.

Materials and methods. Virus strains. P-15 from *Culex* mosquitoes of Pinellas County and the first SLE mosquito isolate from Florida(4), and TBH-28, a subsequent human brain isolate(5), were tested in early mouse brain passage. Each had a titer of $10^{8.5}$ TCD_{50} per 0.1 ml in hamster kidney

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tissue culture (HKTC). The Webster strain (6) and the Hubbard(7), each after a number of mouse brain passages in this laboratory, had CPE titers of $10^{7.0}$ per 0.1 ml and $10^{8.0}$, respectively in HKTC.

Monolayer tissue culture. Primary tissue culture methods for both HKTC and for CETC were similar to those described previously(8). Tubes and 3 ounce prescription bottles were seeded and incubated at 37°C until monolayers were obtained.

The outgrowth medium consisted of 4% calf serum, 1% vitamin stock (Microbiological Associates, 100× concentration), 0.02 g% $NaHCO_3$ (0.5% of a 4% stock solution), 0.0292 g% L-glutamine (1% of a 2.92 g% stock) and 92.5% of a 0.5% solution of lactalbumin hydrolysate in Hanks' balanced salt solution (HBSS) and 1% antibiotic mixture consisting of 100 units of penicillin, 40 μg of streptomycin, 10 units of polymyxin and 25 units of nystatin per ml.

Maintenance medium was the same as outgrowth medium but contained 2% calf serum and 0.1 g% $NaHCO_3$.

Agar overlay medium. The agar overlay medium was made by mixing equal parts (50 ml) of melted 3.0% agar (Difco Bacto-Agar) in sterile distilled water and the following: 2% lactalbumin hydrolysate in HBSS (4× concentrated, without either phenol red or $NaHCO_3$) 25 ml; calf serum 4 ml; antibiotic mixture 1 ml; $NaHCO_3$ (4%), 2.5%; Seitz filtered neutral red, (1:1000), 5.0 ml; sterile distilled water, 12.5 ml. The overlay medium is held at 43°C. The $NaHCO_3$ and neutral red are added to the agar overlay medium just prior to overlaying the tissue. The effect of varying the concentration of neutral red was investigated, from .003 g% to 0.006 g%, and it was observed that 0.005 g% (5.0 ml of a 1:1000 dilution) gave the most satisfactory results, and was selected for these studies.

Plaque assay. After a complete cell monolayer was obtained in bottles, maintenance medium was removed and the cells inoculated with 0.1 ml of appropriate dilutions of virus. The bottles were then incubated at 37°C for 2 hours for adsorption with occasional rocking to improve distribution of the virus and prevent drying of the cells. To each of the bottles of inoculated cells, 8 ml of the agar overlay medium were added. The bottles were inverted after the agar solidified, and incubated at 37°C for 4 to 6 days.

Results and discussion. Comparative tube titrations of SLE virus strains on HKTC and on CETC. As a base line for plaque titers of various strains of virus all pools were titrated in both CETC and HKTC; CPE if observed continued until all cells were destroyed. With virus dosages of 100 to 1000 TCD_{50}, CPE in CETC was noted 1 to 2 days after inoculation. This was manifested by granulation, contraction and rounding of cells with eventual sloughing from the glass. Results of comparative titrations in the 2 cell types are shown in Table I. All strains titered higher in HKTC than in CETC by 2.0 to 5.5 logs. Final reading of CPE was on the fourth day post inoculation with CETC and on the eighth day with HKTC. CETC could not be observed longer because of loosening of the cell sheet in the control tubes. The much lower titers of CPE observed in CETC might well have been due to this time limitation.

Comparative plaque titers and plaque characteristics of several strains. All 4 strains were titrated in 10-fold dilutions in bottles of CETC with agar overlay. Results are presented in Table II. Titers ranged from 7.1 ×

TABLE I. Results of Comparative CPE Titration* of St. Louis Viruses on HKTC and CETC.

Virus strains	HKTC†	CETC‡
P-15	8.5	5.5
P-15 L§	6.5	4.5
TBH-28	8.5	—
Hubbard	8.0	2.5
Webster	7.0	3.5

* All titers are shown as log 10 TCD_{50}. Titration based on inoculation of 2 tubes for each 10-fold dilution.

† HKTC = Hamster kidney tissue culture; final reading 8 days after virus inoculation at 37°C.

‡ CETC = Chick embryo tissue culture; final reading 4 days after virus inoculation at 37°C.

§ P-15 L = Large plaque of P-15 after 3 plaque purification passages.

TABLE II. Plaque Forming Units of St. Louis Viruses in CETC Monolayers.

Virus strain			
P-15	TBH-28	Hubbard	Webster
9.3×10^7	3.2×10^8	1.6×10^8	7.1×10^7

10^7 to 3.2×10^8 PFU per 0.1 ml, similar to those obtained by CPE titration in HKTC, and far higher than those by CPE in CETC. This latter discrepancy again might be due to time limitations for retaining an attached monolayer for those cells not held in place by agar. With the Webster strain plaques were indistinct and counting was difficult. They were slightly better with the Hubbard strain, but varied in size from 0.1 to 0.4 cm. With the two Florida strains, P-15 and TBH-28, two distinct sizes of clear plaques appeared, the small from 0.15 to 0.25 cm and the large from about 0.5 to 0.6 cm. Since results of subsequent plaque work with both strains of virus were essentially identical only those with the P-15 virus are presented.

Cloning of P-15 large and small plaques. Three serial plaque selections were made of the large (L) and of the small (S) plaques of the P-15 strain. In the first passage most but not all of the plaques were of the same size as that of the original plaque. By the third passage the purification appeared quite, but not absolutely, complete. Fig. 1 illustrates the two sizes of plaques. Subsequently 2 more serial plaque pickings were made but never was there complete absence of S plaques from the L line. No tests were carried out to determine the stability of either line.

Identification of L and S plaque lines of P-15. Neutralization tests with an immune rabbit serum prepared against the Webster strain of SLE virus were performed on at least partially purified L and S lines using serial virus dilutions and undiluted serum in CPE tests with HKTC, and by plaque reduction tests in CETC using virus dilutions with a 1:128 serum dilution. In the CPE tests both lines gave a log neutralization index >3.3 (control virus titer of $10^{-7.3}$ for the L plaque and $10^{-6.3}$ for the S plaque). In plaque reduction tests with both L and S lines $>90\%$ reduction was obtained with the 10^{-4} dilution of virus and 100% with the 10^{-5} dilution. It appeared quite certain that both lines were St. Louis virus and should be neutralized by patients' sera.

Other properties of L and S plaque lines of P-15. Pools of virus prepared in HKTC from the first through the third plaque "purification" pickings of P-15 were titrated by CPE in HKTC, by PFU in CETC and the third passage only, by weanling mouse intracerebral (i.c.) inoculation also. Results are presented in Table III. Each pool prepared from the S plaque titered 1 to 2 logs lower in HKTC than did pools of the L plaque but these lower titers of the small plaque pools were not equally reflected by the plaque ti-

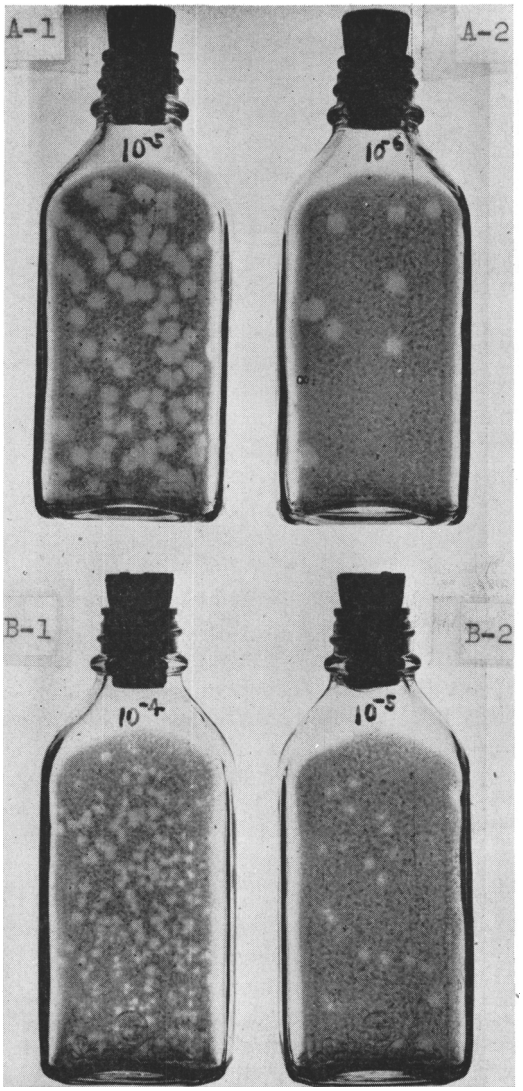

FIG. 1. Plaques produced by the St. Louis P-15 strain on monolayer cultures of chick embryo cells. A. Large plaque formation. A-1: 10^{-5} dilution of St. Louis L-P-15 strain. A-2: 10^{-6} dilution of St. Louis L-P-15 strain. B. Small plaque formation. B-1: 10^{-4} dilution of St. Louis S-P-15 strain. B-2: 10^{-5} dilution of St. Louis S-P-15 strain.

TABLE III. Comparison of 3 Plaque Passages of P-15 Strain for PFU Titers on CETC, for CPE TCD_{50} Titers on HKTC, and for LD_{50} in Weanling Mice.

Serial plaque picking	Large plaque			Small plaque		
	HKTC log TCD_{50}*	CETC number PFU*	Mouse log LD_{50}†	HKTC log TCD_{50}*	CETC number PFU*	Mouse log LD_{50}†
1	6.5	3.5×10^6	—	5.5	5.2×10^5	—
2	7.0	7.7×10^6	—	5.0	1.7×10^6	—
3	6.5	7.6×10^6	6.2	5.5	2.5×10^6	5.4

* Per .1 ml. † Per .03 ml.

trations in CETC. Mouse i.c. titers closely paralleled the CPE titers of HKTC. These results suggest that fewer particles of the L plaque variant than of the S plaque are required to produce CPE in HKTC or death in the mouse.

Plaque reduction neutralization tests. Since the L plaques of P-15 were so much easier to count than those of the mixed types, the L line was used in a series of neutralization tests with human sera‡ from the Tampa Bay area of Florida. Some sera were from patients previously diagnosed serologically as SLE infections, others from normal persons. Tests made with the original mixed virus population, with the L line and by the mouse neutralization test in the standard whole serum virus dilution test(9) confirmed the usefulness of the line. In the plaque test, sera were tested against an estimated 50 PFU, undiluted and at a 1:4 dilution and sometimes also at a 1:2 dilution. A reduction of 50 or 60% in the number of plaques at a 1:4 dilution correlated well with the mouse test when a log neutralization index of 1.7 was considered as positive. A 67% reduction by undiluted sera was either a more sensitive test or gave occasional false positive results. The level of significance at either dilution remains to be determined. In view of all available data obtained with plaque reduction it seems probable that a more sensitive test than the mouse neutralization, yet equally specific, can be developed after more extensive field evaluation. The L plaque line had the expected advantage over that of the mixed population—ease and certainty of reading.

‡ Kindly made available by Dr. James O. Bond, Tampa Bay Regional Encephalitis Laboratory, Tampa, Fla.

Summary. Four strains of St. Louis virus, 2 from the Midwest with many mouse brain passages and 2 from Florida in low mouse brain passage, were tested for CPE and plaque formation in cultures of chick embryo tissue. CPE was readily observed, thus differing from results reported by a number of other workers. Plaque formation also was readily obtained where most others had reported negative results. The 2 newly isolated strains from Florida formed the clearest and most easily counted plaques but both of these strains gave plaques of 2 distinct sizes. Virus lines from the two P-15 strain plaque sizes were clone "purified" and they appeared to represent separate genotypes of St. Louis virus, closely related antigenically if not identical. They appeared to differ significantly, however, in their relative cytopathogenicity for HKTC, in plaque counts in CETC, and in their virulence for mice by the intracerebral route. The line from the large plaque of P-15 gave promise of being very useful in plaque reduction tests with human sera, a test which may be more sensitive than the standard mouse neutralization method.

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Successful Immunization of Primates with Rabies Vaccine Prepared in Human Diploid Cell Strain WI-38.* (30048)

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At present, anti-rabies vaccines used for the immunization of man are produced in either animal brain or in duck embryos and are inactivated by phenol or by betapropiolactone (BPL). Seven to 21 doses of the vaccines have to be given daily, depending on the severity of exposure.

When sensitization occurs during treatment, the vaccine produced in brain tissue can cause postvaccinal neuroparalytic accidents, and the duck embryo vaccine, not completely free from encephalitogenic properties(1,2) has been known to cause on occasion a severe anaphylactic reaction(3).

The Flury HEP strain of rabies grown in chick embryo(4) has been effectively used as a live virus vaccine for booster inoculation of persons who had previously received inactivated vaccine(5). This live virus vaccine had little antigenic value, though, when used for primary immunization of man and monkeys(4,6,7) as compared with the excellent results obtained in other animal species such as dogs and cattle(8,9,10,11,12).

Thus, an urgent need exists for a highly immunogenic anti-rabies vaccine which can be used safely and effectively at low dosages for treatment of humans after exposure as well as for primary immunization of "high risk" groups.

A preliminary investigation(13) showed that the HEP-Flury strain adapted for growth in human diploid cell strains WI-38 (HDCS) had greater antigenic properties than the same strain grown in embryonated eggs. Also, vaccine prepared from Pitman-Moore (PM) and the challenge virus standard (CVS) strains in HDCS and inactivated by BPL was found to be more antigenic than phenolized vaccine in a mouse potency test (13).

These results warranted further investigation of the 2 types of vaccines produced in HDCS which are designed for immunization of man; therefore, an evaluation of their antigenic potency in primates was planned.

Materials and methods. Animals. Seventy Rhesus monkeys *(Macaca mulatta)* which had been maintained in captivity for a number of years at the National Primate Center, University of California, Davis, were used in these experiments. During the last 2 years, these animals had been exposed to repeated intratracheal instillation of DMBA (7,12-dimethyl-(a)-benzanthracene) in an attempt to ascertain whether it had a carcinogenic effect. The results of this experiment were negative.

As shown in Table I, 42 male and 27 female monkeys, weighing from 3.1 to 5.8 kg (average weight 4.0 kg) formed the experimental group. Forty-two of the animals were previously splenectomized when used in an experimental study of malaria.

As shown in Table I, 7 experimental groups were formed each containing 10 animals randomly selected as to sex and weight. Half of the animals were kept inside the animal building and the other half were kept outside in a shaded pen.

All animals were bled before immunization and on the 7th, 14th, 28th, 56th, and 135th day following the first inoculation of vaccine. After the blood was allowed to coagulate at room temperature, the serum was separated

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