August 24, 1954

Dear Professor Lederberg :

Thank you for your letter of August 4. I am very glad to hear that you have been appointed as professor of Genetics. Allow me to congratulate you !

I wish to express my appreciation for your kind suggestions and inquiries. I will do my best to answer. And I do not know how to thank you enough for correcting my papers and even offering to read the galley proof. I could ask for nothing better.

Unfortunately I had an acute attack of appendicitis and have been hospitalized. I hope you will understand the delay.

Thanking you again for everything.

Yours sincerely,

Hisao Uetake

Hisao Uetake
Dept. of Microbiology
Sappro Medical College

HU:tm

page 3 "4". By broth I mean the usual nutrient broth (bouillon). Mixed cultures were incubated at 37°C for 24 hours. They are standing cultures, not aerated.

page 5 "9". The phage suspension used for enzymatic treatments was not an autolysate preparation itself. It was a high titer stock, prepared by propagation by serial transfers of phages on S. anatum.

page 6 first paragraph: When found, altered colonies were found at a rate of one or more out of 20 . It was confirmed that all of the autolysates were capable of forming plaques on S. anatum or S. butantan, though the exact titrations of all the autolysates were not carried out. Serological comparison of the phages and comparison of their host ranges are now under study.

page 11 VIII. Even after one minute exposure, one or more out of 20 colonies were found to be agglutinable by 15 antiserum.

TABLE 3 Rate of phage-sensitive and antigenically altered cells.

One minute exposure is too short to carry out a quantitative determination of phage-sensitive and antigenically altered cells. Then, the quantitative determination was carried out after 30 minutes exposure and the following is an example of data on this series of experiments.

	Dilution	No. of total colonies[x]	No. of translucent colonies[x]	No. of altered colonies among 50 non-translucent colonies
Active phage ($5x10^8$ particles/ml) + S. anatum (10^8 cells/ml)	$1:10^4$	416	20	33
	$1:10^5$	40	2	21
Heat-inactivated phage (same as above) + S. anatum (same as above)	$1:10^6$	104	0	0
	$1:10^7$	7	0	0

The phage particles (from S. canoga) and the E_1 group cells (20-hour-old agar culture of S. anatum) were mixed at the rate of 5:1, kept standing at room temperature for 30 minutes and plated out for viable count. ~~Yielded~~ Surviving colonies were also tested serologically.

x Mean number from 5 plates

This experiment revealed the following.
(i) About 40 out of 700-1040 cells survived after exposure to phage.
(ii) About 40 to 60 per cent ~~among~~ of non-translucent colonies were of antigenic variants.
(iii) All of translucent colonies were found to be agglutinable by 15 antiserum,

2) and 3) that you have been kind enough to point out in the letter have not been made clear as yet. (Thank you for your information on translucent colonies. I am not as yet acquainted with your paper in Genetics, since it is not available here. Though translucent colonies have been found to contain antigenical variant and non-variant cells, ~~other cells,~~ other details are not clear as yet. The details will be studied further.)

page 9 In each of antigenic variants 2 or 3 isolations were tested for lysogenicity and conversion ability.

page 12 As biochemical behaviors the followings were examined.
In peptone broth medium, fermentation of glucose, mannitol, dulcitol, sorbitol, inositol, maltose, arabinose, rhamnose, xylose, trehalose, adonitol, salicin, saccharose and lactose.
In Bitter's medium, fermentation of arabinose, dulcitol, glucose and rhamnose.
Beside the above indole production, gelatin liquefaction, milk coagulation, decomposition of urea and acetyl-methyl-carbinol formation were tested.

page 13 I agree with your opinion "s" should be corrected to direction of antigenic changes found after exposure to antiserum.

page 14 "4)". The experiments for the statements "4)" were as follows.

B. A small number of phages from S. canoga were added to nutrient broth in test tube. In this phage-broth mixture S. anatum was cultivated at 37°C for 20 hours. The bacterial cells were centrifuged, the supernate was removed, filtered through Chamberland L_3 filter and the filtrate was added to broth culture of S. anatum which had been incubated for about 8 to 10 hours. After further incubation for 3 to 4 hours, phage suspension was obtained by centrifugation and filtration and phages were propagated again in the same way as described above. These processes were repeated through several serial passages, and finally phage suspension of relatively high titer was obtained, containing about 4×10^9 particles per ml estimated by plaque count. This phage, which had been serially propagated on S. anatum was still effective in converting S. anatum and S. butantan. Thus, the effectiveness of the phage is independent of the host (contra transduction, Zinder and Lederberg, 1952)

Cells of S. butantan (or S. anatum) were mixed with phage suspension, kept at room temperature for 30 to 60 minutes and plated out on agar plate. Many antigenic variant colonies were found the following day. (~~This method for obtaining high titer stock~~ of phage is so routine ~~that it did not even~~ enter my head to record the above experiment.)

Title : I am all for the title you have suggested.

page 1 and 2, and page 16 section 12). I respect your opinion. Kindly do what you think would be best.

page 18-19 15). You have suggested a shorter summary. I should think that it would not be often for non-member such as I to have his papers accepted. So I feel that I should take advantage of this opportunity and put in as much as I can, for I have no idea when my next chance will come. What is your opinion on this, Professor ? However, I have included a shorter summary in case you think I will be given another opportunity.

Shorter summary of 15) :

Taking H antigens beside O antigens into consideration, when O antigens of S. anatum, S.nyborg, S. meleagridis, [illegible] S. give and S. uganda are altered from 3, 10, to 3, 15, these strains are changed to forms which are indistinguishable from S. newington, S. selandia, S. cambridge, S. [illegible] new brunswick and S. kinshasa respectively. On the other hand comparison of the sources from which they were isolated shows that each pair of S. anatum and S. newington, S. nyborg and S. selandia, S. give and S. new brunswick, and S. lexington and S. illinois has been isolated from similar sources respectively.

Besides, the corresponding two types are identical or almost identical in biochemical behaviors with each other.

These findings may suggest that type strains of E_2 group might have been yielded as a result of infection of E_1 group organisms with phages, in nature, and this might be supported by the fact that more types have been isolated in E_1 group than in E_2 group and each type in E_2 group has its corresponding type in E_1 group.

TABLE 1

Activity of bacteriophages obtained from E_2 group organisms inducing changes in O antigens of E_1 group organisms

E1 GROUP STRAIN		PHAGES							
* No.	Type	S. newington C2	S. selandia 7482	S. newbrunswick 5411	S. cambridge	S. kinshasa	S. canoga	S. illinois	S. thomasville
76	S. london 1446	-	-	+					
77	S. give 316	--	+	+					
78	S. anatum 293	+o	+o	+o	o	o	o	o	o
81	S. amager 2399	+	-	+					
82	S. zanzibar 5628	+	+	+					
83	S. shangani 5630	+	+	-					
101	S. uganda$_a$	+	+		+		+		
189	S. butantan	o	•	o	+o	+o	+o	+o	+o
119	S. vejle				+	+	+	-	-
109	S. meleagridis				+	+	+	-	-
294	S. elisabethville				+	+	+	-	-
282	S. simi				+	+	+	-	+
139	S. weltevreden				+	+	+	-	+
190	S. orion				+	+	+	+	+
123	S. lexington				✚	✚	✚		
273	S. macallen				✚	✚	✚		

+ = Antigenic changes from 3,10 to 3,15
✚ = Antigenic changes from 3,10 to 3,10,15
- = No antigenic change within 6-10 subcultures

* - Standard strain number designated by F, Kauffmann

o = Plaque formation

TABLE 2

Changes in the O antigens of E_3 group organisms, induced by bacteriophages obtained from E_2 group organisms

E_3 GROUP STRAIN		NO. OF CULTURES	PHAGES	ANTIGENIC CHANGES		
No.*	Type				From	To
197	S. chittagong	1 1	S. newington C_2 S. canoga	+ +	1,3,10,19 1,3,10,19	3,15 3,15
88	S. niloese 1236	1 1	mixture# S. canoga	+ -	1,3,19	1,3,15,19
	S. senftenberg Aa'	1	S. canoga	+	3	3,15
140	S. senftenberg-simsbury	1	mixture# S. canoga	+ -	1,3,19	1,3,15,19
	S. senftenberg HS1 - HS10	10	mixture#	-		

\+ Antigenic change
\- No antigenic change within 6-10 subcultures
* Same as in table 1
\# Mixture of phages, obtained from S. newington, S. selandia and S, newbrunswick, and 1,19 antiserum, prepared by absorbing S. niloese "O" antiserum with S. london.

TABLE 3 Comparison of the sources between closely related pairs of types

FIRST REPORT	TYPE	ISOLATED FROM	
		Animal	Human
1919-20	S.anatum	Ducklings (U.S.A.) Retail pork (U.S.A.) Chickens and turkeys (U.S.A.) Amer.spray-dried eggs (G.B.) MLN of normal pigs (U.S.A.;Mex.) Silver fox (U.S.A.) Dog (U.S.A.)	Infantile diarrhoea (Uru.) Feces of healthy persons (Hung.;U.S.A.) Food poisoning (G.B.) Gastroenteritis (U.S.A.)
1937	S.newington	Ducklings (U.S.A.) Retail pork (U.S.A.) Chickens and turkeys (U.S.A.) Amer.spray-dried egg (G.B.) MLN of normal pigs (U.S.A.;Uru.) Silver fox (U.S.A.) Dog (U.S.A.)	Gastroenteritis (U.S.A.) Sewage (U.S.A.) Carriers (U.S.A.)
1936-37	S.nyborg		Gastroenteritis in a child (U.S.A.)
1937	S.selandia		A young sailor with fever, lung symptoms and diarrhoea (Den.)
1937	S.give	MLN of normal pigs (U.S.A.) Retail pork (U.S.A.) Chickens (U.S.A.) Gastroenteritis of dog (U.S.A.) Amer.spray-dried eggs (G.B.) Dog(Mex.)	Long-standing diarrhoea (Spain) Gastroenteritis;Enteric fever (U.S.A.) Carrier (U.S.A.)
1937	S.new brunswick	MLN of normal pigs (U.S.A.) A baby chick (U.S.A.) Dog (U.S.A.;Mex.)	Gastroenteritis in a woman (Den.) A patient who had returned from tropics(Den.)
1941	S.meleagridis	Turkey poults (U.S.A.) MLN of normal pigs (Mex.) Reptiles (U.S.A.) Dog (Mex.;U.S.A.) Amer. dried eggs (G.B.)	Typhoid-like fever (Venezuela;Medit.Area) Infant diarrhoea (Uru.) Sewage (U.S.A.) Gastroenteritis(U.S.A.) Carrier(Medit.Area) German soldier(Norway)Food poisoning(Medit.Area)
1947	S.cambridge		A soldier suffering from Sonne dysentery(G.B.)
1940	S.lexington	Turkeys;MLN of normal pig (U.S.A.)	Carrier (U.S.A.)
1941	S.illinois	Turkeys (U.S.A.) Pigs (U.S.A.) Hungarian partridges (U.S.A.)	Gastroenteritis(U.S.A.) Carrier(U.S.A.)
1940	S.uganda		Pyrexia of unknown origin(Uganda)
1950	S.kinshase		
1949	S.canoga	10-day old poult (U.S.A.)	
1930	S.senftenberg var.newcastle		Carrier (G.B.)
1929	S.senftenberg	Young turkeys(U.S.A.) Chickens(U.S.A.) Chickens egg (U.S.A.;Japan;China) Retail meat (U.S.A.) MLN of normal pigs (Mex.)	Gastroenteritis in a boy (Den.) Gastroenteritis (U.S.A.) Carrier (U.S.A.)
1942	S.simsbury	Turkeys (U.S.A.)	Normal human feces(U.S.A.)Gastroenteritis(Medit.Area)

MLN = Mesenterial lymph nodes

Summarized from: Rubin,H.L.et al. 1942 Am.J.Hyg.,31,43-47;Hormaeche,E.et al.1943 Am. J.Diseases Children,66, 539-551;Edwards,P.R.et al.1943 J.Infectious Diseases,72,58-67;Cherry,W.B.et al.1943 Am.J.Hyg.,37,211-215; Bruner,D.W.et al.1947 Am.J.Hyg.,45,19-24;Wolff,A.M.et al.1948 Am.J.Public Health,38,403-408;Wilson,G.S.et al. 1948;Breed,R.S.et al.1948;Felsenfeld,O.et al.1951 Zentr.Bakteriol.,Parasitenk.Abt.I Ref.,149,351;Ball,M.R. 1952 Zentr.Bakteriol.,Parasitenk.Abt.I Ref.,150,548;Varela,G.et al.1953 Zentr.Bakteriol.,Parasitenk.Abt.I Ref.,151,373;Gorham,J.R.et al.1953 Zentr.Bakteriol.,Parasitenk.Abt.I Ref.,151,373.

Quantitative determinations of the incidence of bacterial survivors and antigenic variants were made after thirty minutes exposure to the phage (table 3). This experiment revealed the following: About one-twentieth of the treated cells survived. About half the surviving colonies formed non-translucent colonies which were antigenic variants. A small number of translucent colonies were also seen, all of these also variants. Studies are in progress to verify whether all lysogenized bacteria are antigenically altered, and vice versa (see section VI).

IX. Effect of host strain on phage specificity. A small number of phage particles from an autolysate of S. canoga were propagated in series on S. anatum. After several passages, a suspension titrating 4×10^9 per ml was obtained. This phage, which had been serially propagated on S. anatum was still effective in converting S. anatum and S. butantan. Thus the effectiveness of the phage is independent of the propagating host (contra transduction, Zinder and Lederberg, 1952).

Many types showing O antigens 3,10 have been isolated which, with respect to their H antigenic complexes, are similar to corresponding 3,15 serotypes (e.g., S. anatum- S. newington; S. nyborg- S. selandia). A consideration of the origins of these strains suggests that they have been found from similar sources. The corresponding pairs also tend to be similar in biochemical behavior. It is therefore suggested that the experimental interconversions have been paralleled in nature, and that each E_1 serotype may be expected to have an E_2 counterpart.

Table 3

Survival and antigenic variation of S. anatum exposed to phage

	Dilution	Colonies (average from 5 plates)	Translucent colonies (all variant)	Antigenic variants (per 50 non-translucent colonies tested)
Active phage, $5x10^8$/ml plus *S. anatum* 10^8/ml	10^{-4}	416	20	33
	10^{-5}	40	2	21
Heat-inactivated phage plus *S. anatum* 10^8/ml	10^{-6}	104	0	0
	10^{-7}	7	0	0

A phage suspension from *S. oanoga* was mixed with cells of *S. anatum* (20 hour agar plate culture) at room temperature for thirty minutes before plating. 50 of each experiment surviving colonies were tested with 15 antiserum.