ment was indicated by a rise in alkaline phosphatase and confirmed at autopsy (dogs 75 and 78, Table II).

Discussion. Our findings in operated dogs show that infarcts in spleen and kidney may cause a significant elevation in serum alkaline phosphatase level. The elevation is considered to be due to absorption of the enzyme from the infarcted areas. This elevation may aid in diagnosis of such infarcts and in following their course. The absence of such an increase, however, does not completely rule out the presence of a renal or splenic infarct. In our studies, some animals with splenic and occasionally with renal infarcts, such as dog 15 in Table I and 66 in Table II, failed to develop an elevation in serum alkaline phosphatase. To determine the causes of these failures requires further investigation.

We have found serum alkaline phosphatase values most useful in experimental endocarditis, in determining during life the occurrence of infarcts and their course. The value of serum alkaline phosphatase studies in man, in endocarditis and in the differential diagnosis of infarcts of spleen or kidney has not been determined.

Summary and conclusions. Infarcts of spleen and kidney were produced by vascular ligation in 13 dogs. The level of serum alkaline phosphatase increased significantly in 3 of 6 dogs with splenic infarcts and in 6 of 7 with renal infarcts. The level reached a maximum in about 24 hours and gradually returned to normal in about a week. An increase in serum alkaline phosphatase level was noted also in dogs with experimental endocarditis. This increase correlated well with the occurrence of infarcts in spleen and kidneys and proved to be of great diagnostic and prognostic value.

We are grateful to Helen J. Burtner for the histochemical preparations, to Judith L. McCarthy for surgical assistance, and to Milton G. Parker for technical aid.

1. Highman, B., Roshe, J., and Altland, P. D., *Circulation Research,* 1956, v4, 250.

2. *Handbook for Leitz Photrometer,* New York, E. Leitz, Inc., 1948.

3. Lillie, R. D., *Histopathologic Technic and Practical Histochemistry,* New York, The Blakiston Co., 1954.

4. Karsner, H. T., and Austin, J. H., *J.A.M.A.,* 1911, v57, 951.

5. Roshe, J., and Morrow, A. G., *Surg., Gynec. and Obst.,* 1955, v101, 305.

Received February 8, 1957. P.S.E.B.M., 1957, v95.

Effect of Beta-Propiolactone on Complement-Fixing Antigens of St. Louis Encephalitis Virus.* (23138)

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A number of laboratory-acquired infections have occurred among individuals processing antigens for use in diagnosis of virus diseases (1,2). The only reported laboratory infection with St. Louis Encephalitis virus(3) occurred in an individual who was preparing complement-fixation antigen by lyophilization and extraction with benzene using the procedure described by Espana and Hammon(4, 5). More recently, a fatal infection with the virus of Russian Spring-Summer Encephalitis occurred as a result of an accident while processing antigens with the same procedure (6). An inapparent infection occurred in a laboratory worker who was working at the same laboratory bench. The present study is concerned with efforts to limit the hazards involved in preparing viral antigens. A recent study by LoGrippo and Hartman(7) has shown that Beta-propiolactone (BPL) promptly inactivates certain viruses without seriously reducing their ability to elicit neu-

* This work was supported by contract with Chemical Corps, Fort Detrick, Frederick, Md.

tralizing and protective antibodies as compared with living vaccines and formalin- and phenol-inactivated vaccines. Similarly, Mack and Chotisen(8) have demonstrated that BPL-treated Newcastle Disease virus is noninfectious for chickens but induces substantial neutralizing and hemagglutination-inhibition antibody formation. A high potency BPL-killed fixed rabies virus vaccine has been reported by Powell and Culbertson(9). *BPL* shows promise for use in sterilizing arterial homografts(10,11,12) and perhaps, coupled with UV irradiation, in sterilization of serum for transfusion(13). These reports suggested to us a possible application of this reagent in rendering viral preparations safe during processing of antigens for use in *in vitro* tests.

Materials and methods. Complement-fixing antigens were prepared from brains of 3-day-old mice inoculated intracerebrally with 0.03 ml of 10^{-4} dilution of SLE virus (Hubbard strain) infected mouse brain according to modification of technic of Havens *et al.* (14). The infant mice were sacrificed by exsanguination after onset of symptoms and their brains removed and frozen at –40°C. Twenty % suspensions were made by thoroughly grinding infected brain tissue in the cold with fine glass beads, after which saline and sufficient M/1 Na_2HPO_4 were added to produce final pH of about 8.0. In the case of antigens treated with Beta-propiolactone,† sufficient BPL from freshly prepared 10% stock solution was incorporated in the saline to give a final concentration of 0.2%. Preparations of normal infant mouse brains were similarly treated for complement-fixation test controls. Infected brain suspensions, BPL-treated and control, were stored 2 hours at 4°C or 37°C when aliquots were removed for infectivity titrations in 12 to 15 g mice. The suspensions were centrifuged in the cold at 3000 rpm for 15 minutes to remove gross sediment and then the supernates were centrifuged at 13,000 rpm in Spinco centrifuge for 1 hour. Merthiolate (1:10,000) was added to supernates which were stored at 4°C in screw cap tubes. The hyperimmune sera were prepared by immunization of guinea pigs with hamster brain infected with St. Louis Encephalitis (SLE), Murray Valley Encephalitis (MVE), Japanese Encephalitis (JE), and West Nile (WN) viruses as previously described(15). The SLE antiserum used for the investigation of the reactivity and stability of antigen preparations fixed complement at a dilution of 1:64 when tested against a commercial SLE CF antigen (Lederle) and showed no fixation with normal mouse brain antigen. All sera were inactivated at 56°C for one-half hour prior to use in complement-fixation tests. Paired human sera from patients with St. Louis Encephalitis were received from the following persons to whom we are indebted: SLE sera nos. 1447, 1448, 1449, 1469, 1503 and 1504 were provided by Dr. S. S. Kalter, CDC, Montgomery, Ala.; nos. 1, 2, and 3 and horse Western Equine Encephalitis (WEE) serum from Dr. J. V. Irons, Texas Department of Health, Austin; and sera from patients with Eastern Equine Encephalitis (EEE) from Dr. Roy F. Feemster and Mrs. J. B. Daniels, Mass. Dept. of Public Health, Boston. The technic for the complement-fixation test was modified from Casals(16). Complement titrations were performed in the presence of dilutions of each antigen. The final volume of reactants was 1.5 ml including 0.25 ml each of appropriate antigen and serum dilutions, 0.5 ml of guinea pig complement (Sharp and Dohme) diluted to contain 2 exact units, and equal volumes of rabbit anti-sheep erythrocyte hemolysin (3 units) and 3% sheep erythrocytes in 0.5 ml. The antigens, serum and complement were incubated overnight at 4°C before addition of the hemolytic system. Tests were then incubated for 30 minutes at 37°C and read visually. In some cases, owing to a scarcity of serum, CF tests were performed with half the above volumes of reactants.

Results. The LD_{50} titer of SLE stock suspensions prepared in the described manner was around 7.22 calculated by the method of Reed and Muench(17). After BPL treatment, either at 4°C or 37°C for 2 hours, no infectious virus could be recovered in undi-

† BPL was obtained through the courtesy of T. L. Gresham, B. F. Goodrich Co., Cleveland, O.

TABLE I. Complement-Fixation with BPL-Treated and Control SLE Antigens.

Hyperimmune serum		CF titer* SLE (1:8)	BPL-SLE (1:8)
Guinea pig	SLE	128	128
	MVE	16	16
	WN	32	32
	JE	8	8
	Normal	<4	<4
Horse	WEE	"	"
Human (conv.)	EEE	"	"

* Reciprocal of highest serum dilution showing 50% fixation.

luted stock suspensions or serial dilutions thereof by intracerebral inoculation of 12 to 15 g mice.

The reactivity of BPL-treated antigens with SLE antiserum was essentially the same as control preparations. CF titers as high as 1:256 were achieved with BPL-treated and untreated preparations although a one tube loss in titer was occasionally encountered with BPL treatment. Since BPL hydrolyzes to yield acidic products, care must be taken to maintain the pH by the addition of M/1 Na_2HPO_4. The antigens are relatively stable on storage at 4°C for at least 3 months. In addition, BPL-treated and control antigens were lyophilized and reconstituted after one month storage at 4°C with only a one tube drop in titer in each instance.

In the data presented in Table I, it can be seen that the degree of cross reactivity of the BPL-treated SLE antigen with sera of the JE-WN-SLE-MVE group is the same as the untreated antigen and similar to that reported in a previous study(15) in which benzene extracted antigens were used. In addition, tests with paired human patients' sera (Table II) demonstrated that the BPL-treated antigen was equally effective in detecting complement-fixing antibodies as its infectious counterpart and seems to be equally as sensitive as the antigens used in the laboratories from which the sera were obtained.

Summary. A method is described for preparing a complement-fixing antigen of SLE virus which is non-infective for mice. The antigen is treated in an early stage in its preparation with Beta-propiolactone, a potent virucidal agent. The liquid antigen is relatively stable on storage at 4°C and may be lyophilized without significant loss of reactivity. The BPL-treated antigen exhibits the same degree of cross-reaction with other members of the encephalitis group as untreated preparations and previously described antigens. Tests with paired patients' sera have indicated its usefulness as a diagnostic aid. The use of BPL as a virucidal agent in the preparation of non-infectious inactivated viral antigens should constitute a distinct advantage over previously described technics.

TABLE II. Complement-Fixation Tests with BPL-Treated SLE Antigen and Human Immune Sera.

Patient serum No.	Time after onset		CF titer SLE	BPL-SLE
1447	Acute	(8)*	8	8
	Conv.	(8)	16	16
1448	Acute	(2)	<4	<4
	Conv.	(8)	16	16
1449	Acute	(<2)	8	4
	Conv.	(8)	32	32
1469	Conv.	(16)	32	32
	Post conv.	(4)	16	16
1503	Acute	(2)	16	16
	Conv.	(32)	64	64
1504	Acute	(<2)	<4	<4
	Conv.	(64)	128	128
1	Acute	(±4)	<4	<4
	Conv.	(32)	16	16
2	Acute	(4)	8	8
	Conv.	(32)	32	32
3	Acute	(<4)	<4	<4
	Conv.	(128)	64	64

* Complement-fixing titer obtained by laboratory donating the serum.

1. Sulkin, S. E., and Pike, R. M., *New England J. Med.*, 1949, v241, 205.

2. ———, *Am. J. Public Health*, 1951. v41, 769.

3. von Magnus, H., *Acta path. et microbiol. Scandinav.*, 1950, v27, 276.

4. Espana, C., and Hammon, W. McD., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 101.

5. ———, *J. Immunol.*, 1948. v59, 31.

6. Haymaker, W., Sather, G. E., and Hammon, W. McD., *A.M.A. Arch. Neurol. and Psychiat.*, 1955, v73, 609.

7. LoGrippo, G. A., and Hartman, F. W., *J. Immunol.*, 1955, v75, 123.

8. Mack, W. N., and Chotisen, A., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 288.

9. Powell, H. M., and Culbertson, C. G., *Bact.*

Proc., 1956, p. 70.

10. Szilagyi, D. E., Overhulse, P. R., Shonnard, C. P., and LoGrippo, G. A., *Surgical Forum of the Am. Coll. Surg.*, 1954, p. 244.

11. Trafas, P. C., Carlson, R. E., LoGrippo, G. A., and Lam, C. R., *A.M.A. Arch. Surg.*, 1954, v69, 415.

12. LoGrippo, G. A., Overhulse, R. P., Szilagyi, D. R., and Hartman, F. W., *Lab. Invest.*, 1955, v4, 217.

13. Smolens, J., and Stokes, J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 538.

14. Havens, W. P., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, v77, 139.

15. Sulkin, S. E., Wallis, C., and Allen, R., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 79.

16. Casals, J., *J. Immunol.*, 1947, v56, 337.

17. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

Received November 2, 1956. P.S.E.B.M., 1957, v95.

Studies in Marine Biology. II. *In vitro* Culture of Zooxanthellae.* (23139)

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In November, 1956, the authors examined a wide variety of marine invertebrates from the waters around Bimini, B.W.I. We were interested in the algal cells—the so-called symbiotic zooxanthellae—which live within the tissues of many radiolarians, coelenterates, molluscs, bryozoa, worms, ascidians, etc. (1,2). With a few exceptions, firm taxonomic placement of zooxanthellae has been difficult because of their somewhat generalized morphology and because of absence or elusive nature of a motile phase(3,4); only Kawaguti(5) has reported seeing motile forms. Furthermore, whether zooxanthellae of various host animals fall into one or into multiple taxonomic groups has not been established. As to their role as symbionts, one school(6,7) holds that the zooxanthellae do not appreciably contribute to nutrition of the host; another, based largely on recent work of Sargent and Austin(8) and of Odum and Odum (9), suggests that the abundant presence in coral reef communities of photosynthetic algae (including both interstitial zooxanthellae and intraskeletal filamentous algae) may well be crucial to growth and maintenance of the entire reef biotope.

If, following on the work of Kawaguti, zooxanthellae could be isolated from the host animal, grown serially in bacteria-free culture, and their nutritive and reproductive characteristics studied, not only should their identity in each case be ascertainable, but also possibly clues as to their trophic relationship with the host animal. The aim of the present study was to isolate certain coelenterate zooxanthellae and to culture them *in vitro*.

Materials and methods. A large zooxanthellae-containing Scyphozoan (*Cassiopeia* sp.) and a large zooxanthellae-containing anemone (*Condylactis* sp.) were selected. *Cassiopeia* abounded in waters around S. Bimini, B.W.I. *Condylactis* was similarly abundant at E. Bimini. The brown-green coloration of both these animals reflects the presence, mainly in endodermal tissues, of densely packed layers of zooxanthellae (Plate I, Fig. 1 and 2). To secure zooxanthellae free from host tissue for *in vitro* culture, an entire live specimen of either *Cassiopeia* or *Condylactis* was washed successively in tapwater and sterile seawater, then macerated in a Waring blendor with approximately 100 ml of sterile seawater. Within a few minutes tissues were

* We are grateful to Dr. C. M. Breder, director of the Lerner Marine Laboratory, Bimini, B.W.I., for having made facilities of that institution available to us. Acknowledgment is made of partial support of this work under grants from the Office of Naval Research and the National Science Foundation. Our thanks go to Andrew N. Nowak for valued technical assistance. The second-named author wishes to express deep gratitude to the National Geographic Society of Washington, D.C. for aid in field work which led to initiation and development of this program.