TABLE I.
Effect of Diethylstilbestrol and Estrone on the Development of Eggs of *Rana pipiens.*

Treatment* γ/cc	No. eggs	% abnormal	% survival
D 27.0	154	95.5	12.3
E 27.0	146	100.0	7.5
D 13.5	152	54.6	71.1
E 13.5	152	99.3	6.6
D 6.8	152	34.9	97.4
E 6.8	150	100.0	0
D 2.7	61	4.9	100.0
E 2.7	117	100.0	0.9
E 1.35	123	90.2	26.0
E 0.68	121	26.4	90.1

* Eggs were exposed to the solutions for a period of 2½ hrs beginning 1 hr after fertilization. Development was observed for 5 days.
D = diethylstilbestrol. E = estrone.

R. pipiens, with only one hour's exposure, 27γ of diethylstilbestrol per cc produced approximately 100% abnormal embryos and high mortality; 4 hours with 6.8γ per cc produced less than 40% abnormality and low mortality. 13.5γ per cc for 2½ hours, however, gave approximately 55% abnormality and 30% mortality. Eggs of *R. clamitans* showed a similar variation in response according to concentration and period of exposure but were more sensitive. 1 to 12% of control embryos were abnormal.

On the basis of these results the 2½ hour period of exposure was used for a comparison of the effects of estrone and diethylstilbestrol on eggs of *R. pipiens.* Concentrations ranging from 27 to 0.68γ per cc together with appropriate control solutions were employed. The data in Table I show that the effects obtained with estrone were much greater than those produced by diethylstilbestrol, and that when the ratio of abnormal embryos was about 50% or less the percentage survival was high. Abnormalities in the controls ranged from 1.3 to 10%. The stronger concentrations of both diethylstilbestrol and estrone produced a large number of abnormal embryos which did not develop beyond the blastula stage. Severe exogastrulation and various degrees of spinal bifida were the most common abnormalities observed.

Since it has been shown that estrogens inhibit cytochrome oxidase *in vitro,* further work is being done to determine whether this enzyme system can be inhibited in frog embryos by estrogens and thereby offer a possible explanation for the effects reported herein.

Summary. Abnormalities were produced in the developing eggs of *Rana pipiens* and *Rana clamitans* by exposure to diethylstilbestrol and to estrone in varying concentrations and for varying lengths of time. Higher concentrations of both substances produced more abnormalities, and estrone was found to be more effective than diethylstilbestrol.

16001 P

A Simple Method of Producing Control Guinea Pig Immune Sera for Use with Complement Fixing Antigens for the Arthropod-Borne Virus Encephalitides.*

W. McD. HAMMON AND CARLOS ESPANA.†

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, California.

It was noted in this laboratory that the sera of guinea pigs, following the mild infection resulting from an intracerebral inoculation of St. Louis virus,[1] had good neutral-

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† Recipient of a predoctoral fellowship from the National Foundation for Infantile Paralysis, Inc.

izing titers. Later, when complement fixation with this virus and the Japanese B virus was undertaken, it was noted that sera of guinea pigs recovering from infection with either virus, had moderate complement fixing antibody titers. Howitt[2] and later Brown[3] prepared such sera for equine viruses. However, most workers, following the recommendation of Casals and Palacios[4] and of Casals,[5] used hyperimmune mouse sera. Havens *et al.*[6] used hyperimmune hamster sera. Mice and hamsters have the unique advantage of being susceptible to fatal infections with all members of this group of viruses. Because of this, they can be given a long series of inoculations of any quantity of homologous brain-tissue virus. Disadvantages are that several months of immunization are required to obtain suitable titers; each animal when bled yields a very small quantity of serum, and because of anti-complementary or a non-specific type of reaction inherent in sera from these species, the sera require inactivation at temperatures of 60°C (mice) to 65°C (hamsters). Guinea pigs are susceptible to fatal infection only to the equine viruses. Using guinea pigs in long immunological procedures with mouse brain (other viruses) could be expected to result in sera reacting to all mouse brain antigens. Therefore, even though guinea pig sera require inactivation at only 56°C, they have been avoided in the past.

The following simple technic, however, produces specific sera of high titer in a very short period of time, with minimal work and with a relatively large yield per animal.

Western and Eastern Equine Viruses. 0.5 ml of a suspension of infected guinea pig brain of such dilution (10^{-2} to 10^{-4}) as will produce a paralytic, but non-fatal infection is given subcutaneously to each of a group of guinea pigs. Ten days later 0.5 ml of a 10^{-2} dilution is given intraperitoneally and after 7 to 10 days, 0.15 ml of a 10^{-1} dilution is given intracerebrally. After 2 weeks the animals are bled to death. The serum is pooled and frozen in CO_2 ice in a large number of small ampoules, or lyophilized in small tubes. The titer of this serum will fall quite rapidly when stored as a liquid at 5°C.

St. Louis, Japanese B, West Nile, Russian Spring-summer and Hammon-Reeves California virus. After a few serial passages in hamsters, ampoules of hamster brain suspension are frozen and stored in CO_2 ice. From the stock source guinea pigs are inoculated. Two intracerebral inoculations are given, 0.15 ml each, of 10% brain suspension at an interval of 10 days. Fever occurs in the guinea pig after the first injection, but rarely are other signs of infection observed. The animals are bled to death 10 or 15 days after the second injection, and the sera preserved as in the case of the equine viruses.

Characteristics of sera. These sera have been tested extensively over a period of several years with many types of brain antigen. A detailed report of the results is being published elsewhere. In no instance has a serum from any of these animals reacted with normal mouse-brain antigen, either before or after these immunization procedures. Japanese B and St. Louis sera usually have titers of 1:256, occasionally 1:512; Eastern equine sera are generally in the range of 1:128 to 1:256, while serum titers to the Western equine and California viruses are of the lowest order, usually 1:64 to 1:128. All are in a range where they can be used in relatively high dilutions as positive controls, or for the standardization of antigens.

When used with mouse-brain antigens made by the Casals technic or by our modification of that of Havens *et al.* (centrifuged at 16,000 r.p.m.), some minimal overlapping occurs between the Western and Eastern equine sera as reported for monkey and hamster sera by Havens *et al.*, and between the members of the St. Louis-West Nile-Japanese B complex. A slight but less pronounced over-

[1] Hammon, W. McD., unpublished data.

[2] Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 526.

[3] Brown, C. G., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 91.

[4] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

[5] Casals, J., *J. Bact.*, 1945, **50**, 1.

[6] Havens, W. P., Jr., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, **77**, 139.

lapping occurs with sera of the latter group to the California virus antigen, but not in the reverse direction. With DeBoer and Cox[7] benzene extracted antigens, and our recent modification of their method,[8] much less overlapping occurs and when our antigen is employed in its optimal dilution (the highest antigen dilution which gives the highest serum titer in a combined antigen-serum titration), there is absolutely no overlapping detectable between any heterologous serum and antigen in original serum dilutions as low as 1:4.

Summary. Herein is described a simple, economical and quick method for preparing highly specific, high titer guinea pig immune sera for use as positive controls, and for standardizing antigens in the complement fixation test. This method has been used for producing sera for Western and Eastern equine, St. Louis, Japanese B, West Nile, Russian Spring-summer and the Hammon-Reeves California virus.

[7] DeBoer, C. J., and Cox, H. E., *J. Immunol.*, 1947, **55**, 193.

[8] Espana, C., and Hammon, W. McD., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 101.

16002

A Suitable Current Stabilizer for the Tiselius Apparatus.*

MARK S. LEVINE AND JOHN T. MCCARTHY. (Introduced by E. E. Ecker.)

From the Institute of Pathology, and the Department of Physics, Western Reserve University, Cleveland, Ohio.

It has become evident that present methods of current control for the electrophoretic apparatus of Tiselius can be improved. It is common practice to use a voltage-stabilized power supply to furnish the current. While this power supply gives a constant voltage across the whole cell, it does not furnish a constant current because of fluctuations of resistance within the cell. Most of these fluctuations probably arise within the electrode vessels. Inasmuch as the resistance of the central part of the cell is not a constant fraction of the total resistance of the cell, the voltage drop across this part and the field strength within it do not remain constant. Because of this it is customary[1] to measure the current and to calculate the field strength by means of the equation: $X = I/qk_s$.

X = field strength in volts per cm.

I = current in amperes.

q = cross-sectional area of the central part of the cell.

k_s = specific conductance of sample in reciprocal ohms.

In order to apply this equation to apparatus employing a voltage-stabilized power supply it is necessary for the operator to make frequent observations of the current and to compensate for fluctuations by altering the applied voltage. This frequent adjustment is tedious as well as time-consuming.

It is preferable that current-stabilization rather than voltage-stabilization be used. The simple circuit which has been found suitable for the Tiselius apparatus is herein given. It is based on the principle that the plate current of a pentode vacuum tube remains constant despite changes in plate voltage. Further stabilization is obtained by the degenerative effect of the resistance in the cathode circuit.

In operation the switch S_2 is connected to the dummy load R_3 and then the switch S_1 is closed. This dummy load serves two purposes: first, the tubes are brought to normal operating temperatures in order to avoid subsequent fluctuations due to thermal changes; and secondly, the dummy load has approxi-

* Aided by a grant from the Commonwealth Fund. The materials needed for this investigation were supplied by the Charles F. Kettering Foundation.

[1] Abramson, H. A., Moyer, L. S., and Gorin, M. H., *Electrophoresis of Proteins*, p. 61, Reinhold Publishing Co., New York, 1942.