TABLE I.
Growth of the Lobster.

Stage x	Observed y	Calculated y	Stage x	Observed y	Calculated y
0	8.2	8.02	18	162.0	153.81
1	9.6	9.55	19	180.0	175.72
2	11.4	11.39	20	200.0	199.51
3	13.5	13.57	21	222.0	224.99
4	16.0	16.16	22	247.0	251.90
5	18.8	19.23	23	275.0	279.92
6	22.5	22.74	24	300.0	308.63
7	26.5	27.13	25	327.0	337.60
8	32.0	32.16	26	356.0	366.37
9	37.9	38.08	27	380.0	394.50
10	45.0	45.00	28	406.0	421.57
11	53.0	53.07	29	431.0	447.26
12	62.0	62.44	30	457.0	471.28
13	73.0	73.26	31	480.0	493.44
14	86.0	85.69	32	505.0	513.66
15	102.0	99.85	33	525.0	531.89
16	121.0	115.88	34	546.0	548.17
17	141.0	133.85	35	568.0	562.39

but instead a logarithmic parabola. For this difference in the form of the growth curve in the lobster, when body length is plotted against (a) temporal and (b) biologic age, I have at present no explanation, but the fact seems worthy of record.

[1] Hadley, Philip P. 1906. 36th Ann. Rep. Comm. Inland Fish, Rhode Island, 153-226, pls. xxvi-xxxvii and xl. The same data are quoted in Herrick, Bul. U. S. Bur. Fish., 1911, xxix, 747.

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Study of Alpha and Beta Units of an Anti-Paratyphoid Bacteriophage.

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Under the influence of a fresh, sewage filtrate we isolated a lytic agent for an old laboratory strain of *B. paratyphosus A,* in the S cyclostage. This filtrate, which was first active in F_3, was enhanced to a high titer by alternate feeding and filtration in series. When tested by the plate method against the homologous culture there first appeared only large plaques. Later (F_6), the filtrate gave small plaques in addition to the large. With continued propagation there was a tendency for the large plaques to be replaced by the small.

The large lytic areas possessed an average diameter of 6 to 7 mm. when mature. As a rule, there were no areas of intermediate size. A few that measured from 3 to 4 mm. possessed the chief characteristics of the large plaques, and on further propagation they always reproduced the large area type. The small lytic areas were sharp and clearcut, while the large areas were usually surrounded by a hazy zone of imperfectly lysed culture. As a rule the principle determining the large areas permitted the appearance of a relatively larger number of secondary, resistant colonies than did the small-area principle. Frequently, under conditions of crowding, the large areas enclosed one or more of the small. In this case the latter were especially distinct if they happened to lie in the marginal zone of the large areas; for, here, the small areas completed the lysis which had been left imperfect by the large areas.

Separate, pure line, lytic filtrates were prepared through isolations performed upon discrete large and small areas respectively, and these filtrates were built up to a moderately high titer by alternate feeding and filtration in series. The large area principle will hereafter be termed the α principle and the small area principle will be termed the β. The large and the small areas will receive the same respective designations (α and β). When the alpha filtrate was tested against the homologous S type culture it gave only large (α) areas. Such areas were reproduced constantly through 15 plate-generations by the following method: A straight needle, moistened in sterile broth, was touched lightly to the center of a discrete area, then dipped in a tube containing 5 cc. of sterile, beef infusion broth of pH 7.8. One loop (4 mm.) of the broth was then spread over the surface of an agar plate previously seeded with 3 drops of a young broth culture of the original strain. The number of lytic areas arising on such a plate, after an incubation of 24 hours or less, was usually 10 to 100. After the pure-line alpha filtrate had been propagated in broth by alternate feeding and filtration through 6 to 8 series, the β principle made its appearance and registered on agar plates by the production of small areas in addition to the large. In other words, the α pure-line principle did not "breed true."

When β filtrate was tested on plates against the S type culture only small (β) areas were produced. This principle was not propagated by "colony-to-plate" transfers as in the case of the α principle; but when the filtrate was propagated by alternate feedings and filtrations for 12 generations it still produced only the β areas. This has also been the experience of one of the writers

working with a β principle of the Shiga dysentery bacillus. The pure-line β principle has thus appeared to "breed true." Moreover the β principle of the Shiga bacillus has been found to retain its characteristics after being sealed in glass ampoules over a period of 3 years.

The points of thermal inactivation of the 2 principles were roughly ascertained. In this connection it may be recalled that different investigators have reported the thermal "death point" of the bacteriophage at various levels—from 62 to 74° C. A fraction of a cc. of each principle was taken into a thin, drawn-out capillary tube and the tips sealed 2 inches from the ends of the column. The capillaries were then immersed in a water bath and heated at various temperatures. After cooling, the contents were expelled on agar plates previously seeded with 3 drops of the S type culture. If lysis did not occur it was assumed that the heated principle had been inactivated. No attempt to cause the regeneration of a "latent" principle was made. By means of these tests it was ascertained: (1) that the α principle was rendered inert by 30 minutes heating at 63° C., but not at 60° C. (2) that the β principle was rendered inert by heating for 30 minutes at 75° C., but not at 70° C. The inactivation points were thus sharply distinct.

A study was conducted on the action of the 2 principles on their reciprocal, secondary, resistant cultures. When the α principle was permitted to act on the homologous, S type culture secondary colonies, resistant to this principle, arose. Some of these were cultured and, when tested against the β principle, were found to be readily susceptible. When the β principle was permitted to act on the homologous, S type culture secondary, resistant colonies arose in relatively smaller number. These embraced at least 2 types: One, although resistant to the β principle, was susceptible to the α principle. The other, whose mucoid form of growth suggested the intermediate or O form of culture, was resistant to both principles.

Cross-tests were performed between the α and β principles of *B. paratyphosus B* and an S type of *B. typhosus*. When the α paratyphoid principle was permitted to act on the typhoid culture on agar plates, no lytic areas appeared. When the β paratyphoid principle was similarly used only small areas in the typhoid culture film developed. When the α and β paratyphoid principles were mixed in a tube and then streaked against the typhoid culture, only the β paratyphoid principle registered; that is, there were only small areas.

A small area (β) typhoid principle, when brought into contact with the original paratyphoid culture on plates, gave only small

areas. The same result was obtained when this typhoid principle was streaked against paratyphoid resistants that had been developed at the expense of the α principle of *B. paratyphosus* acting on the original paratyphoid culture. The β typhoid principle had no influence on paratyphoid resistants that had been developed at the expense of the β paratyphoid principle acting on the original S type of *B. paratyphosus B.*

An attempt was made to prepare antilytic serums for each principle and to study the influence of such serums on the behavior of the respective principles in the course of lysis. Rabbits were immunized and the serums collected. Each serum, in a dilution of 1:10, was allowed to stand in contact for 2 hours with both principles. The influence of these mixtures on lysis was then observed by the agar plate method. The serum immune to the α principle showed a strong inhibiting influence against this principle when the latter was allowed to act on the original culture. The same serum seemed to have little or no effect on the β principle. The serum immune to the β principle had no effect on lysis by the α principle, and apparently little on lysis by the β principle itself. The last result was unexpected and may have been due to incomplete immunization of the rabbits. This work will be repeated.

The results of these studies, taken as a whole, both confirm and extend certain findings reported earlier by Bail,[1] Bail and Watanabe,[2] Watanabe,[3] Gratia,[4, 5] Hadley,[6] and Kline.[7] They are also in harmony with later observations soon to be reported from this laboratory and involving *B. paratyphosus A.*

Conclusions: From the results reported in this paper, taken in conjunction with those of Bail, Watanabe, Gratia and Kline, it is strongly suggested that the bacteriophage possesses a dual nature. It may exist in the form of either the alpha or the beta units, the former producing the large lytic areas, the latter producing the small. There are no true intermediates, for the variation is discontinuous. While the continued propagation of the alpha principle at the expense of the S type culture results eventually in the generation of the β units, in addition to the α units, the continued propagation of the beta principle, either at the expense of the S type culture or at the expense of the alpha resistants, seems never to determine the generation of the α units. The mode of action of the 2 principles is, in a measure, reciprocal, as pointed out by Gratia[4, 5] for *B. coli.* The alpha principle is active on at least one of the beta resistants; and the beta principle is active on at least one of the alpha resistants. Both principles, on the other hand, act on the

S type culture—which we know commonly possesses a double antigenic configuration. But neither principle acts on certain mucoid resistants arising from the action of the alpha principle on the S type culture.

These observations are impossible of reconciliation with d'Herelle's filtrable virus theory of bacteriophage action, in which the differences in size of the lytic areas (assumed to represent a continuous variation) are correlated with differences in "virulence" of the bacteriophage. And they can be reconciled no more readily with Bordet's theory of nutritive viation. Their significance is still far from clear, but we believe that they offer more substantial support to a conception of the bacteriophage as comprising 2 functionally reciprocal units which may be regarded provisionally as complementary cyclostages in the developmental history of the culture concerned. This conception is developed in greater detail in a series of papers soon to appear.[8]

[1] Bail, O., *Wien. klin. Wchnschr.*, 1922, xxxv, 722.
[2] Bail, O., and Watanabe, T., *Wien. klin. Wchnschr.*, 1922, xxxv, 362.
[3] Watanabe, T., *Zeitschr. f. Immunitätsf.*, 1923, xxxvii, 106.
[4] Gratia, A., *Compt. rend. Soc. biol.*, 1923, lxxxix, 821.
[5] Gratia, A., *Compt. rend. Soc. biol.*, 1923, lxxxix, 824.
[6] Hadley, Ph., *J. Bact.*, 1924, ix, 397.
[7] Kline, G. M., *J. Am. Pub. Health Assn.*, 1927, xii, 1074.
[8] Hadley, Ph., *J. Inf. Dis.*, 1928, (in press).

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Anemia Following Splenectomy in White Rats.

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Numerous European workers have observed that rats frequently develop a severe anemia following removal of the spleen. That this is not always the case, however, is shown by the fact that in certain laboratories splenectomy of rats has not been followed by anemia. Lauda[1] made an extensive study of this problem and found that in approximately 75% of his splenectomized rats, a very severe hemolytic type of anemia developed. He proved it to be of an infectious nature and transmissible to other splenectomized rats. Shortly af-