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"The Bacteriophage of Pasteurella Pestis:
General Characteristics of Bacteriophage"

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Dec 1942.

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Introduction

Felix Hubert d'Herelle[1] was the first to report on the bacteriophage of Pasteurella pestis (hereafter abbreviated merely 'phage'). He succeeded in isolating this phage which ~~possessed a~~ acted specifically ~~action~~ against Pasteurella pestis from rodent stools while in Indo-china in 1920. ~~It was reported that~~ d'Herelle ~~obtained~~ reported excellent therapeutic results from four glandular plague cases by injecting this phage directly into the buboes. Since then number of other scientists also carried out research on this phage. Based on ~~the bibliographies~~ data on the isolation of bacteriophages and their characteristics, we find that Hauduroy and Ghalib[2] ~~carried out research~~ attempted the isolation of this phage and detected ~~this phage~~ it in blood sera and stools of rodents that were captured in the sewers of Paris. We also know that Pokrovskaya[3] succeeded in isolating this phage along with Pasteurella pestis from dead shrews. Others like Advier[4] isolated this phage from the blood of plague cases and Harvey[5] also proved that it was possible to isolate this phage from the intestinal contents,

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stools and bubonic tissues of infected cases.

Girard[6] isolated this "phage" from the stools of rodents that were captured in ~~an~~ epidemic area and also from the Xenopsylla cheopis found on these rodents. On the other hand, Girard failed to detect this "phage" in the Xenopsylla cheopis taken from rodents captured in areas free from plague epidemic. Based on these facts, Girard claimed ~~that~~ the "phage" that was isolated from the Xenopsylla cheopis actually ~~came from these~~ originated in rodents and considered this a very vital epidemiologic ~~significance~~ fact.

Other scientists, aside from those mentioned above, like Flu[7][8], detected this "phage" in the water of the Leyden Canal in Holland, and reported that this "phage" ~~possessed~~ bacteriolyzed ~~actions against~~ Escherichia coli, Shigella dysenteriae and Pasteurella pestis, while SUGINO[9] reported that he succeeded in isolating a "phage" ~~possessed~~ that has of a specific bacteriolytic ~~action~~ against Pasteurella pestis ~~through~~ by the protamylase-method.

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Based on ~~the~~ studies on the characteristics of these isolated "phages", Hauduroy, Pokrovskaya, SUGINO and Flu ~~[illegible]~~ learned that the bacteriolytic ~~action~~ of these "phages" could be ~~[illegible]~~ increased by ~~a~~ combined transplantation with Pasteurella pestis; ~~and~~ based on ~~the~~ studies on the specificity of the~~s~~ bacteriolytic action by many scientists, (d'herelle, Hauduroy and Pokrovskaya), it was proven that these "phages" ~~possessed~~ had a higher specificity against Pasteurella pestis. Couvy (10) proved that this specificity existed even among ~~the~~ different types of Pasteurella pestis strains and that each "phage" ~~possessed~~ bacteriolyzed only ~~action~~ ~~[illegible]~~ its original strain, ~~alone and non against other strains~~. Advier also proved that the "phage" which he ~~had~~ isolated from a plague case ~~possessed a~~ specifically bacteriolyzed ~~action only against~~ only the Pasteurella pestis and ~~none against~~ had no effect on Corynebacterium pseudo tuberculosis rodentium. SUGINO also proved that the "phage" which he ~~had~~ isolated from Pasteurella pestis by the protamylase method ~~possessed a~~ acted specifically ~~action only against~~ Pasteurella pestis and ~~none against~~ had no effect against the Corynebacterium pseudotuberculosis rodentium and others of the genus Pasteurella.

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The majority of these scientists have thus proved the existence of the specificity of pestis phage, ~~but~~ and Flu ~~proved~~ claimed that the phage which he ~~has~~ isolated from the canal water ~~[illegible]~~ ~~a~~ bacteriolyzed ~~action~~ not only ~~against~~ the Pasteurella pestis but also ~~against~~ Escherichia coli and Shigella dysenteriae. Flu also ~~proved~~ claimed that a combined transplantation of this phage with Pasteurella pestis ~~and~~ or Escherichia coli for several generations did not inhibit ~~the~~ its bacteriolytic ~~[illegible]~~ power ~~of the phage~~ against Pasteurella pestis, Escherichia coli and Shigella dysenteriae. Based on these findings, Flu claimed that these phages did not exist in combined forms.

Research on the isolation and studies on characteristics of Pasteurella pestis phage, as those mentioned above, ~~is~~ are few and many of ~~these~~ the points mentioned above need further clarification. I isolated Pasteurella pestis phage by the protamylase method and ~~have~~ investigated its characteristics. I also verified the relation between Pasteurella pestis variants and ~~the~~ bacteriolytic ~~actions~~ action of ~~the~~ phages of these variants ~~for the~~ particularly ~~object of investigating~~ to determine the specificity of the

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was transferred on identical agar slant cultures to determine the presence of phage in these suspensions. Furthermore, ~~with the object of~~ to detect ~~ing~~ the presence of this phage ~~by promoting its growth~~ through rapid growth promotion, approximately 1cc of this bacterial suspension was added to 9cc of ordinary bouillon and another platinum loopful ~~of~~ from an identical strain was transplanted together. This preparation was then incubated at 37°C for 45 hours, ~~This culture was~~ and then heat-treated at 60°C for 60 minutes until the bacteria were destroyed and then tested for bacterio~~phagic~~lytic action. This procedure was repeated over and over ~~for~~ with 3-4 generations and tested for the presence of phages.

Preparation of Slant Agar Culture

The entire surface of ~~a~~ ordinary agar slant ~~medium~~ ~~was~~ was smeared uniformly with Pasteurella pestis. Then a platinum loopful of ~~the~~ a phage preparation was dropped in the center of the slant surface (in this particular case a large platinum loop was specially prepared for ~~and~~ use). The test tube ~~was~~ was then immediately placed upright and incubated at 37°C for 45 minutes.

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Table I

A Table of Bacterial Strains used in Tests

No.	Identification No. of Bacterial Strains	Source of Strains	Date of isolation	Ordinary Agar Slants: Growth	Ordinary Agar Slants: Viscosity	Type of Colony	Virulence	Glycerine Analysis
1	86	adenoma of glandular plague case	2 Jul 1940	+++	+++	S	Strong	+
2	87	adenoma of glandular plague case	15 Jul 1940	++	++	S	"	+
3	101	liver of glandular plague case	28 Oct 1940	++	++	S	"	+
4	106	spleen of glandular plague case	11 Oct 1940	++	++	S	"	+
5	108	adenoma of glandular plague case	26 Oct 1940	++	++	S	"	+
6	142	blood from the heart of glandular plague case	4 Oct 1940	++	++	S	"	+
7	143	blood from the heart of glandular plague case	2 Oct 1940	++	++	S	"	+
8	151	blood from the heart of glandular plague case	2 Oct 1940	++	++	S	"	+
9	152	spleen of glandular plague case	10 Oct 1940	++	++	S	"	+
10	153	spleen of glandular plague case	4 Oct 1940	++	++	S	"	+
11	159	spleen of sewer rats during the epidemic in Hsin-ching	12 Oct 1940	++	++	S	"	+

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12	177	spleen of sewer rats during the epidemic in Hsin-ching	8 Oct 1940	+	+	S	"	+
13	184	Xenopsylla cheopis caught during the epidemic in Hsin-ching	10 Oct 1940	+	+	S	"	+
14	191	Xenopsylla cheopis caught during the epidemic in Hsin-ching	14 Oct 1940	+	+	S	"	+
15	1004	glandular plague case	1933	+	+	S	"	—
16	43	glandular plague case	1938	+	+	S	"	+
17	7	plague case (this specimen was kept at the Army Medical College for a long while)	?	+	+	S	weak	—
18	1	Otten's 'Tjiwidej' strain	1929	+	+	S	"	—
19	2	Girard's 'E. V.' strain	1926	+	+	S	"	—

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bacterial strains are given in Table 1. All these strains do not possess bacteriophage in their natural forms.

Table 1. A Table of Bacterial Strains used in Tests

(Table on separate paper) (See next page)

2. Isolation Methods

In accordance with the protamylase method an approximate 1% of protamylase was added to ordinary bouillon ~~with~~ pH, 7.2. This preparation was then agitated and filtered through Chamberland's L_3 filter. Approximately 10cc of this filtrate was then poured into test tubes and examined for possible contamination by ~~other~~ various microorganisms. After this sterility test, a platinum loopful of a 45 hour cultured test strain ~~bacteria~~, cultivated on ordinary agar and incubated at 37°C, was transplanted into this filtrate. These test tube cultures were then incubated at 37°C for a predetermined number of days ~~then~~ after which these cultures were heat treated at 60°C for 60 minutes. After the Pasteurella pestis was definitely destroyed, the bacterial suspension

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bacteriolytic action. I investigated also the bacteriolytic action of this isolated 'phage' against other types of bacteria and the bacteriolytic action~~s~~ of ~~bacterio~~phages of various origin against Pasteurella pestis. I found that the pestis phage ~~possessed~~ bacteriolyzed ~~actions~~ ~~against~~ certain types of Corynebacterium pseudotuberculosis rodentium and the R-type Bacillus metadysenteriae, and that the ~~bacterio~~phage of Corynebacterium pseudotuberculosis rodentium ~~possessed~~ bacteriolyzed ~~actions~~ only ~~against~~ the R-type variant of Pasteurella pestis. I herewith submit my reports ~~on~~ these interesting results and request your comments. ~~on them~~.

I. Isolation of Bacteriophage from Pasteurella pestis ~~Bacteriophage~~

1. Test Bacterial Strains and their Biological Characteristics

These bacterial strains were isolated during the plague epidemic in Hsin-ching and Nung-an in Oct, 1940, from plague cases, rodents ~~in~~ from these epidemic areas, fleas found on these rodents, and ~~these~~ strains that were kept in our laboratory. The source and general biological characteristics of these

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The resulting growth was then examined for possible bacteriolytic action.

3. Test Results

Based on the above isolation method, the presence of phage in these test strains was determined but none indicated positive results in the first series. This was assumed to be caused by the use of an old supply of protamylase in consideration of the positive test results obtained in the second series.

Table 2 gives test results in the second series, and according to this table, phages were detected in 2 of the 5-day old strains, 1 of the 7-day old strains, 1 of the 14-day old strains and 2 of the 21-day old strains.

Table 2. Results in phage detection

(Table given in separate paper)

Table 3 gives test results in the third series and according to this Table, phages were detected in 1 of the 5-day old strains, 1 of the 7-day old strains and 1 of the 14-day old strains.

Table 3. Results in phage detection

(Table given in separate paper)

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Table 2. Results in 'phage' detection

Age of cultures	3-day				5-day				7-day				14-day				21-day			
Number of generations	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Identification No. Bacterial Strains																				
86																				
87																				
101																				
106																				
108																				
142																	+	+	+	+
143																				
151																				
152	+	+	+	+																
153																				
129																	+	+	+	+
177																				
184	+	+	+	+																
191																				
1004																				
43																				
7																				
1					+	+	+	+	+	+	+	+								
2																				

Notes: — + is positive
blank spaces indicate negative results

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Table 3. Results in 'phage' detection

Age of cultures	5-day			7-day			14-day		
Number of generation	1	2	3	1	2	3	1	2	3
Identification No. of bacterial strains									
86									
87									
101									
106									
108									
142									
143									
151									
152									
153							+	+	+
157									
177									
184	+	+	+						
491									
1004				+	+	+			
43									
7									
1									
2									

Notes:—
+ is positive
blank spaces indicate negative results.

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Tables 2 and 3 show that phages were detected in 7 of the 19 test bacterial strains, and according to these results the age of the culture had no great effect upon detection of the phage.

Even in those cases where phages were detected (none of these indicated the presence of phages in) the first generation, nor produced positive results even after these cultures were cultured for 2 and 3 generations.

Aside from the protamylase method which was mentioned previously, I attempted to detect pestis phage in chicken droppings and intestinal contents of test animals (mice and guinea pigs) that died from plague but failed.

4. Summary

I detected phages in 7 of the 19 Pasteurella pestis strains by using agar slants and proceeding according to the protamylase method.

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II. Characteristics of Pasteurella Pestis phage

1. Tests on Increasing Bacteriolytic Power

During the initial stage immediately after isolation from their respective bacterial strains, these phages indicated very weak bacteriolytic action and barely produced bacteriolysis in 10^{-3} concentrations. However, when these phages were transplanted for generations with their respective bacterial strains (approximately 0.1cc of bacteriophagic suspension was added to approximately 10cc ordinary bouillon. Then a platinum loopful of the original strain that was cultured on ordinary agar for 45 hours and incubated at 37° was transplanted into this bouillon preparation. This preparation was then incubated at 37°C for 45 hours. Then this bouillon preparation was heat-treated at 60°C for 60 minutes until the bacteria were killed and then bacteriolytic characteristics were studied. Their bacteriolytic action rapidly increased and indicated bacteriolyses even in 10^{-7} and 10^{-8} concentrations (as shown in Table 4).

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These bacteriophagic suspensions were transplanted with various other bacterial strains and their bacteriolytic action found to be identically increased (as shown in Table 5).

Table 4. Tests on Increasing Bacteriolytic Action (Part 1)

(Table 4 is given in separate paper)

Table 5. Tests on Increasing Bacteriolytic Action (Part 2)

(Table 5 is given in separate paper)

The bacteriolytic action of each of these various phages were tested on different type of bacterial strain and results are given in Table 6. According to this Table these test phages bacteriolyzed all the virulent Pasteurella pestis strains. During the inital stage after isolation, these phages indicated weak bacteriolytic action and bacteriolyzed only their identical bacterial strains and had no effect against other strain. However, even these weak phages bacteriolyzed other type of strain after they were transplanted for generations as mentioned above, and thereby increasing their bacteriolytic potency (as shown in Table 7).

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Table 1. Tests on ~~the production~~ increasing of Bacteriolytic Action (Part 1)

Types of phage	Concentration / No of generation	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Phage 1004	1	+++	++	+	—	—	—	—	—	—	—
	2	+++	+++	+++	++	++	++	+	—	—	—
	3	+++	+++	+++	++	++	++	++	+	—	—
	5	+++	+++	+++	+++	++	++	++	+	—	—
Phage 1	1	+++	++	+	—	—	—	—	—	—	—
	2	+++	+++	+++	+++	++	++	+	+	—	—
	3	+++	+++	+++	++	++	++	+	+	+	—
	5	+++	+++	+++	+++	++	++	++	+	+	—
Phage 153	1	+++	++	+	—	—	—	—	—	—	—
	2	+++	+++	++	++	++	+	+	—	—	—
	3	+++	+++	+++	++	++	++	+	—	—	—
	5	+++	+++	+++	++	++	++	+	+	—	—

Notes: — 1. +++ complete bacteriolysis; ++ bacteriolytic vacuolar count 10-100; + bacteriolytic vacuolar count less than 10; — negative bacteriolysis. These definitions are applicable to all the following Tables
2. Phage 1004 was isolated from 1004 bacterial strain, etc.

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Table 5. Tests on ~~the promotion of~~ Increasing Bacteriolytic Action (Part 2)

Type of Phage	Type of Bacterial strain used to promote Bacteriolytic activity	No of generation	concentration 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Phage 1004	1	1	+++	++	+	—	—	—	—	—	—	—
		2	+++	+++	++	++	++	++	+	—	—	—
		3	+++	+++	+++	+++	+++	++	+	—	—	—
Phage 1	1004	1	+++	++	—	—	—	—	—	—	—	—
		2	+++	+++	++	++	++	++	++	+	—	—
		3	+++	+++	+++	++	++	++	++	+	—	—

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Table 6. Bacteriolytic test against different types of strain (Part 1)

Phage \ Bacterial strains	86	87	101	106	108	142	143	151	152	153	159	177	184	191	1004	143	7	1	2
phage 142	++	+++	+++	+++	++	++	++	++	+++	++	++	+++	+++	++	+++	++	+++	+++	++
" 152	++	++	+	+	+	++	+++	+++	++	++	+++	+++	+++	+++	++	+++	++	++	++
" 153	++	++	+	++	++	++	++	+++	++	+++	++	+++	++	+++	++	++	++	++	++
" 159	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	++
" 184	—	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—
" 1004	—	+	—	+	—	—	+	—	++	++	++	++	+	+	++	—	—	+	—
" 1	++	+++	+++	+++	++	++	++	++	++	++	+++	+++	++	+++	++	++	+++	+++	++

Table 7. Bacteriolytic test against different types of strains (Part 2)

Type of phage	Bacterial strains / Phage generation	86	87	101	106	108	142	143	151	152	153	159	177	184	191	1004	143	7	1	2
Phage 184	1	—	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—
	2	++	++	+	+++	++	++	+++	++	++	++	++	++	+++	+++	++	+++	++	+	+++
	3	++	+++	++	+++	+	++	++	+++	++	++	+++	+++	+++	+++	+++	+++	++	++	+++
Phage 1004	1	—	+	—	—	—	—	+	+	+	+	++	++	++	—	++	—	—	+	—
	2	++	++	++	++	+++	++	+++	++	+++	++	++	++	++	+++	+++	+++	++	++	++
	3	++	+++	+++	+++	++	+++	+++	+++	++	+++	++	+++	++	+++	++	+++	+++	++	++

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Table 8 - Resistance against Heat of Vaporization

Temperature / Input Phage	65°C	70°C	75°C
Phage 142	+++	+	—
" 152	++	—	—
" 153	+++	+	—
" 159	++	+	—
" 184	+++	—	—
" 1004	+++	+	—
" 1	+++	+	—

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Table 9. Experiments on "Elementary phage" Part 1

Bacterial Strain / Type of phage	1	1004	153	184	159
Phage 1	med. sm.	med. sm.	med. sm.	med. sm.	lg. med. sm.
" 1004	lg. med. sm.	med. sm.	med. sm.	lg. med. sm.	med. sm.
" 153	lg. med. sm.	lg. med. sm.	med. sm.	med. sm.	med. sm.
" 184	med. sm.	med. sm.	lg. med. sm.	med. sm.	med. sm.

lg. = large; med = medium; sm = small

Notes: — large = vacuolar formations larger than 0.5 cm diameter
small = " " smaller " 0.1 cm "
medium " " " between 0.5 — 0.1 cm

These explanations are applicable to the following table on elementary phage

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Table 10. Experiments on 'Elementary phage' Part 2

Bacterial strain / Type phage		1	1004	153	184	159
Phage 1	large	lg. med. sm.	med. sm.	med. sm.	med. sm.	lg. med. sm.
	small	med. sm.	" "	" "	lg. med. sm.	med. sm.
1004	large	lg. med. sm.	" "	lg. med. sm.	med. sm.	" "
	small	med. sm.	" "	med. sm.	" "	" "
153	large	med. sm.	" "	lg. med. sm.	" "	" "
	small	med. sm.	" "	" " "	" "	" "
184	large	med. sm.	" "	" " "	" "	lg. med. sm.
	small	med. sm.	" "	med. sm.	lg. med. sm.	med. sm.

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2. Tests on Heat Resistance

Test tubes were filled with approximately 5cc each of the original bacteriophagic suspensions and immersed in double boiler and then heated for 30 minutes at 65 - 70 - 75°C. The bacteriolytic ~~action~~ of these various bacteriophagic suspensions in vitro were tested after each series of heating and the resulting readings are given in Table 8. According to this Table, these [redacted]phages were more resistant to vaporization heat than Pasteurella pestis in general and were found to be wholly unaffected after an immersion for 30 minutes at 65°C and indicated some life even after an immersion for 30 minutes at 70°C. This proved that the resistance of these phages to heat differed according to their respective bacterial strains.

(insert Table 8 on pp 472-8 of original)

(Table 8 given in separate paper)

3. Tests on 'Elementary phage'

Bail (11) detected the [redacted]phage of Shigella dysenteriae in the filtrates of the

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stool and urine specimens from an afflicted case. He smeared this phage together with Shigella dysenteriae on the agar surface, resulting formations of bacteriolytic vacuoles. He classified the into large, medium, and small virgin groups and studied the biological and immunological characteristics of each. He found that phages forming small vacuoles and those forming medium and large vacuoles were different types and that these phages obtained from filtrates of stool and urine specimens were so-called 'mixed phages'. Bail advocated the practice of dividing all other types of phages as an essential factor in the study of phages.

Numerous scientists carried out similar research since Bail. Janzen and Wolff (12) studied the phage of Eberthella typhosa and came to a different conclusion, claiming that the formation of the large, medium and small vacuoles was not brought about by the action of phages but due to the action of the bacterial strains used. Seiffert (13) also came to a similar conclusion.

KUWANO isolated phages

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by the protamylase method and on the strength of his detailed research claimed that Bail's so-called 'division of phages' was not composed of independent groups of various types. He claimed that the formation of these various vacuoles was merely a phenomenon due to a change in the action of phages variously influenced by the interrelationship of these phages, and the toxicity of the strains used, the various characteristics of culture media, and the themselves.

(15)

On the other hand, KUROZAWA studied the Eberthella typhosa and Shigella dysenteriae phages which he obtained from the stool and urine specimens of a case and claimed that these were mixed phages as reported by Bail, and succeeded in classifying these phages into divisions.

(16)

YAGI also studied the phages of the genus Salmonella and succeeded in classifying these into divisions, and concluded that the grouping of these phages according to their vacuolar sizes was not a difficult matter if appropriate bacterial strains and phages were

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used. HANABU also isolated the bacteriophage of Salmonella typhimurium by the protamylase method and verified the existence of Bail's so-called 'elementary phage' by isolating a pure culture of large and small vacuolar forming phages.

I also investigated the existence of the 'elementary phage' in the Pasteurella pestis phage obtained by the protamylase method.

One minim each of these phages and a platinum loopful of a 45-hour old ordinary agar cultures of the test bacterial strain incubated at 37°C were transferred on ENDO's flat plate and uniformly smeared on the surface with Conradi's rod. These plates were then incubated at 37°C for 45 hours until vacuoles were formed. The resulting readings are given in Table 9. According to Table, most of the phages formed medium and small vacuoles in the test bacterial strains. Some formed large vacuoles. I isolated the large and small vacuoles and attempted to produce pure phage specimens of each.

(18)

(insert Table 9 given in separate paper)

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These small and large vacuolar formations were separately transplanted into ordinary bouillon together with their surrounding Pasteurella pestis furs and then cultured at 37°C for 45 hours. These cultures were then immersed in 60°C bath for 30 minutes until the Pasteurella pestis was destroyed. These were then transplanted with test bacterial strains and the resulting vacuolar formations were examined. Results of tests are given in Table 10 and according to this Table, small and medium vacuolar formations and at times even large vacuoles were present in the cultures prepared from small vacuolar formations, and large, medium and small vacuolar formations were found in cultures prepared with large vacuolar phages.

(insert Table 10 given in separate paper)

Repeated tests were made in my attempt to produce pure cultures of phage forming small and large vacuolar but failed. In other words, I failed to isolate Bail's so-called 'elementary phage' from this

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particular phage. Furthermore, the large, medium and small vacuolar formations found in Pasteurella pestis phage cultures were not various types of individual phages, as reported by KUWANO, but a temporary phenomenon.

4. Relation between Temperature used for culture and Bacteriolytic Action

The Pasteurella pestis formed an envelope when cultured at 37°C and its fur became viscous. However, when cultured below 26°C it did not produce an envelope and the fur became non-viscous. I therefore proceeded to determine whether there existed a relationship between the bacteriolytic action of the phages and the formation of this envelope. This phage was transplanted on slant agar and incubated at 37° and 20°C for 45 minutes. Then they were examined to determine their bacteriolytic action and the result of this test given in Table 11. According to this Table, there was no difference in the bacteriolytic action of both cultures. These cultures were also transplanted on flat plates but indicated no difference in their bacteriolytic action.

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Table 11. Relation between ~~Cultured~~ the Temperature used for culture and Bacteriolytic Action

~~Cultured~~ Temperature of culture	Bacterial Strains / Type of Phages	1	1004	153	143	159
37°C	Phage 1	+++	+++	+++	+++	+++
	" 1004	+++	+++	+++	+++	+++
	" 153	+++	+++	+++	+++	+++
20°C	" 1	+++	+++	+++	+++	+++
	" 1004	+++	+++	+++	+++	+++
	" 153	+++	+++	+++	+++	+++

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5. Summary

Phages that were isolated from the Pasteurella pestis not only ~~produced~~ showed bacteriolytic ~~process~~ action against their original strains, but also against other bacterial strains. It was also observed that these phages indicated an increase in ~~their~~ bacteriolytic ~~action power~~ action when transplanted in their original strains, or other bacterial strains.

The resistance of the phages ~~against~~ to steam is comparatively stronger than the Pasteurella pestis and it was observed that there existed a difference in this resistance according to their original strains.

We failed to obtain the 'elementary phage' from the pestis phage.

We also observed that there existed no relation between the temperature used in cultivation and ~~the~~ bacteriolytic action.

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III. Relation between the Pasteurella pestis bacteriophages and variants

Based on our research, we have already reported that Type E ⟶ Type N variant and Type S ⟶ Type R ⇄ Type RS variants existed. We therefore investigated ~~as to what sort of~~ the relationship that existed between the pestis phage and these variants.

1. Isolation of ~~the~~ bacteriophages from variants

Based on the method mentioned in Chapter 1 ~~Paragraph~~ Section 2, I isolated the phages ~~from~~ of No 145 and No 3 strains of Type N bacillus which bacteriolyze their respective strains and also from the R-variants of No 100, No 1 and No 145 strains and from the RS variants of No 1004 strain.

2. Characteristics of the bacteriophages isolated from the variants

These ~~various~~ phages that were isolated from the various variants showed increase in ~~their~~ bacteriolytic action ~~strength~~ ~~if~~ when they were ~~are~~ transplanted in their respective strains and also showed ~~indicated~~ an increase ~~in their bacteriolytic strength~~ when they were transplanted in other strains (in stock strains and other variants, in stock strains ~~strains~~ and variants of other bacilli). (Table omitted).

These phages were also tested ~~against~~ ~~host~~ and were found to be identically resistant ~~against~~ to

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heat as the phage that was isolated from the stock strain.

The relation between the bacteriolytic action of the phage and the temperature used in the cultivation of these phage cultures was investigated and ~~it was found~~ proved that 30°C and 20°C produced no change.

3. ~~The~~ Interaction of the bacteriophages

Phages that were isolated from the stock strain and variants were used in testing their bacteriolytic action~~s~~ against the stock and variant bacilli by the flat agar plate method ~~as~~ mentioned in Chapter ~~3~~ Sec from 3 ~~and~~ IN forming vacuoles in the media. The conclusive results from this test are given in Table 12, showing that the phages obtained from both the stock and variant strains acted upon these strains and produced identical results.

(insert Table 12 on page 472-12 of original TRANSLATED ON SEPARATE SHEET)

~~Only through a close observation of the~~ bacteriolytic condition, however, will reveal that both phages of the stock and variant strains produced, in most cases, medium and small vacuoles and at times large vacuoles against the stock strain (Type ES) and the Type N variant strain (Type NS). The borders of the vacuoles are translucent and the semi-clear portion developing between the boundries of the clear bacteriolyzed section and the bacterial fur is narrow

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Table 12. Relation between Variants and Bacteriolytic Action

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Type of Variants		E							
		S		R		RS		RT	
Type of Bacterial Strains		1	100L	1	100L	1	100L	1	100L
Type of Phage: ES	Phage 1	med. sm.	med. sm.	lg. med. sm.	lg. med. sm.	med sm	med sm	med sm	med sm
	" 100L	med. sm.	lg. med. sm.	lg med sm	med sm	med. sm	lg med sm	med sm	med sm
ER	" 1R	lg. med sm.	med sm.	lg. med sm	lg med sm	lg med sm	med sm	lg med sm	med sm
	" 100L R	med. sm.	lg. med sm.	lg med sm	lg med sm	med sm	med sm	med sm	med sm
NS	" 145	med. sm.	med. sm.	lg med sm	med sm	med sm	med sm	med sm	med sm
	" 3	med sm.	med. sm.	med sm	lg med sm	med sm	med sm	med sm	med sm
NR	" 145 R	med. sm.	lg med sm	lg med sm	med sm	med sm	lg med sm	med sm	med sm
	" 3R	med. sm.	med. sm.	lg med sm	lg med sm	med sm	lg med sm	med sm	med sm
RS	" 100L RS	med. sm.	med. sm.	lg med sm	med sm	med sm	med sm	med sm	med sm

Type of Variants		N							
		S		R		RS		RT	
Type of Bacterial Strains		145	3	145	4	145	3	145	3
Type of Phage: ES	Phage 1	lg med sm	med sm	lg med sm	lg med sm	med sm	med sm	med sm	med sm
	" 100L	med sm	med sm	lg med sm	med sm	med sm	med sm	med sm	med sm
ER	" 1R	med sm	lg med sm	lg med sm	med sm	med sm	med sm	med sm	lg med sm
	" 100L R	lg med sm	med sm	lg med sm	lg med sm	med sm	lg med sm	med sm	med sm
NS	" 145	med sm	med sm	med sm	lg med sm	med sm	med sm	med sm	med sm
	" 3	med sm	med sm	lg med sm	lg med sm	med sm	med sm	med sm	med sm
NR	" 145 R	med sm	med sm	lg med sm	med sm	med sm	med sm	lg med sm	med sm
	" 3R	med sm	lg med sm	med sm	lg med sm	med sm	lg med sm	med sm	med sm
RS	" 100L RS	med sm	med sm	lg med sm	med sm	med sm	med sm	med sm	med sm

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A close observation of the bacteriolytic characteristics indicated no relation between phages of the original and variant strains. However, these phages produced medium and small vacuolar formations in most cases when they were transplanted with their original strains(ES variant) and the N variant (NS variant) and at times produced large vacuolar formations. These vacuolar fomrations were clear along the borders and the partially transparent section forming the boundary between the transparent bacteriolyzed section and the bacterial fur (this section indicated weak bacteriolysis with a thin furry growth) followed the borderline of the vacuole in thin and uniform order. In this respect, the ES and the NS variants were identical.

Investigation of the bacteriolytic actions against the R variant showed that these phages, irrespective of their E or N, variant origin, produced large, medium and small vacuolar formations. There were more large vacuolar formation in this case in comparison to the test made on the S variant, and the bordline of the vacuoles slightly turbid.

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The partially transparent boundary between the transparent bacteriolyzed section and the furry growth was wider and followed the borderline of the vacuole irregularly and this characteristic differentiated the R Variant from the S variant. However, this difference was negligible and could not be clearly ascertained in some cases.

Many of the RS variants indicated bacteriolysis similar to that seen where the R variant was used.

The RT variant indicated bacteriolysis identical to that seen where the original S variant was used.

In summarizing these results, it was found that phages obtained from both the original and variant strains produced identical bacteriolytic results. However, a close investigation on these results indicated that some difference in the formation of vacuoles and in the vacuolar characteristics in the original S strain and the R variant existed.

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In other words, it was possible to find different bacteriolytic conditions in existence depending on the difference in the characteristics of the bacterial strains used.

4. ~~Resume of this Chapter~~ Summary

Phages obtained from both the variants and original strains of Pasteurella pestis indicated identical characteristics.

Investigation of the bacteriolytic phenomena of these phages against the original and variant strains of Pasteurella pestis indicated that the N and the original strains produced identical results but the R variant, in comparison to the original S variant, indicated some difference in the vacuolar formation and bacteriolytic characteristics.

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IV. Inter~~relationship~~action of ~~Bacteria~~ phages of Pasteurella pestis and other Bacteria

1. ~~Experiments~~ Tests on Corynebacterium pseudotuberculosis rodentium

~~Upon~~ From my investigation on the bacteriolytic action of the pestis phage against Corynebacterium pseudotuberculosis rodentium (Pfeiffer) through the use of slant agar, ~~[illegible]~~ I obtained ~~the~~ the results ~~[illegible]~~ given in Table 13. According to this Table, the pestis phage produced bacteriolysis against certain strains of Corynebacterium pseudotuberculosis rodentium ~~[illegible]~~ and none against others. Although the bacteriolytic action of these phages were about the same there ~~[illegible]~~ still existed ~~some~~ some ~~[illegible]~~ difference ~~[illegible]~~ depending on the type of phage.

(insert Table 13. ~~[illegible]~~ on page 472-13 of original)

Keys to Table 13

1. Table 13. Bacteriolytic action against Corynebacterium pseudotuberculosis rodentium (Part 1)
2. Bacterial Strain
3. phages

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4. Bacterial Strain

5. NISHI (TN: abbreviation of Personal Name)

The pestis phage was transplanted together with its original strain for generations and its action increased. The resulting phage was then tested on Corynebacterium pseudotuberculosis rodentium to determine the presence of bacteriolysis giving the results as shown in Table 14. According to this Table, there was in the bacteriolytic action practically no change although its bacteriolytic power had been increased by transplantation. Two strains of phages obtained from Corynebacterium pseudotuberculosis rodentium by the protamylase method, as in the case of the Pasteurella pestis, (one of these strains was donated by Capt TANAKA) were tested on Pasteurella pestis and their bacteriolytic action investigated, giving the results shown in Table 15. According to this Table, these phages had no effect on E and N variants of Pasteurella pestis, showed no bacteriolytic action against the original S type, but produced active bacteriolysis against the R variant. They also bacteriolyzed the RS variant and yet had no effect against the RT type. As I have previously mentioned,

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in the subject 'Research on Pasteurella pestis Variants', RS variant grows more readily than the R variant and the biological and serological characteristics of this RS variant is very similar to the R variant. On the other hand, the RT variant closely resembles the original S variant in its biological and serological charcteristics, although the RT variant originates from the R variant. If both these facts on their characteristics and their relation to the bacteriolytic action were considered together the ensuing result would prove very interesting.

(insert Table 14 on pp 472-14 of original)

Keys to Table 14

1. Table 14. Bacteriolytic Action against Corynebacterium pseudotuberculosis rodentium (Part 2)
2. phage
3. generation
4. bacterial strain

(insert Table 15 on pp 472-15 of original)

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Keys to Table 15

1. Bacteriolytic Action of Corynebacterium pseudotuberculosis podentium phage against Pasteurella pestis (Part 1)
2. phage
3. bacterial strain
4. type of bacteria

The Corynebacterium pseudotuberculosis rodentium phage indicated no bacteriolytic action against E and N variants as shown in Table 16.

(insert Table 16 on pp 472-15 of original)

1. Table 16. Same as Table 15 above (Part 2)
2. phage
3. bacterial strain
4. type of bacteria

The relative bacteriolytic action of the Corynebacterium pseudotuberculosis rodentium phage against Pasteurella pestis showed no change even after this phage was transplanted for generations with original strains and strengthened, its as shown in Table 17.

(insert Table 17 on pp 472-15 of original)

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Keys to Table 17

1. Table 17. Same as Table 15 above (Part 3)
2. phage
3. generation
4. bacterial strain

Next, Pasteurella pestis phage was transplanted with variants of Corynebacterium pseudotuberculosis rodentium (~~both~~ ~~the~~ variants which were bacteriolysed by this phage and variant which were not, ~~that~~ ~~become bacteriolysed and the variant that do not become bacteriolysed by this pestis~~ ~~phage~~ were both used) for several generations. The resulting phages were then tested ~~to determine their bacteriolytic actions~~ against Pasteurella pestis and Corynebacterium pseudotuberculosis rodentium. Results of these tests are given in Table 18. According to this Table, the phage that was transplanted with Corynebacterium pseudotuberculosis rodentium variant ~~which~~ and ~~showed~~ ~~become~~ bacteriolysis ~~by this phage~~ thrived for several generations ~~and did not indicated~~ with no indication of a decrease in its bacteriolytic power ~~potency~~ while ~~the phage that was~~ the one transplanted with the variant ~~which becomes~~ that showed no bacteriolysis did not ~~thrive.~~

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The Corynebacterium pseudotuberculosis rodentium phage was also transplanted together with the S and R variants of Pasteurella pestis (this S variant was not bacteriolyzed by the Corynebacterium pseudotuberculosis rodentium phage while the R variant was bacteriolyzed) for several generations. The resulting ~~bacterio~~phages were then tested ~~for their bacteriolytic actions~~ against Pasteurella pestis and Corynebacterium pseudotuberculosis rodentium and the results are given in Table 19. According to this Table, the Corynebacterium pseudotuberculosis rodentium phage that was transplanted with the R variant thrived ~~[redacted]~~ for generations and showed no decrease in its bacteriolytic action while the phage that was transplanted with the S variant not only retained its bacteriolytic action but also bacteriolyzed the S variant.

Summarizing the above results, it was found that the pestis phage possessed no specificity against Pasteurella pestis and indicated bacteriolytic action against

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certain variants of Corynebacterium pseudotuberculosis rodentium. The Corynebacterium pseudotuberculosis rodentium phage on the other hand, produced bacteriolysis only against the R variant of Pasteurella pestis.

The above facts proved that the Pasteurella pestis phage had the ability to thrive for several generations when transplanted with a variant of Corynebacterium pseudotuberculosis rodentium which this phage could bacteriolyze but could not thrive when transplanted with another variant which it could not bacteriolyze. On the other hand, the Corynebacterium pseudotuberculosis rodentium phage had the ability to thrive for several generations when transplanted with the R variant of Pasteurella pestis which this phage could bacteriolyze but when this phage was transplanted with the S variant of Pasteurella pestis, which it could not bacteriolyze, it not only thrived for several generations but also generated to the point where it bacteriolyzed this S variant.

These facts could be considered significant in regards to studies on the characteristics and true identity of phages.

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(insert Table 18 on pp 472-17 of original)

Keys to Table 18.

1. Table 18. Results of the transplantation of Pasteurella pestis phage with Corynebacterium pseudotuberculosis rodentium for generations
2. phage
3. Variants of Corynebacterium pseudotuberculosis rodentium used for transplantation
4. number of generations
5. Bacterial strains
6. Pasteurella pestis
7. Corynebacterium pseudotuberculosis rodentium
8. NISHI (TN: abbreviation of personal name)

(insert Table 19 on pp 472-18 of original)

Keys to Table 19

1. Table 19. Results of the transplantation of Corynebacterium pseudotuberculosis rodentium phage with Pasteurella pestis for generations
2. phage
3. Variants of Pasteurella pestis used for transplantation
4. number of generations
5. bacterial strains

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6. Pasteurella pestis

7. Corynebacterium pseudotuberculosis rodentium

2. ~~Experiments~~ Data on Bacillus metadysenteriae (OHARA Bacillus)

The bacteriolytic ~~potency~~ action of Pasteurella pestis phage against Bacillus metadysenteriae ~~was~~ has not yet been investigated by any one ~~as yet~~. I carried out an investigation on this and obtained results as given in Table 20. According to this Table, the pestis phage indicated absolutely ~~completely~~ no bacteriolytic action against the S variant of Bacillus metadysenteriae but ~~displayed a~~ showed very ~~conspicuous~~ active bacteriolytic ~~action~~ against the R variant.

(insert Table 20.on pp 472-19 of original)

Keys to Table 20.

1. Table 20. The bacteriolytic action of Pasteurella pestis phage against Bacillus metadysenteriae
2. bacterial variants
3. phage
4. bacterial strains (variants of Bacillus metadysenteriae

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[redacted] Phages of Bacillus metadysenteriae were isolated by the protamylase method and tested for bacteriolytic action against the Bacillus metadysenteriae. According to these tests the phages that were isolated from the S variant bacteriolytic only while these isolated from the R variant bacteriolytic only the R variant. Results are given in Table 21. These phages were then tested against Pasteurella pestis and the bacteriolytic results are given in Table 22 showing completely negative results.

(insert Table 21 on pp 472-19 of original)

Keys to Table 21.

1. Table 21. The bacteriolytic action of Bacillus metadysenteriae phages against the variants of Bacillus metadysenteriae
2. phages
3. Variants of Bacillus metadysenteriae

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(insert Table 22 on pp 472-20 of original)

Keysto Table 22

1. Table 22. The bacteriolytic ~~potency~~ action of Bacillus metadysenteriae against Pasteurella pestis

2. phages

3. Variants of Pasteurella pestis

4. Type of Bacterila Strains

Contrary to the Corynebacterium pseudotuberculosis rodentium phage having a somewhat identical bacteriolytic action as the pasteurella pestis phage against Bacillus metadysenteriae (shown in Table 23), the Bacillus metadysenteriae phage indicated absolutely no bacteriolytic action against Corynebacterium pseudotuberculosis rodentium (as shown in Table 24).

(insert Table 23 on pp 472-20 of original)

Keys to Table 23

1. Table 23. The bacteriolytic ~~potency~~ action of Corynebacterium pseudotuberculosis rodentium against Bacillus metadysenteriae

2. phages

3. bacterial variants

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(insert Table 24 on pp 472-20 of original)

Keys to Table 24

1. Table 24. The bacteriolytic ~~function~~ action of Bacillus metadysenteriae phage against Corynebacterium pseudotuberculosis rodentium

2. phages

3. bacterial variants

4. NISHI ~~(TN: abbreviation of personal name)~~

The pestis phage which failed to ~~indicate~~ show bacteriolytic ~~function~~ action against the Bacillus metadysenteriae, due to its weakness during the initial stage after ~~the~~ isolation, ~~indicated~~ showed bacteriolytic ~~function~~ action against the R variant of Bacillus metadysenteriae ~~after~~ ~~it~~ after it was transplanted for generations with ~~its~~ its original strain ~~and~~ which increased its power.

~~Considering these facts~~ In accordance with these facts, if ~~the~~ Bacillus metadysenteriae phage ~~become~~ were to be mixed with Pasteurellla pestis phage and ~~then~~ these mixed phages ~~were to be~~ transplanted together with Pasteurella pestis, then the Bacillus metadysenteriae phage should gradually become weakened and finally ~~dissolved~~ disappear; but on the contrary, the intensified bacteriolytic ~~function~~ action of ~~the~~

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Pasteurella pestis phage against Bacillus metadysenteriae signify ~~the fact~~ that ~~the~~ Bacillus metadysenteriae phage did ~~was~~ not mix with the Pasteurella pestis phage. There ~~were~~ some irregular type of phages like the one isolated from the RS variant of Type 1004 strain that showed ~~indicated~~ bacteriolytic action ~~function~~ against Bacillus metadysenteriae (results given in Table 25).

(insert Table 25 on pp 472-21 of original)

Keys to Table 25

1. Table 25. ~~The~~ Bacteriolytic action ~~function~~ of Pasteurella pestis phage against Bacillus metadysenteriae
2. phages
3. number of generations
4. bacterial strains (variants)

On the other hand, the Bacillus metadysenteriae phages showed ~~indicated~~ no bacteriolytic action ~~function~~ against ~~the~~ Pasteurella pestis even though they were transplanted and strenghtened for many generations (results given in Table 26.)

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(insert Table 26 on pp 472-22 of original)

Keys to Table 26

1. Table 26. ~~The~~ Bacteriolytic ~~function~~ action of Bacillus metadysenteriae phage against Pasteurella pestis

2. phages

3. number of generations

4. bacterial strains (variants)

~~The~~ Pasteurella pestis phage was transplanted with the R variant of Bacillus metadysenteriae for generations and the bacteriolytic ~~function~~ action of the resulting phage was tested ~~for~~ against Pasteurella pestis and Bacillus metadysenteriae with results ~~given~~ shown in Table 27. According to this Table, the Pasteurella pestis phage thrived for several generations and indicated no decrease in ~~the~~ bacteriolytic ~~function~~ action against Pasteurella pestis and the R variant of Bacillus metadysenteriae. When this phage was transplanted with the S variant of Bacillus metadysenteriae and Shigella dysenteriae, both of which were not bacteriolyzed by the pestis phage, ~~then~~ it lost its power to bacteriolyze ~~lytic function against~~ Pasteurella pestis and

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the R variant of Bacillus metadysenteriae during the 5th and 6th generation. (results given in Table 28).

(insert Table 27 on pp 472-23 of original)

Keys to Table 27

1. Table 27. Transplantation of Pasteurella pestis phage with Bacillus metadysenteriae for generations (Part 1)

2. phages

3. Bacterial strains used for transplantation

4. number of generations

5. bacterial strains

6. Pasteurella pestis

7. Bacillus metadysenteriae

8. No 9 strain of Bacillus metadysenteriae

9. No 5 strain of Bacillus metadysenteriae

(insert Table 28 on pp 472-24 of original)

Keys to Table 28

1. Table 28. Transplantation of Pasteurella pestis phage with Bacillus metadysenteriae and Shigella dysenteriae for generations (Part 2)

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2. phages

3. bacterial strains used for transplantation

4. number of generations

5. bacterial strains

6. Pasteurella pestis

7. Bacillus metadysenteriae

8. Shigella dysenteriae

9. HIGASHI (TN: abbreviation of personal name)

10. No 4 strain of Bacillus metadysenteriae

11. F type Shigella dysenteriae

12. No 4 strain of Bacillus metadysenteriae

When the Bacillus metadysenteriae phage was transplanted with Pasteurella pestis for several generations, it lost its power to bacteriolyz~~ic~~ ~~function~~ ~~against~~ Bacillus metadysenteriae after the ~~its~~ 4th or 5th generation and ~~indicated~~ showed absolutely no bacteriolytic action ~~function~~ also against Pasteurella pestis (results given in Table 29).

(insert Table 29 on pp 472-25 of original)

Keys to Table 29

1. Table 29. Transplantation of Bacillus metadysenteriae phage with Pasteurella pestis

2. phages

3. bacterial strains used in transplantation

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4. number of generations

5. bacterial strains

6. Pasteurella pestis

7. Bacillus metadysenteriae

In summarizing the above results, it was found that the Pasteurella pestis phage ~~possessed~~ bacteriolyzed ~~function against~~ the R variant of Bacillus metadysenteriae and although it retained its bacteriolytic action ~~function~~ through transplantation with the R variant of Bacillus metadysenteriae it ~~was unable to~~ could not retain its bacteriolytic ~~power~~ action when transplanted with the S variant of Pasteurella pestis and Shigella dysenteriae. Contrary to this, the Bacillus metadysenteriae phage was transplanted for 25 generations with its original strains but showed no bacteriolytic action ~~function~~ against Pasteurella pestis. When this Bacillus metadysenteriae phage was transplanted with Pasteurella pestis for several generations, however, it was found that this phage not only failed to produce bacteriolytic action ~~function~~ against Pasteurella pestis but also lost its power to bacteriolyze ~~function against~~ Bacillus metadysenteriae.

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According to the above results, phages of Pasteurella pestis and the R variant of Bacillus metadysenteriae had different characteristics, although their bacteriolytic action against the R variant of Bacillus metadysenteriae remained the same. The Pasteurella pestis phage, which was isolated by the protamylase method, bacteriolyzed and thrived for several generations when transplanted with the R variant of Bacillus dysenteriae. Consequently, this pestis phage could be considered as a type of Bacillus metadysenteriae phage. However, it is a very significant fact that the isolation of such a phage directly from Bacillus metadysenteriae can not be done without the use of Pasteurella pestis as an agent. Such data is of interest to those studying the genetic order of phages that were isolated by the protamylase method.

Formerly, there was doubt as to whether the Bacillus metadysenteriae which was used in the transplantation of Pasteurella pestis phage contained phages, but subsequent research proved that this was not so according to results obtained (Table 30).

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(insert Table 30 on pp 472-26 of original)

Keys to Table 30

1. Table 30. Tests in the determination of the presence of phages in Bacillus metadysenteriae

2. phages

3. bacterial strains

3. ~~Experiments~~ Tests on Other Bacteria

Bacteriolytic ~~functions~~ action of Pasteurella pestis phage against other type of bacteria were investigated since it was proven that it ~~possesses~~ bacteriolytic ~~its functions~~ not only ~~against~~ the Corynebacterium pseudotuberculosis rodentium but also the R variant of Bacillus metadysenteriae, as mentioned previously.

This pestis phage had no bacteriolytic effect upon Shigella dysenteriae regardless of ~~the type~~ its source and the Shigella dysenteriae phage also had no effect on Pasteurella pestis (results given in Tables 31 and 32).

(insert Table 31 on pp 472-27 of original)

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Keys to Table 31

1. Table 31. ~~The~~ Bacteriolytic ~~function~~ action of Pasteurella pestis phage against Shigella dysenteriae

2. bacterila strains

3. phages

4. type of bacteria

5. Shigella dysenteriae

6. Shigella of Flexner (Shigella paradysenteriae)

7. Bacillus 'Y'
8. SUGI
9. KUNI/ KOKU
10. HIGASHI/ TO
11. FUJI/ TO
12. KAWA

(TN: abbreviations of personal names)

(insert: Table 32 on pp 472-27 of original)

Keys to Table 32

1. Table 32. ~~The~~ Bacteriolytic ~~function~~ action of Shigella dysenteriae phage against Pasteurella pestis

2. phages

3. bacterial strains

4. type of bacteria

5. Shiga

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6. F (TN: Flexner)
7. Y (TN: Bacillus 'Y')
8. SUGI
9. KUNI
10. HIGASHI
11. FUJI
12. KAWA

(TN: abbreviations of personal names)

The relation between Shigella dysenteriae phage and Corynebacterium pseudotuberculosis rodentium was the same as ~~the relation~~ ~~was~~ between Shigella dysenteriae phage and Pasteurella pestis (results given in Tables 33 and 34) (insert Table 33 on pp 472-27 of original)

Keys to Table 33.

1. Table 33. ~~The~~ Bacteriolytic ~~function~~ action of Corynebacterium pseudotuberculosis phage against Shigella dysenteriae
2. phages
3. bacterial strains
4. SHI ~~(TN:~~
5. SHUGI
6. KUNI
7. HIGASHI
8. FUJI
9. KAWA

(TN: abbreviations of personal names)

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(insert Table 34 on pp 472-28 of original)

Keys to Table 34

1. Table 34. ~~The~~ Bacteriolytic action ~~function~~ of Shigella dysenteriae phage against Corynebacterium pseudotuberculosis rodentium

2. phages

3. bacterial strains

4. NISHI
5. SHI
6. SUGI
7. KUNI
8. HIGASHI
9. FUJI
10. KAWA

(TN: abbreviations of personal names)

Tests on the bacteriolytic action ~~function~~ of Pasteurella pestis phage against Eberthella typhosa, Brucella, Salmonella paratyphi, Salmonella schottmuelleri, Salmonella hirschfeldii and Salmonella enteritidis indicated that it ~~the phage~~ had no effect on these bacteria (Table sowing results of these tests are omitted).

The bacteriolytic interaction ~~relationship~~ between Bacillus metadysenteriae and Shigella dysenteriae phages is given in Table 35 for reference.

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(insert Table 35 on pp 472-28 of original)

Keys to Table 35

1. Table 35. ~~The~~ Bacteriolytic inter~~re~~action ~~lationship~~ between Bacillus metadysenteriae and Shigella dysenteriae phages

2. phages
3. bacterial strains
4. Bacillus metadysenteriae
5. Shigella dysenteriae
6. Shiga
7. SUGI
8. KUNI
9. HIGASHI
10. FUJI
11. KAWA
12. Bacillus metadysenteriae
13. Shigella dysenteriae
14. Shiga
15. SUGI
16. KUNI
17. HIGASHI
18. FUJI
19. KAWA

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4. ~~Resume! of this Chapter~~ Summary

I investigated the bacteriolytic ~~function~~ action of pestis phage against other type of bacteria and also the bacteriolytic action of phages of other ~~phages of other~~ types of bacteria against Pasteurella pestis. I found that the pestis phage bacteriolyzed certain types of Corynebacterium pseudotuberculosis rodentium and the R variant of Bacillus metadysenteriae.

The Corynebacterium pseudotuberculosis rodentium phage ~~indicated~~ showed bacteriolytic ~~function~~ action only against the R variant of Pasteurella pestis and the Bacillus metadysenteriae phage ~~indicated~~ showed absolutely no bacteriolytic ~~function~~ action against Pasteurella pestis.

Several tests were made to determine the inter~~relationship~~action of the bacteriolytic characteristics of Bacillus metadysenteriae and Pasteurella pestis phages. According to the results ~~from~~ of these tests, it was possible by the protamylase method to isolate directly from Bacillus metadysenteriae and indirectly from Pasteurella pestis phages ~~with~~ having specificity against Bacillus metadysenteriae. These phages

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may be trans lanted for generations with Bacillus metadysenteriae but ~~it proved~~ it was impossible to isolate identical phages directly from Bacillus metadysenteriae unless Pasteurella pestis was once used as an agent. It is a very interesting fact that this certain type of Bacillus metadysenteriae phage can be isolated only ~~when~~ after Pasteurella pestis ~~is~~ was used as an agent.

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Summary and Considerations

My attempts in carrying out research on Pasteurella pestis phage resulted in the detection of phage in 7 of the 19 Pasteurella pestis strains by the protamylase method. I found that the use of old protamylase made the detection of phages difficult and also that the detection rate was unchanged by the period of time required by the protamylase to act against Pasteurella pestis.

I also attempted to detect pestis phage in chicken droppings and in the intestinal contents and droppings of test animals (mice and guinea pigs) that died from plague, but failed to isolate any. This proved that it was comparatively more difficult to detect phages in the Pasteurella pestis than in Enterobacteriaceae.

Next, I investigated the characteristics of pestis phages which were isolated by the protamylase method. These phages indicated bacteriolytic not only against their original strains but also against other strains in general, and their bacteriolytic power increased when transplanted with their original bacterial strain or other type of

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strains. Moreover, phages that ~~indicated~~ showed bacteriolytic ~~function~~ action only against their original strains during the initial stage after they were isolated were found to act also against other ~~type of~~ strains ~~after~~ after they were transplanted for generations and their ~~potency~~ power increased.

The Pasteurella pestis phage indicated comparatively stronger resistence against vaporization heat than the Pasteurella pestis and showed ~~a little~~ some difference in resistence ~~according to~~ depending on the original bacterial strain.

Bail's so-called 'elementary phage' phenomenon was not detected in the pestis phage and there existed no relation between the degree of temperature used in the cultivation of bacteria and their bacteriolytic action.

The ~~relation between the~~ variants of Pasteurella pestis ~~phage~~ and the bacteriolytic functions of pestis phage was investigated and ~~it~~ was found that there existed no relation between the E→N variants of Pasteurella pestis and the pestis phage. Phages that were isolated from E and N variants ~~were~~ had identical characteristics and ~~there were~~ showed absolutely no difference in ~~the~~ their bacteriolytic action. ~~of~~ In consideration

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of these facts, it could be assumed that the existence or non-existence of antigenic envelopes had no influence on the bacteriolytic action of the pestis phage.

Relation between the S → R ⇄ RS variants of Pasteurella pestis and the pestis phage was investigated and found that phages isolated from the R and RS variants were characteristically identical to that isolated from the S variant. Investigation of their bacteriolytic phenomena indicated that phages of the S, R and RS variants all produced in most cases medium and small vacuoles against the S variant and at times produced large vacuoles. Borders of these vacuoles were clear. On the other hand, these phages produced large, medium and small vacuoles against the R and RS variants, and in comparison to the S variant, produced large vacuoles more often. The borders of these vacuoles differed from that of the S variant and were slightly turbid.

It could be clearly seen from the above results that pestis phages isolated from the S and the R variants cannot be diffe-

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rentiated. However, these phages clarified the existence of dissimilar bacteriolytic characteristics against the S variant in comparison to the R and RS variants. It was also found that the Corynebacterium pseudotuberculosis rodentium phage ~~indicated~~ showed bacteriolytic ~~function~~ action only against the R and RS variants of Pasteurella pestis and none against the S variant, and [redacted] the RT variant.

These investigational results [redacted] clarify [redacted] the existence of the S, $\rightarrow$ R $\rightleftarrows$ RS variants of Pasteurella pestis all the more.

The specificity of the pestis phage was next investigated and it was found that this phage ~~indicated~~ showed bacteriolytic ~~function~~ action only against certain types of Corynebacterium pseudotuberculosis rodentium strains. ~~Considering~~ Both the above results [redacted] and the fact that the Corynebacterium pseudotuberculosis rodentium phage ~~indicated~~ showed bacteriolytic ~~function~~ action only against the R variant of Pasteurella pestis [redacted] proved very interesting. The question of [redacted] what difference exists between the Corynebacterium pseudotuberculosis rodentium that ~~becomes~~ is bacteriolyzed by the pestis phage

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and the one that does not ~~become bacteriolyzed~~, ~~by the pestis phage~~ is a very interesting ~~problem~~ subject which should be studied and clarified in the future.

The Pasteurella pestis phage also ~~indicate~~ showed bacteriolytic ~~function~~ action only against the R variant of Bacillus metadysenteriae and none against the S variant. This ~~fact~~ result also ~~proved that~~ clarified the point that ~~it~~ this phenomenon was not brought about by the possible infiltration of Bacillus metadysenteriae phage into the pestis phage. However, both these phages that were isolated from the S and R variants of Bacillus metadysenteriae ~~indicated~~ showed absolutely no bacteriolytic action against Pasteurella pestis.

Aside from the above tests, the pestis phage was also tested against Shigella dysenteriae, Eberthella typhosa, Brucella, Salmonella paratyphi, Salmonella Schottmülleri, ~~and~~ Salmonella hirschfeldii and Salmonella enteritidis and was found ~~that it had~~ to have no bacteriolytic ~~action~~ action against any of these bacteria.

Synthetic phages isolated by the protamylase method were principally used in

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these tests. However, it is my belief that when natural phages ~~could be~~ are isolated in the future and ~~and their characteristics~~ studied, the charcteristics of Pestis phage ~~could~~ will be further clarified. ~~all the more and very interesting, data could be obtained.~~

Conclusions

I isolated pestis phages from 7 out of the 19 Pasteurella pestis strains by the protamylase method, investigated their characteristics, and in particular, clarified the relation between the variants of Pasteurella pestis and the bacteriolytic ~~functions~~ action of the [redacted] phages.

I investigated also the specificity of the pestis phages and also the bacteriolytic ~~functions~~ action of [redacted] other bacterial phages against Pasteurella pestis. The pestis phage ~~indicated~~ showed bacteriolytic action against certain [redacted] variants of Corynebacterium pseudotuberculosis rodentium and the R variant of Bacillus metadysenteriae, while the Corynebacterium pseudotuberculosis rodentium phage ~~indicated~~ showed bacteriolytic ~~function~~ action only against the R variant of Pasteurella pestis.

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