The electrocardiogram of a guinea pig is very similar to that of other laboratory animals. Therefore analytical studies of electrocardiograms must consider more carefully than heretofore the anatomical patterns of the conducting system.

Summary. In the guinea pig heart, the A-V conducting tissue is readily differentiated under the microscope from the myocardial tissue. The distribution of the right and left branches of the A-V bundle is similar to that described for other mammalian hearts. In addition there are other septal branches in this heart which are not seen in most mammalian hearts. The significance of these findings is pointed out.

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Vaccination with Various Western Equine Encephalomyelitis Viruses; Comparison as Antigens and as Test Inocula.*

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Vaccination of mice and guinea pigs with various strains of Western equine encephalomyelitis (W.E.E.) virus, carried out recently in this laboratory, induced a satisfactory degree of immunity to all except the current Rockefeller Institute (R.I.) strain. Indeed, mice immunized with the latter to such a degree as to show immunity to other W.E.E. viruses, failed to resist comparable amounts of the homologous R.I. strain.

Sabin reported[1] earlier that certain strains employed in his laboratory may possibly have different antigenic constitutions. Hammon cited[1] experiences similar to ours with respect to the use of current R.I. strains in challenge tests for immunity.

A study was undertaken to determine whether the various strains of W.E.E. virus which were available showed immunological differences; as a corollary, to select strains best suited not only for preparation of vaccines but also for use as standard in challenge tests for induced immunity.

The W.E.E. strains of the present investigation comprised the following:

The *R.I.* strain was obtained in 1933 from Miss B. F. Howitt, then at the George Williams Hooper Foundation, San Francisco. Since that time it has undergone frequent intracerebral passages in mice at the Rockefeller Institute. Three samples were available, hereafter referred to as (a) "R.I."—the virus currently used; (b) "R.I. 1939"; and (c) "R.I. 1941," the dates referring to the year in which the strains were frozen and kept so until required for use as fresh virus when an occasional mouse-passage was made.

The *Lederle* strain was sent to us in the form of guinea pig brain on Sept. 8, 1943, by Miss F. L. Clapp of the Lederle Laboratories, Pearl River, N.Y. It was in the 2nd guinea pig passage and had ultimately been derived from horse brain obtained by the Lederle Laboratories from the Bureau of Animal Industry.

The *Mosquito* strain was received as a mouse-brain passage of Strain F-199, on Oct. 19, 1943, from Dr. Hammon of the Hooper Foundation. It had been isolated by Dr. Hammon from *Culex tarsalis* in the Yakima Valley, Washington.

The *Kelser* strain was sent by Brigadier General R. A. Kelser, Chief of the Veterinary Division, U.S.A., to Dr. C. TenBroeck, who offered it to Dr. J. Zichis of the Illinois Department of Public Health, Chicago, and he in turn gave it to us in 1942. The strain has undergone many guinea pig passages and

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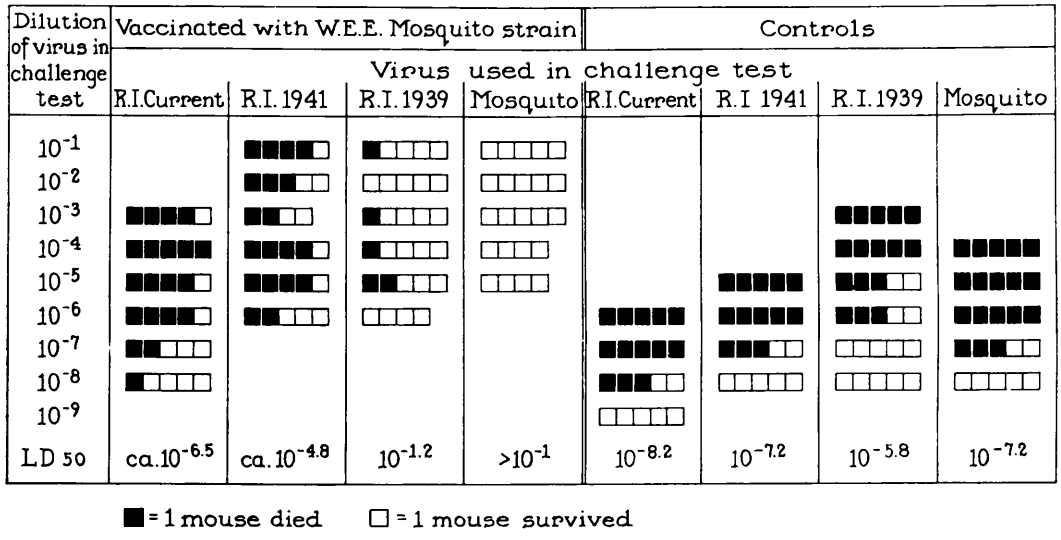
[1] By personal communication.



GRAPH 1.

Intensive vaccination with mosquito strain and challenge tests with different samples of R.I. virus and with the homologous strain.

(The 1939 R.I. strain as used here was derived from frozen mouse-brain virus—as were the other viruses—and the prior two determinations of its LD_{50} titer were $10^{-7.6}$ and $10^{-7.3}$.)

was used earlier in this laboratory and described in studies on serotherapy.[2]

Method of Vaccination. Vaccines consisted of 10% virus-infected chick-embryo containing 0.1 to 0.4% formalin and were stored at 4°C. Different procedures of immunization were carried out in several series of repeated experiments; in one, mice received subcutaneously 0.1 cc of each of the vaccines to be tested twice at a week's interval, the total amount given being 0.2 cc. The test for immunity was applied about 14 days after the first dose. This is designated as a "light" type of immunization. In other procedures, more intensive vaccination ("intermediate" and "intensive") was carried out, *e.g.*, 2 courses of intraäbdominal or subcutaneous inoculations given on 3 consecutive days with a week's interval between the courses: the total amount of vaccine per mouse varied from 0.6 to 1.5 cc. Two weeks after the first dose the mice were tested for immunity over a range of serial tenfold virus (mouse-brain) dilutions given intracerebrally. Thus differing shades of response were elicited to differing degrees of immunization.

2 Olitsky, P. K., Schlesinger, R. W., and Morgan, I. M., *J. Exp. Med.*, 1943, **77**, 359.

Demonstration of Strain Differences. Three typical experiments, each representing one of the immunization schedules described, are shown in Table I.

Results indicate that, no matter which strain served as vaccine, within each experiment the degrees of resistance to the Lederle, Mosquito, and Kelser strains were of the same order of magnitude. In contrast, resistance to the current R.I. strain was either not demonstrable or of much lower degree. The outcome of these and other similar experiments furthermore suggests that the Mosquito strain, *i.e.*, the one with the shortest laboratory history, may be somewhat superior to the others in immunogenic potency.

The following studies were undertaken in an attempt to determine any factor responsible for the failure of the current R.I. strain to indicate in full measure the immune state of a vaccinated animal.

Immunological Check on Identity of the Current R.I. Strain. The tests already described furnished evidence that the current R.I. strain was immunologically related to the other 3 strains of W.E.E. virus used. By immunizing mice with Eastern or Western inactivated virus and testing with Eastern or cur-

TABLE I.
Response of Mice Immunized by Various Procedures to Several Strains of Virus.

Exp. 1. Immunity to intracerebral challenge dose of W.E.E. viruses after "light" type of immunization (2 doses each 0.1 cc vaccine subcutaneously).

	Tested with active virus intracerebrally			
Vaccinated with	R.I. (current)	Lederle	Mosquito	Kelser
R.I.	0;10*	0;10	0;10	10
Lederle	0;0	10;10	>10;0	10
Mosquito	0;0;10	1000;100	>10;0;10	100;10
Kelser	0;0	100;40	>10;0	100;10

Exp. 2. Immunity to intracerebral challenge dose of W.E.E. viruses after "intermediate" type of immunization (6 doses each 0.1 cc subcutaneously).

	Tested with active virus intracerebrally			
Vaccinated with	R.I. (current)	Lederle	Mosquito	Kelser
R.I.		0		
Lederle	0	10	>10	0
Mosquito	0	>1000	>10	>1000
Kelser	0	100	>10	100

Exp. 3. Immunity to intracerebral challenge dose of W.E.E. viruses after "intensive" type of immunization (6 doses each 0.25 cc intraäbdominally).

	Tested with active virus intracerebrally			
Vaccinated with	R.I. (current)	Lederle	Mosquito	Kelser
R.I.	$400;10^3$	$>10^5$	$>10^5$	$>10^5$
Lederle	$300;10^3$	$>10^5;>10^5$	$>10^5;>10^5$	$>10^5;>10^5$
Mosquito	$>4000;10^3$	$>10^5;>10^5$	$>10^5;>10^5$	$>10^5;>10^5$
Kelser	$600;10^3$	$>10^5;>10^5$	$>10^5;>10^5$	$>10^5;>10^5$

* 0;10 = Results of repeated tests. The figures represent the resistance index: ratio of titer in the vaccinated to that in the control mice.
> = more than the number of m.l.d. stated.

rent R.I. strain, it was found that the latter is a Western virus which shows no cross-reaction with the Eastern. This was also confirmed by Dr. Casals of this laboratory by means of active immunization of guinea pigs and by complement-fixation and neutralization tests.

The possible existence of strain-specific neutralizing antibody was tested by vaccinating 2 groups of mice, one with the R.I. (current) strain and the other with Kelser virus. After light type of immunization as described, a minimal amount of immunity to each strain was demonstrated in each case (undiluted serum neutralized 10 m.l.d. of virus by intracerebral test in mice). Thus no evidence was derived through vaccination to show strain-specificity for the 2 viruses.

Comparison of Subcutaneous and Intraabdominal Immunization. A Lederle-strain vaccine was injected into mice following the pattern of the intensive type of immunization. They were challenged by an intracerebral test of Lederle and of current R.I. viruses. Mice injected subcutaneously failed to resist 100 LD_{50} of the R.I. strain (smallest amount tested); those treated intraäbdominally resisted 300 LD_{50} of it. Mice vaccinated by either route and tested with the homologous Lederle strain withstood at least 10,000 LD_{50}. Although not all the tests showed end-points, no striking difference between intraäbdominal and subcutaneous routes of vaccination was demonstrable; the difference according to the strain used for the challenge dose was, however, marked: little or no immunity to the R.I. current strain yet solid immunity to the Lederle.

Changes in the R.I. Strain. Graph 1 illustrates one of repeated tests in which mice intensively immunized with a single strain (Mosquito strain) were challenged with the 3 available samples of the R.I. strain and with the homologous virus. To the latter, the Mosquito strain, the vaccinated showed complete protection; to the current R.I. strain, only to a slight degree, or 50 LD_{50}. A possibly

TABLE II.
Properties of the Various Strains.

Virus in form of infected	Host species and route	Property compared	Strain of virus			
			R.I. current	Lederle	Mosquito	Kelser
Mouse brain	Mice intracerebral	Titer (inverse log)	7.2-9.0	6.6-7.4	5.0-7.2	6.5-7.3
Whole chick embryo			7.5	7.5	7.5	7.5
Mouse brain	Mice intracerebral	Virus dilution	10^{-7}	10^{-5}	10^{-5}	10^{-5}
Whole chick embryo	Chick embryo; toward embryo	Avg interval between inoculation and death — Days	2.5	5	5.5	5.5
		Hours	20	20	40	20

somewhat higher degree of protection was exhibited by the mice tested with the R.I. 1941 sample, and almost complete protection by those challenged with the R.I. 1939 sample (Graph 1). The odd animal which succumbed in the latter group, died (with one exception in the 10^{-3} dilution) after a prolonged incubation period. Moreover, this unevenness of response to the 1939 virus may possibly be a reflection of the development of a change in this sample in a direction toward the type of reaction induced by the 1941 strain and even more strikingly by the current R.I. strain. As has been pointed out, the 3 samples of the R.I. strain differ in that between 1939 and 1941, and again to the present time frequent additional, consecutive, intracerebral passages in mice have been carried out. These passages may have brought about the change suggested by the relative difficulty encountered in immunizing effectively against the later samples. It is noteworthy that the incubation period after intracerebral inoculations in mice of the current R.I. strain is now 2 to 3 days as compared with that of earlier samples of 3 to 4 days, and that its titer is generally somewhat higher (LD_{50} ranging from $10^{-7.2}$ to $10^{-9.0}$) than the present titers of the 1941 ($10^{-6.8}$ to $10^{-7.8}$) and 1939 ($10^{-5.8}$ to $10^{-7.6}$) samples. Table II shows certain properties of the current R.I. strain in comparison with those of other W.E.E. viruses.

It is of interest in this connection that Burnet[3] concluded from his experience that influenza virus, well-adapted to growth in the allantoic cavity of chick embryos, after 42 and 60 passages, is more difficult to neutralize by antiserum than virus with only a few passages (4 and 1, respectively) in this medium.

Conclusions. (1) The immunological identity of 4 strains of W.E.E. virus was confirmed by tests in mice. (2) Results of comparative tests for their antigenic potency suggested that the one most recently isolated may be somewhat superior to the older laboratory strains. (3) Mice vaccinated with comparable doses of either of the 4 strains showed equal degrees of resistance to 3 of the strains, while tests with

[3] Burnet, F. M., *Australian J. Exp. Biol. and Med. Sci.*, 1943, **21**, 231.

the 4th, the current R.I. strain, indicated that this strain is not a sensitive indicator of the degree of immunity of vaccinated mice. (4) Parallel tests in which vaccinated mice were challenged intracerebrally with the current and with earlier samples of the R.I. strain indicate that after its 9th or 10th year of propagation in laboratory mice, certain changes may have taken place which rendered this strain increasingly less suitable for use in challenge tests for immunity.