tary liver necrosis in rats(14) and exudative diathesis in chicks(15). It is therefore possible that the basal diet was deficient in certain factors involved in prevention of muscular dystrophy (Vit. E, selenium and perhaps methionine and cystine) as well as containing inhibitors or antagonists that may enhance the occurrence of muscular dystrophy.

Summary. Rabbits maintained on a torula yeast, Vit. E deficient diet developed a severe and rapidly progressing muscular dystrophy. This condition was not prevented by addition to the diet of 1 ppm selenium or of supplements of dl-alpha tocopheryl acetate alone or in combination. Additions of natural feedstuffs (wheat bran, linseed oil meal or kidney beans) to the semi-purified diet did not fully prevent the condition, although they did lessen the severity of the lesion and increase the average number of days of survival indicating deficiencies other than selenium and Vit. E were involved in the particular experimental diets used. A marked alopecia was observed in all animals fed diets not containing a natural feedstuff.

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1. Andersson, P. O., M.S. Thesis, 1956, Cornell Univ., Ithaca, N. Y.

2. Cartan, G. H., Swingle, K. F., *Am. J. Vet. Res.,* 1959, v20, 235.

3. Day, P. L., Dinning, J. S., *Am. J. Clin. Nutr.,* 1956, v4, 386.

4. Hjarre, A., Lilleengen, K., *Ein Beitrag Virchows Archiv.,* 1936, v297, 565.

5. Hogue, D. E., Warner, R. G., *J. Anim. Sci.,* 1960, v19, 1267.

6. Hove, E. L., Copeland, D. H., Salmon, W. D., *J. Nutr.,* 1954, v53, 377.

7. Hove, E. L., Herndon, J. F., *ibid.,* 1954, v55, 363.

8. Hove, E. L., Copeland, D. H., Herndon, J. F., Salmon, W. D., *ibid.,* 1957, v63, 289.

9. MacKenzie, C. G., McCollum, E. V., *ibid.,* 1940, v19, 345.

10. Morgulis, S., Spencer, H. C., *ibid.,* 1936, v11, 573.

11. Muth, O. H., Oldfield, J. E., Remmert, L. F., Schubert, J. R., *Science,* 1958, v128, 1090.

12. Proctor, J. F., Hogue, D. E., Warner, R. G., *J. Anim. Sci.,* 1958, v17, 1183.

13. Schofield, F. W., *Can. J. Comp. Med.,* 1953, v17, 422.

14. Schwarz, K., Foltz, C. M., *J. Am. Chem. Soc.,* 1951, v79, 3292.

15. Scott, M. L., Hill, F. W., Norris, L. C., Dobson, D. C., Nelson, T. S., *J. Nutr.,* 1955, v56, 387.

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Immune Response to Actinophage in the Mouse.* (26851)

S. G. BRADLEY, B. W. PAPERMASTER,† D. W. WATSON AND R. A. GOOD‡
Departments of Anatomy, Microbiology and Pediatric Heart Hospital Research Laboratories, University of Minnesota, Minneapolis

Recently about 25 actinophages virulent for members of the genera Streptomyces, Nocardia, Micromonospora, Actinoplanes and Mycobacteria have been isolated and characterized with respect to host range(1). The conditions for growth of these bacterial viruses, their antigenic relationships to one another and their distribution in nature have been partially determined(2). Moreover, actinophage plaque-forming units can be enumerated easily, quickly and accurately(3). Therefore, actinophages, as well as bacteriophages of *Escherichia coli*(4), possess many desirable properties required of an antigen used in the study of onset of antibody formation. This report is concerned with the antigenicity of actinophage and uptake and retention of the viral particles by organs of the mouse.

Uptake and retention of actinophage. Inbred, male, adult, Bagg albino mice (a sub-

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† U.S.P.H.S. Medical Graduate Student Trainee, Dept. Anatomy.

‡ American Legion Memorial Heart Professor of Pediatrics.

TABLE I. Appearance of Actinophage in Organs after Intraperitoneal Presentation.

Organ	No. samples	Log No. plaque-forming units/g tissue After 5 min.	After 3 hr
Spleen	14	8.9	>9.0
Lung	21	6.3	8.4
Testis	14	7.8	8.5
Brain	4	4.2	6.2
Liver	8	7.6	>8.0
Kidney	10	7.4	7.9
Whole blood	17	4.3	4.8
Serum		4.2*	4.6*
Buffy coat		2.7*	3.2*
Washed red cells		3.3*	2.9*

Balb mice were inj. intraper. with 10^{10} actinophage MSP8. Subsequently, organs were excised, washed, homogenized and assayed for viable virus. Data are presented as geometric means.

* Virus content of fraction derived from 1 g of whole blood.

line of BALB/Sy) were injected intraperitoneally with 10^{10} plaque-forming units of actinophage MSP8. Blood samples were collected and lungs, liver, brain, kidney, spleen and testis were removed 5 and 180 minutes after injection. Serum was obtained after centrifugation in capillary tubes. Blood was mixed with an inoculative suspension of the virus-host *Streptomyces venezuelae* S13 in molten peptone-yeast-extract agar at 48°C and poured into petri plates containing a basal layer of the same medium(3). The excised organs were washed in peptone-yeast-extract broth, dispersed with a tissue grinder, diluted appropriately, mixed with molten, seeded overlay medium and dispensed into petri plates. Viable actinophage was recovered from all of the organs tested (Table I). Viral content of blood collected from the retro-orbital plexus, tail and toes of the fore and hind limbs was the same. Actinophage appeared in the blood immediately after injection; a sample taken as quickly as possible (less than 15 seconds) contained in excess of 5×10^3 particles/ml. It should be noted that the virus was in the serum; the cellular fraction of the blood was essentially virus-free (Table I) Phage could not be isolated regularly from the blood 2 days after presentation of antigen, but was detected in lung, liver and kidney for 6 days, and in testis and spleen after 10 days (Fig. 1). When 10^7 or fewer virus particles were administered, they were not recovered in the blood but were found in the lung and testis. The lung did not retain as many phage particles or hold them as long as the testis (Table II).

Antibody production. Mice injected with 10^{10} MSP8 were bled daily for 4 days and the titer of neutralizing antibody in each serum determined by the method of Lennox *et al.*(5). Specificity of the reaction was established by including bacteriophage T2, which lysed *Escherichia coli* but not *S. venezuelae,* in the assay mixture. Plaque-forming capability of MSP8 was reduced markedly by serum collected 4 days after presentation of antigen, but T2 was unaffected. Substantial antibody activity was detected in the serum taken 48 hours after antigenic stimulation (Fig. 2). Antibody production was influenced by the amount of antigen administered. Mice challenged with fewer than 10^7 MSP8 did not produce measurable antibody. The optimal concentration for provocation of neutralizing activity fell between 10^{10} and 5×10^{10}; doses of 10^{11} and 2×10^{11} evoked no better or an inferior response (Table III).

Discussion. Many years ago, Buxton demonstrated that living typhoid bacilli, introduced into the peritoneum of the rabbit, enter the general circulation almost immedi-

TABLE II. Effect of Actinophage Dose on Virus Uptake.

No. virus injected	Sample taken, min. after inj.	Log No. plaque-forming units/g tissue Blood	Lungs	Testis
10^{10}	5	4.4	6.4	8.0
"	180	4.3	8.4	8.7
10^9	5	2.4	5.3	7.3
"	180	3.5	7.2	7.8
10^8	5	1.7	4.6	5.9
"	180	3.0	5.4	6.8
10^7	5	<1.3	<3.0	5.1
"	180	<1.3	5.0	5.3
10^6	5	<1.3	<3.0	3.8
"	180	<1.3	<3.0	4.9

Balb mice were inj. intraper. with actinophage MSP8. Subsequently organs were excised, washed, homogenized and assayed for viable virus. Data are presented as geometric means of samples taken from 15 mice.

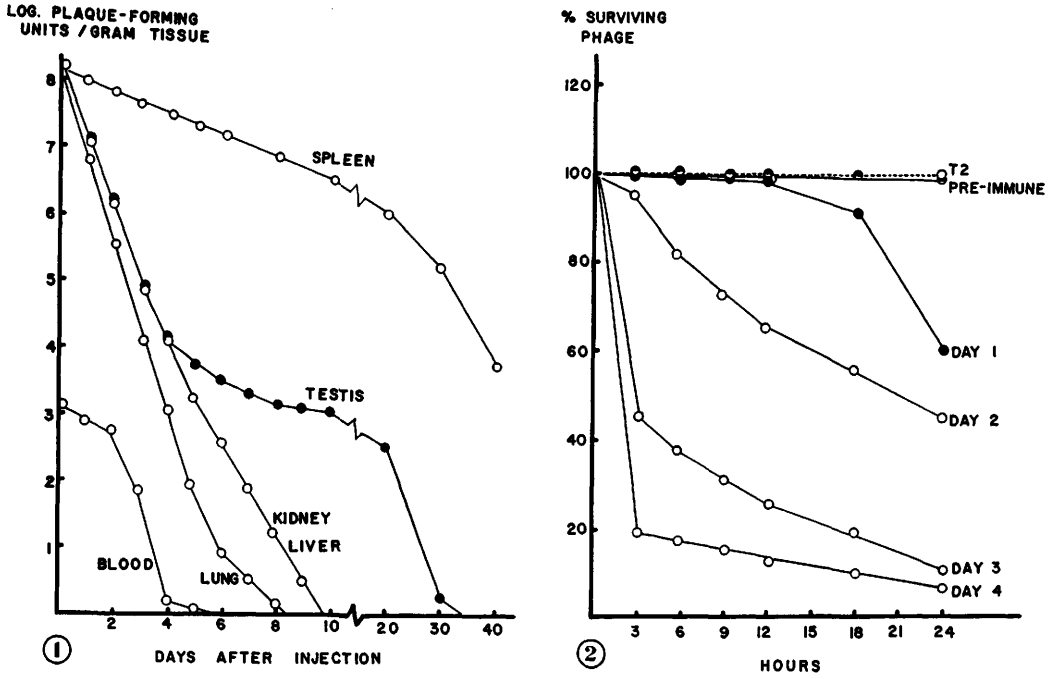

FIG. 1. Retention of viable actinophage in organs of Balb mice inj. intraper. with 10^{10} plaque-forming units. Each point is the geometric mean of samples from 2-15 mice (mode = 5).

FIG. 2. Onset of antibody production. Balb mice were inj. with 10^{10} MSP8, bled daily for 4 days and neutralizing activity of serum diluted 1:15 determined with respect to MSP8 and *E. coli*-phage T2. Phage + serum mixtures were incubated at 30°C. Each point is the arithmetic mean of samples from 25 mice.

ately and concentrate in the liver and spleen subsequently(6). Similarly, actinophage administered intraperitoneally is found in the circulatory system in less than 15 seconds and is distributed throughout the body of the mouse within 5 minutes. Route of entry of the bacterial virus has not been determined, but may be through the peritoneal capillary circulation or possibly through the lymphatic vessels. Viable actinophage is retained in the animal, in particular by the spleen, for more than 6 weeks. Prolonged persistence of other antigens(7,8) is now well established; however its significance for genesis and maintenance of antibody is not apparent. It is interesting that 10^7 phage particles were sufficient to invoke measurable antibody production. This amount of actinophage contains less than 0.01 μg protein, or assuming an average molecular weight of 10^5 for protein, fewer than 6×10^{10} molecules. Bacteriophage contains at least 6 distinct components(9), most of which probably are not concerned with neutralization. If we assume that the mouse contains 10^{10} reticuloendothelial cells, then there is only one "neutralizing antigen" molecule per RE cell. In our experiments, it is possible that many

TABLE III. Effect of Actinophage Dose on Antibody Production.

No. MSP8 inj.	% survival of virus after incubation with 1:20 serum			
	2-day sample		4-day sample	
	MSP8	T2	MSP8	T2
2×10^{11}	76	100	9	99
10^{11}	47	101	5	100
4×10^{10}	32	100	2	98
10^{10}	61	98	6	98
4×10^{9}	83	98	22	97
10^{9}	88	99	57	98
10^{8}	98	101	63	100
10^{7}	104	100	71	99
10^{6}	101	100	96	98

Balb mice were inj. intraper. with actinophage MSP8 and bled on day 2 and 4. Serum was heated 56°C for 30 min. and mixed with actinophage MSP8 and *E. coli*-phage T2. The virus + antiserum mixture was incubated for 24 hr at 30°C, diluted and plated with *Streptomyces venezuelae* S13 or *Escherichia coli* B. Data are presented as arithmetic means of samples taken from 15 mice.

immunologically competent cells were not exposed to antigen. Equally significant, detectable specific antibody is made within 24 hours after antigenic stimulation. Similarly, Lennox and Avegno (private communication) has found neutralizing antibody 24 hr after antigenic stimulation of rabbits with coliphage T2. As measurements become more sensitive, it may be possible to demonstrate antibody production immediately after presentation of antigen. It should be emphasized that this possibility does not disprove the inductive hypothesis for genesis of antibody. The enzyme-forming system for synthesis of adaptive (induced or repressed) enzyme is present in the unadapted cell. In systems where the inducer is not a substrate, enzymic activity increases almost instantaneously and linearly after addition of inducer(10). Our results do not support preferentially the clonal selection, natural selection or inductive theory of antibody synthesis. This study does establish that the actinophage-neutralizing antibody system in the mouse is an excellent tool for determination of the minimum time and antigenic dose necessary for antibody synthesis.

Summary. Actinophage injected intraperitoneally into inbred Balb mice appeared almost instantaneously in blood, brain, liver, kidney, spleen, lung and testis. Viable virus was isolated from the blood for 2 days following challenge, from lung, liver and kidney for 6 days, and from testis and spleen for more than 10 days. Mice produced neutralizing antibody within 48 hours after antigenic stimulation. Antibody production was elicited by as few as 10^7 actinophage particles.

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1. Bradley, S. G., Anderson, D. L., Jones, L. A., *Devel. Indust. Microbiol.,* 1961, v2, 223.

2. Bradley, S. G., *Mycologia,* 1959, v51, 9.

3. Jones, L. A., Bradley, S. G., *Devel. Indust. Microbiol.,* 1961, v3, in press.

4. Attardi, G., Cohn, M., Horibata, K., Lennox, E. S., *Bact. Rev.,* 1959, v23, 213.

5. Cohn, M., Lennox, E. S., *J. Immunol.,* 1961, v87, in press.

6. Buxton, B. H., *J. Med. Res.,* 1906, v15, 18.

7. McMaster, P. D., Kruse, H., *J. Exp. Med.,* 1951, v94, 323.

8. Garvey, J. S., Campbell, D. H., *J. Immunol.,* 1956, v76, 36.

9. Brenner, S., Streisinger, G., Horne, R. W., Champe, S. P., Barnett, L., Benzer, S., Rees, M. W., *J. Mol. Biol.,* 1959, v1, 281.

10. Cohn, M., *Bact. Rev.,* 1957, v21, 140.

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Excision of Infarcts of the Left Ventricle and Replacement by Teflon Prostheses. (26852)

P. CRASTNOPOL, A. DOUGLAS, I. BUMIN, N. ERTUG AND A. KLEIN
(Introduced by B. W. Volk)
Isaac Albert Research Institute and Department of Surgery, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

Murray(1) and Bailey(2) have demonstrated that excision of a myocardial infarct in the dog can be successfully performed. However, the latter showed that the procedure usually resulted in a reduction in left ventricular capacity and cardiac failure. To obviate this, we undertook a series of experiments to determine the practicability of replacement of the excised myocardium with a teflon prosthesis. The results of 10 experiments are reported here.

Procedure. We attempted first to develop a technic which would have an acceptably low morbidity and mortality. In a pilot series of experiments, operations on the left ventricle were performed under cardiopulmonary bypass and aortic occlusion. Later, we added systemic, and then selective (cardiac) hypothermia, but none of these animals survived. It was apparent that a simpler technic, avoiding extracorporeal circulation and hypothermia must be employed. The following pro-