radioactive component(s) in the aqueous phase has been noted in plasma and liver from estrone-16-C^{14} treated rats. These results indicate that the rat is capable of degrading the steroid nucleus at ring D. Heard, *et al.*(6) has reported the presence of radioactive CO_2 after administration of isotopic estrone. however, other workers have been unable to detect any respiratory $C^{14}O_2$(2,7). A more definite identification of the radioactive compound(s) in the aqueous residue is now in progress.

The lack of any appreciable steroidal radioactivity or biological activity in either luminal fluid or uteri seems to indicate that once the steroid has produced its physiological effect in the uterus, it either leaves the uterus to enter the general circulation or is degraded.

Summary. The partitioning of radioactivity in uterine luminal fluid and uteri from rats injected with estrone-16-C^{14} is described. Though a minute amount of radioactivity is present in the phenolic fraction, the major portion is present as a water soluble component. No steroidal radioactivity was present as either a conjugated or protein-bound form.

1. Budy, Ann M., *Arch. Inter. Pharm. et de Therapie,* 1955, C111, 435.

2. Blockage, B. C., Nicholas, H. J., Doisy, E. A., Jr., Elliot, W. H., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.,* 1953, v202, 27.

3. Hanahan, D. J., Daskalakis, E. G., Edwards, T., and Dauben, H. J., Jr., *Endocrinol.,* 1953, v53, 163.

4. Berliner, D. L., and Wiest, W. G., *J. Biol. Chem.,* 1956, v221, 449.

5. Astwood, E. B., *Endocrinol.,* 1938, v23, 25.

6. Heard, R. D. H., Jacobs, R., O'Donnell, V., Peron, F. G., Saffran, J. C., Solomon, S. S., Thompson, L. M., Willoughby, H., and Yates, C. H., *Rec. Prog. in Hormone Res.,* 1954, v9, 383.

7. Gallagher, T. F., Bradlow, H. L., Fukushima, D. K., Beer, C. T., Kritchevsky, T. H., Stokem, M., Eidinoff, M. L., Hellman, L., and Dubriner, K., *Rec. Prog. in Hormone Res.,* 1954, v9, 411.

Received May 17, 1957. P.S.E.B.M., 1957, v95.

Adaptation of Equine Abortion Virus to HeLa Cells.* (23270)

CHARLES C. RANDALL

Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tenn.

Goodpasture(1) has presented histological evidence that equine abortion virus (EAV) is infectious for the human amnion. The agent has been propagated in tissues obtained from the natural host(2). This reference contains a suitable review of the literature. A search for readily susceptible cells to investigate further the nature of chemical changes induced in hamster liver cells infected with EAV(3) led to the use of HeLa cultures. In the present communication the propagation of the virus is reported.

Materials and methods. The tissue culture cells used have been adapted to horse serum in this laboratory for over 3 years. Stock cultures were maintained in a mixture of 40% horse serum, 2% chick embryo extract, and 58% Earle's balanced salt solution (BSS), and were subcultured approximately every 10 days. The virus strain used in this study deserves some comment. Preliminary experiments indicated that "native" virus from infected horse tissue could not be propagated in HeLa cells. Therefore, it seemed advisable to employ a strain modified to another host. The virus (strain F)(4) used to initiate this study had been adapted to hamster liver through 86 passages. Subsequently, the agent had been subjected to 2 alternate passages in young hamsters and HeLa cells maintained in ascitic fluid, followed by 12 successive passages in HeLa cells. Material from the last trial was reinoculated into hamsters. The infected liver used as inoculum was frozen and ground, diluted 1:10 in physiological saline and centrifuged for 5 minutes at 5,000 rpm.

* Supported in part by a grant from the Grayson Foundation.

Prior to inoculation tissue cultures in 30 ml serum bottles were washed 3 times with BSS. Three or more cultures were inoculated with 0.2 ml of 1:10 virus suspension and allowed to stand at room temperature for 30 minutes. Control cultures received 0.2 ml of BSS. All cultures were incubated at 37°C with 2 ml of a 50-50 mixture of human ascitic fluid and BSS, which was changed every other day. The cultures were incubated from 3-7 days, harvested, ground and restored to volume with the last change of nutrient fluid. Fresh cultures were inoculated with 0.2 ml of the previous passage. For histological studies coverslips, 11 x 22 mm, were introduced into the culture vessels at the time of subculture. Suitable inoculated cultures and controls were fixed in Zenker-acetic acid and stained with H.&E. *Complement-fixation methods.* The conventional method used in this study has been utilized elsewhere for identification of the agent(5,6). The origin and preparation of the various control sera and antigens have been previously documented(2,6). Hence, only the barest essentials will be mentioned. The tissue culture cells used as antigens, and all other reagents, were stored at −50°C. Immediately prior to use the cells were thawed, washed free of nutrient fluid, ground in physiological saline, and clarified by centrifugation at 2,500 rpm for 10 minutes. In the test proper 0.25 ml of a 1:10 dilution of antiserum was pipetted into tubes containing 2-fold 0.25 ml of serial dilutions of antigen and 2 full units of guinea pig complement in 0.5 ml. Following overnight fixation at 4°C, 0.5 ml of sensitized sheep red cells were added and the tubes incubated for ½ hour at 37°C. Adequate controls were included for each reagent.

Results. Serial propagation. In very early passages there was considerable variation in the gross evidence of infection. Some cultures appeared normal, others showed focal to general aggregation into masses of oval dark cells. This process of retraction and clumping formed spaces simulating plaques. Beginning with the 16th passage these cytopathogenic effects have been fairly constant. The incubation period gradually decreased from 7 to 3 days as the virus became more cytopathogenic. When the culture showed general clumping of cells into necrotic masses which were readily detachable from the glass, the culture was terminated. The identification of intranuclear inclusions characteristic of the infection in susceptible species(2) is of some interest. Characteristic inclusions can be identified in 24 hour cultures stained at random including the 1st and 35th. Older cultures were unsatisfactory because cells would not adhere to the cover slips.

TABLE I. Complement-Fixation Reactions.

Antigens	Serial dilution of virus	Antigen titration end-points
EAV original inoculum		128
Normal HeLa cells		0
8th passage tissue culture virus	10^{-8}	8
27th passage *Idem*	10^{-27}	8
35th passage "	10^{-35}	16

Identification of tissue culture virus. Hamster propagated EAV is lethal for 3-week-old hamsters with LD_{50} titers varying from 10^{-6}-10^{-8} and inclusions were demonstrated in practically all the parenchymal cells of the liver. HeLa cell cultures beginning with the 3rd and subsequently with every other passage have been pathogenic for young hamsters; the titer varying from passage to passage. Undiluted material from some cultures, in spite of pronounced cytopathogenic effects in tissue culture, will not kill all inoculated animals. A rare passage will yield a LD_{50} titer of 10^{-4}. Data from passages 3-35 apparently indicate no real decrease or increase in virulence of tissue culture virus for hamsters. The dilution of the original inoculum, as a result of serial passage, has exceeded 10^{-35}. If dilution due to change of nutrient fluid is taken into consideration, then the dilution exceeds 10^{-80}.

The cultured virus appears to be adequately identified by the complement fixation reaction. The data shown in Table I are adequate proof that the serological specificity of the agent has not been altered by passage in HeLa cells. The antigen titration end-point is defined as the highest dilution of antigen which results in complete fixation of complement with a 1:10 dilution of antiserum.

Summary. The adaptation of equine abortion virus to HeLa cells through 35 serial passages is reported. Characteristic intranuclear inclusions occur in cultures selected at random. The agent fixed complement in significant dilutions and remained pathogenic for hamsters.

1. Goodpasture, E. W., and Anderson, J., *Am. J. Path.*, 1942, v18, 563.

2. Randall, C. C., Ryden, F. W., Doll, E. R., and Schell, F. S., *Am. J. Path.*, 1953, v29, 139.

3. Bracken, E. C., and Randall, C. C., *Am. J. Path.*,

4. Doll, E. R., Richards, M. S., and Wallace, M. E., *Cornell Vet.*, 1953, v43, 551.

5. Randall, Charles C., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 176.

6. Randall, C. C., McVickar, D. L., and Doll, E. R., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 176.

Received May 17, 1957. P.S.E.B.M., 1957, v95.

Influence of Stress on Distribution of Endotoxin in RES Determined by Fluorescein Antibody Technic.* (23271)

NATALIE CREMER AND DENNIS W. WATSON

Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

The site and mode of primary action of endotoxin of Gram negative bacteria in animal tissue is unknown. In an attempt to elucidate this problem, results of parenteral injection of toxin have been studied under a variety of conditions. Among the many experiments, which have been performed, are studies of the effect of toxin after a single injection in normal animals and in animals treated with cortisone, thorotrast, x-irradiation and after two spaced injections(2,8,13,14). Since cortisone and thorotrast can substitute for the preparatory dose of toxin in the generalized Shwartzman reaction, it was considered of interest to determine how animals dispose of toxin under these conditions. Therefore, distribution and retention of the endotoxin of *Salmonella typhosa* after intravenous injection in normal animals and in animals stressed by the aforementioned agents was followed by the Coons' fluorescein technic.†

Materials and methods. The direct fluorescein tagging of gamma globulin according to the method of Coons and Kaplan(5) was employed. Normal and typhoid immune gamma$_2$ globulin fractions were purified by the method of Nichol and Deutsch(11). The endotoxin of *Salmonella typhosa,* strain Edward, was purified by the method of Webster, *et al.* (15). All experiments were performed in American Dutch rabbits weighing approximately 1 kg. The deposition of toxin was studied in 15 normal rabbits after a single intravenous injection of 2 mg of toxin. The animals died or were killed between 20 minutes and 47 hours after injection. In the cortisone study, 15 rabbits were injected intramuscularly daily for 3-4 days with 25 mg of cortisone acetate.‡ After third injection of cortisone, 2 mg of toxin was injected intravenously. The animals died or were killed between 30 minutes and 46 hours after toxin injection. Fourteen animals were injected intravenously with 3 ml of thorotrast§/kg body weight. Six hours later, 2 mg of endotoxin was injected intravenously. The animals died or were killed between 3 hours and approximately 15 hours after toxin injection. Fourteen animals were subjected to 400 roentgen units, total body irradiation. Twenty-four hours later, 2 mg of toxin was injected intravenously. The animals died or were killed

* Supported by Commission on Immunization of the Armed Forces Epidemiological Board.

† The light source was an Osram mercury arc in a Reichert housing equipped with Schott BG 12 filter, 3-3½ mm thick, and a Wratten 2A and 15G filter. We are indebted to Dr. George Price for his assistance in selection of the optical system used.

‡ Cortisone acetate, aqueous suspension, Upjohn Co., Kalamazoo, Mich.

§ Thorotrast, 24-26% thorium dioxide by volume, Testagar and Co., Detroit, Mich.