Observations on Flea Transfer between Hosts; a Mechanism in the Spread of Bubonic Plague

A particularly important problem in plague epidemiology is concerned with the method of transfer of the disease from one rodent species to another. Since enzootic plague is known to be established in at least 15 of our western states, in western Canada, and in Mexico, the mechanism of plague transfer from wild rodents to domestic rats in the vicinity of human habitations is a particularly important question (*1*). Many investigators have suggested that the primary mechanism of plague spread is by transfer of infected fleas between hosts (*2*). Convincing circumstantial evidence that, in rural areas with a large rat population, the infection chain of "squirrel–squirrel flea–rat" may operate was obtained in studies of a plague epizootic in Ventura County, California (*3*). Although the evidence has been highly suggestive, the actual transfer of fleas from host to host has never been demonstrated.

The means for evaluating ectoparasite transfer more precisely were obtained through the development of a method for tagging fleas and other arthropods with Ce^{144} (*4*). By use of the California vole (*Microtus californicus*), domestic rats (*Rattus norvegicus*) and radioactively tagged wild rodent fleas (*Malaraeus telchinum*), a preliminary study of flea transfer was conducted in experimental plots simulating field conditions. These plots were enclosed by steel screens and provided soil and native grasses for the establishment of the rodents. In one type of experiment, male and female *Microtus* were toe-clipped for identification and allowed to establish nests; then tagged fleas were placed on certain individuals. Traps were set each day, and the captured animals were lightly anesthetized. The fleas were removed, and checked for radioactivity, and then returned to the hosts. In another experiment, three rats were maintained in a closure adjacent to three *Microtus* harboring tagged fleas, or the rats were allowed to enter the vole enclosure after the wild rodents had been killed or while they were alive. In all cases, a survey meter, Nuclear model 2612 equipped with a mica end-window probe, was used to scan the animals and fleas removed from them. The radioactivity of each flea was checked at the end of each trial with a RIDL scaler equipped with an end-window counter, Scott type.

Table 1 summarizes typical data on the movement of tagged fleas between individual *Microtus*. It should be noted that fleas transferred between animals, were found in nests, and were eaten by the animals, as was shown by radioactive feces. Thirty to sixty percent of the fleas were recovered from *Microtus* and their nests after periods ranging from 13 to 21 days.

The movement of radioactively tagged wild rodent fleas from the voles to the rats may be summarized as follows: 30 tagged fleas were introduced via the *Microtus*; while the *Microtus* were alive, none of these were found on the separated rats; after the *Microtus* were killed by snap-trapping, seven flea transfers were noted when the rats had entered the area with the dead voles; no transfers were noted on three new *Microtus* placed in the enclosure after the rats were again separated; 30 tagged fleas were placed on the new *Microtus*, and 12 transfers to rats were noted when the rats were allowed in the area with the live voles. Of the 60 fleas placed on the *Microtus*, none was found in *Microtus* nests, and 27 were recovered from the rats' nests. Radioactivity was found in *Microtus* feces twice, once in rat feces. Of the total fleas added, 49, or 81.6 percent, were accounted for during a period of 56 days. It should be noted that over 50 percent of the fleas accounted for were recovered between days 50 and 56. The fleas showed an initial average count of $(6.2 \text{ to } 8.5) \times 10^2$ counts per flea per minute, and after 56 days, $(3.6 \text{ to } 5.1) \times 10^2$ counts per flea per minute.

Under actual plague epizootic conditions, the coexistence of *Microtus*, *Malaraeus*, and *Rattus* has been postulated to be a significant relationship in a complex ecological situation in which other flea and rodent species are involved (*5*). Further studies on flea transfer under actual field conditions are being planned to confirm and extend the obserevations reported here (*6*).

Table 1. Movement of radioactively tagged wild rodent fleas, *Malaraeus telchinum*, between one male and two female voles, *Microtus californicus*.

Trial No.	No. fleas added	Sex of host	Days of trial	No. flea transfers	No. times radioactive feces found	No. fleas recovered		Percent fleas accounted for
						Animal	Nest	
1	40	M	18	21	4	11	16	67
2	10	F	21	9	not checked	5	no nest	50
3	10	F	18	21	not checked	3	no nest	30
4	12 ♂	F	6	8	1	0 ♂	0 ♂	36
	13 ♀	F				4 ♀	4 ♀	
5	15 ♂	F	13	40	not checked	2 ♂	3 ♂	62
	11 ♀	F				6 ♀	5 ♀	

W. V. Hartwell
S. F. Quan
Communicable Disease Center, U.S. Public Health Service, San Francisco, California
K. G. Scott
Department of Radiology, University of California, School of Medicine, San Francisco
L. Kartman
Communicable Disease Center, U.S. Public Health Service, San Francisco

References and Notes

1. A discussion of flea transfer in plague ecology is given in L. Kartman, F. M. Prince, S. F. Quan, and H. E. Stark, *Ann. N.Y. Acad. Sci.*, in press. A review of the literature may be found in R. Pollitzer, *World Health Organization Monogr. No. 22* (1954).
2. G. W. McCoy and M. B. Mitzmain, *Public Health Repts.* 24, 1013 (1909); W. C. Jacobsen, *Calif. State Comm. Hort. Bull.* 7, 721 (1918); W. M. Dickie, *Proc. Conf. State and Provincial Health Authorities N. Am.* (1926), p. 30; F. M. Prince, *Public Health Repts.* 58, 700 (1943).
3. K. F. Meyer and R. Holdenried, *Puerto Rico J. Public Health Trop. Med.* 24, 201 (1949).
4. S. F. Quan *et al.*, *Trans. Roy. Soc. Trop. Med. Hyg.* 51, 87 (1957).
5. V. I. Miles, A. R. Kinney, H. E. Stark, *Am. J. Trop. Med. Hyg.* 6, 752 (1957); L. Kartman, V. I. Miles, F. M. Prince, *Am. J. Trop. Med. Hyg.*, in press.
6. Acknowledgment is made to the following for aid and advice in these experiments: F. M. Prince, A. R. Kinney, R. L. Martin, and H. E. Stark.

18 November 1957

Presence of Polyamines in Certain Bacterial Viruses

Recent studies from this and other laboratories have shown the wide distribution of the polyamines putrescine [$NH_2(CH_2)_4NH_2$], spermidine [$NH_2(CH_2)_3NH(CH_2)_4NH_2$], and spermine [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] in nature (*1–3*). Little is known of their function, but their importance is implied by their roles as growth factors for several microorganisms (*4*) and as substrates for amine oxidases (*5*), and by their pharmacological effects (*1*). A possible functional connection with nucleic acids is suggested by the in vitro affinity of spermidine and spermine for nucleic acids and by the recovery of a considerable quantity of these bases from the nuclear fraction of liver cells (*3*). The studies on polyamines and the unanswered question about what compounds neutralize the negatively charged phosphate groups of the deoxyribonucleic acid (DNA) in bacteriophage (*6*) led to the present study of the polyamine content of phage.

Putrescine and spermidine are present in phage T_4 of *Escherichia coli* B in quantities sufficient to neutralize much

of the viral DNA; these polyamines appear to be the unidentified compounds recently found by Hershey to be injected into *E. coli* along with the DNA of phage T_2, while the phage protein remained outside (*7*).

Carbon-14 labeled putrescine is incorporated into spermidine in *E. coli* B, and these amines are then transferred to the phage. These findings are consistent with previous work showing that putrescine and spermidine are present in considerable amounts in *E. coli* (*2, 8*) and that spermidine is synthesized from putrescine and methionine (*9, 10*).

Cultures of *E. coli* B were grown in minimal medium (*11*), and were either harvested or infected with phage T_4r^+ while they were in the logarithmic phase. Putrescine and spermidine were extracted from suspensions of washed bacteria or purified phage (*12*) with hot 5 percent trichloroacetic acid. The bases were separated by ion-exchange chromatography on Dowex 50 (X2, 200–400 mesh) and were assayed colorimetrically as the dinitrophenyl derivatives, as previously described (*1*). Phosphate analyses were done on the phage after ashing (*13*).

The identity of the compounds recovered from the phage was established by ion-exchange and paper chromatography and by recrystallization with carrier to constant specific activity. Both compounds were eluted from Dowex 50 in the expected fractions, and the putrescine peak was further characterized by rechromatography on Amberlite XE-64 (*1*). Paper chromatography was performed in an isopropanol-HCl solvent (*7, 14*), which separates putrescine, spermidine, spermine, agmatine, and propanediamine. The putrescine and spermidine peaks from the Dowex eluates each showed single ninhydrin spots on the paper chromatograms. These spots had the same R_f values, respectively, as synthetic putrescine and spermidine (0.30 and 0.18) and contained all the applied radioactivity. Both compounds showed constant specific activity upon recrystallization with synthetic carrier (*3*).

Table 1 summarizes the results of several experiments. It can be seen that the putrescine and spermidine recovered from the phage are comparable in quantity to that recovered from either the uninfected or the infected bacteria. A comparison of the DNA phosphorus content with the polyamine content of the phage (noting two amino groups for putrescine, three for spermidine), shows that the neutralization of one-third to one-half of the DNA phosphate can be accounted for by polyamines. In four of the runs C^{14}-putrescine (*15*) was added with the bacterial inoculum, and from these it can be seen that the degree of dilution of the radioactivity is greater for the putrescine than for the spermidine; this is consistent with the higher rate of turnover of putrescine previously noted (*10*). The dilution of both polyamines is slightly greater in the phage than in the bacteria; this may be related to the length of time required after infection for complete lysis. In experiment 3 radioactive putrescine was added only after lysis of the bacteria; the absence of radioactivity from the phage recovered from this lysate rules out the direct exchange of the putrescine in the medium with that of the phage or the contamination of the phage with external putrescine. The variations among the several runs may be due in part to variations in growth conditions for the bacteria. It has been shown that the polyamines in *E. coli* vary markedly with growth conditions (*3*), and work is in progress to relate more precisely the host polyamines to those of the phage.

Table 1. Putrescine and spermidine content of *E. coli* B and phage T_4r^+. In experiments 1, 2, 3, and 4 the host bacteria were infected with about six phage each, and 5.5 hours later, when lysis was complete, the progeny phage were isolated; each run yielded about 4×10^{13} phage. The host bacteria were grown at 37°C with shaking in minimal medium (*11*) (1 lit except where noted) from a titer of about 10^6/ml to titers of 2.3, 1.5, 2.4, and 1.9×10^8/ml, respectively. Experiments 1A and 4A paralleled 1 and 4; the bacteria from the former two were harvested at the same time that phage were added to the latter two. Experiment 1B also paralleled 1, but here the bacteria were harvested 19 minutes after infection (before lysis had occurred). Analytical values for the purified phage were corrected for losses during purification.

Experiment	Putrescine (μmole)	Spermidine (μmole)	DNA phosphorus (μmole)	Ratio of specific activity of isolated material to that of added putrescine	
				Putrescine	Spermidine
1* (phage)	11.7	1.3		0.10	0.64
2* (phage)	4.9	1.3	46	0.22	0.65
3† (phage)	5.5	0.5	30	0.00	0.00
4 (phage)	6.1	1.2	43		
1A* (uninfected bacteria)	3.7	0.49		0.26	0.81
4A (uninfected bacteria)	4.1	0.61			
1B* (infected bacteria)	3.4	0.41		0.26	0.85

* C^{14} Putrescine (47,500 count/min μmole) added with bacterial inoculum: experiments of series 1, 4 μmole in 800 ml of medium; experiment 2, 11 μmole in 2 lit.
† 2.4 μmole of C^{14} putrescine was added to the liter of bacterial lysate after the first (low-speed) centrifugation and was incubated with the phage for 1 hour at 35°C and then overnight in the cold.

Hershey (*7*) has recently described two unidentified compounds A_1 and A_2 comprising about 1.5 percent of the total carbon in phage T_2; these compounds now appear to be spermidine and putrescine. Compounds A_1 and A_2 were labeled from C^{14}-arginine, which is a reasonable metabolic precursor of putrescine. Hershey reported that A_1 and A_2 were extractable from the phage with cold trichloroacetic acid, were unaffected by heating with 6*N* HCl, were ninhydrin-positive, appeared in the phage unchanged after assimilation by the bacteria, and seemed to be normal constituents of the bacteria. These properties are shared by the polyamines. In addition, spermidine was found to have the same R_f values as A_1 (the minor component) and putrescine as A_2 (the major component) in the solvents used by Hershey.

Paper chromatography of whole phage has also been performed in the isopropanol-HCl solvent. If 5×10^{10} purified T_4 phage (or T_2 phage) is applied to the paper, the strongly acid solvent extracts the amines from the phage, and two spots corresponding to putrescine and spermidine are revealed by developing with ninhydrin; the only other ninhydrin spot is at the origin and presumably is phage protein. It is interesting that the *Salmonella* phage PLT-22 and 98 (*16*) show quite different ninhydrin patterns from that of T_4. Chromatography of purified PLT-22 on paper and on Dowex 50 shows spermine but little if any putrescine or spermidine. However, these latter two types of phage were grown in broth, and the possible effect of different media on amine content remains to be assessed. At present the high amine content has been established only for certain types of coli phage, and further work is in progress on other viruses (*17*).

BRUCE N. AMES
DONALD T. DUBIN
SANFORD M. ROSENTHAL
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda Maryland

References and Notes

1. S. M. Rosenthal and C. W. Tabor, *J. Pharmacol. Exptl. Therap.* 116, 131, 139 (1956). (Earlier references cited.)
2. E. J. Herbst, R. H. Weaver, Donald L. Keister, *Federation Proc.* 16, 195 (1957).

3. C. W. Tabor, S. M. Rosenthal, H. Tabor, unpublished observations.
4. H. Kihara and E. E. Snell, *Proc. Natl. Acad. Sci. U.S.* 43, 867 (1957); E. J. Herbst and E. E. Snell, *J. Biol. Chem.* 181, 47 (1949). (Earlier references cited.)
5. C. W. Tabor, H. Tabor, S. M. Rosenthal, *J. Biol. Chem.* 208, 645 (1954); H. Tabor, *Pharmacol. Revs.* 6, 299 (1954). (Earlier references cited.)
6. We wish to thank Dr. C. Levinthal for calling this problem to our attention.
7. A. D. Hershey, *Virology* 4, 237 (1957).
8. H. Tabor, S. M. Rosenthal, C. W. Tabor, *Federation Proc.* 15, 367 (1956).
9. R. C. Greene, *J. Am. Chem. Soc.* 79, 3929 (1957).
10. H. Tabor, S. M. Rosenthal, C. W. Tabor, *ibid.* 79, 2978 (1957).
11. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* 218, 97 (1956). Glucose was added to the medium to make a 0.5 percent solution.
12. Prepared by successive 4000, 20,000, 5000, and 20,000 *g* centrifugations. The optical density at 260 mμ of 10^{11} phage per milliliter was about 1.2/cm.
13. The phage were ashed with $Mg(NO_3)_2$ by the procedure of Assoc. of Off. Agr. Chemists (1955). Inorganic phosphate, which was negligible before ashing, was assayed by the procedure of C. H. Fiske and Y. Subbarow [*J. Biol. Chem.* 66, 375 (1925)].
14. G. R. Wyatt, *Biochem. J.* 48, 584 (1951).
15. Prepared by H. Tabor, S. M. Rosenthal, C. W. Tabor (unpublished synthesis).
16. We wish to thank Dr. P. Hartman for a preparation of PLT-22 and Dr. L. Baron for phage 98.
17. We wish to thank Drs. H. Tabor and C. W. Tabor for their advice and helpful criticism.

24 February 1958

Gibberellin-Induced Systemic Fruit Set in a Male-Sterile Tomato

Although recent reports (*1–3*) suggest that gibberellin or gibberellin-like substances (hereafter referred to as gibberellin) occur naturally in plants, investigators differ in their opinions regarding the movement of these compounds. The probability that gibberellin, like auxin, produces physiological effects distal from the site of synthesis indicates the need for further investigations of its movement in plants (*4, 5*). Hitchcock and Zimmerman (*6*) and Ferri (*7*) have demonstrated the movement of auxin through the plant by application to the soil, to roots, or to cuttings.

In the present study (*8*) the ability of gibberellin, applied as flower sprays, to set fruit parthenocarpically (*9, 10*) or to increase the growth of "dormant" tomato fruit (*11*) was regarded as a biological assay of its systemic movement.

The technique was refined by using male-sterile tomato plants (*Lycopersicon esculentum*) of the variety Earlypak (*12*) which were identified and selected at anthesis from a segregating backcross generation. These plants were normal in every respect except that the pollen grains were aborted (as indicated by an acetocarmine test). A few parthenocarpic fruit may set naturally on this mutant, but any appreciable increase in numbers of fruit under isolated greenhouse conditions could be attributed to applied gibberellin.

Table 1. Effect of gibberellin applied both to the foliage and soil on induction of fruit set in male-sterile Earlypak tomato.

Gibberellin per plant	Place of application	Average per plant			
		Total No. of clusters with fruits	Total No. of fruits	No. of fruits on treated lateral	No. of fruits on untreated lateral
100 μg	Expanded leaves	6.4	14.8	10.8	4.8
100 μg	Stem apices	7.0	12.0	7.0	5.0
100 μg	Flower peduncles	2.0	5.0	5.0	0.0
100 mg	Soil	8.0	36.0		
0	Control (untreated)	0.3	0.7		

Immediately preceding anthesis of the first flower cluster, the main stems of the tomato plants were pruned in order to stimulate the growth of two lateral branches from the cotyledonary axils. These branches were nearly alike with regard to time of flowering, number of flowers per cluster, and number and length of internodes. Basipetal and acropetal movement from a treated lateral would be reflected in a stimulation of fruit set on an untreated lateral. The plants were grown during the spring and summer in a greenhouse held at approximately 65°F at night. Day temperatures were held between 65° and 85°F.

In preliminary experiments to confirm previous results (*13*), floral sprays containing 500 μg of gibberellin per milliliter resulted in characteristic parthenocarpic fruit development. Subsequently, the effect of gibberellin (*14*) on inducing systemic fruit set was evaluated (i) by applying, with a micropipette, 100 μg per plant to the first or second fully expanded leaf above the second open flower cluster, to stem apices, and to the peduncle of a single inflorescence (*15*) and (ii) by applying 100 ml of a solution containing 1000 μg/ml (100 mg) to the soil (Table 1). An excess of gibberellin was applied in order to compensate for the rapid degradation in the soil reported by Brian *et al.* (*16*). One milliliter of polyoxyethylene sorbitan monolaurate (Tween-20) per 100 ml of solution was added as a wetting agent for both plant and soil treatments.

Increased parthenocarpic fruit set on both treated and untreated laterals was induced by applying gibberellin to the foliage, but not by treating peduncles (Table 1). Greatest fruit set resulted from the soil application. Fruit from these treatments in every way resembled that resulting from direct floral sprays. All treatments, in addition to floral sprays, resulted in significant increases in size of "dormant" fruits. Johnson and Liverman (*11*) reported that a "dormancy" of developing fruits induced by high temperature or by far red irradiation could be overcome by spraying them with gibberellin. No quantitative studies were made to determine whether promotion of fruit growth in our experiments was comparable to that reported by Johnson and Liverman.

The marked increase in fruit set on an untreated lateral of a male-sterile plant indicates that gibberellin initiates a physiological response distant from the point of treatment. The results do not necessarily imply that gibberellin per se is directly responsible for systemic induction of fruit set. The use of male-sterile plants to assay for systemic fruit setting may have application for evaluating other growth-regulating substances.

ARNULF PERSSON
LAWRENCE RAPPAPORT
Department of Vegetable Crops, University of California, Davis

References and Notes

1. B. O. Phinney *et al.*, *Proc. Natl. Acad. Sci. U.S.* 43, 398 (1957).
2. M. Radley, *Nature* 178, 1070 (1956).
3. F. Lona, *Ateneo Parmense* 28, 111 (1957).
4. R. Watanabe and N. J. Scully, *Plant Physiol.* Suppl. 32, lvi (1957).
5. P. M. Neely and B. O. Phinney, *ibid.* Suppl. 32, xxi (1957).
6. A. E. Hitchcock and P. W. Zimmerman, *Contribs. Boyce Thompson Inst.* 7, 447 (1935).
7. M. G. Ferri, *ibid.* 14, 51 (1945).
8. This investigation was supported in part by a research grant from Merck, Sharp and Dohme, Inc.
9. S. H. Wittwer *et al.*, *Plant Physiol.* 32, 39 (1957).
10. L. Rappaport, *Calif. Agr.* 10, 4 (1956).
11. J. L. Johnson and S. P. Liverman, *Science* 125, 1086 (1957).
12. Appreciation is expressed to Dr. C. M. Rick for supplying seeds of ms_{24} [C. M. Rick, *Rept. Tomato Genetics Coop.* 6, 26 (1956)].
13. L. Rappaport, *Plant Physiol.* 32, 440 (1957).
14. The preparation, supplied by Merck, Sharp and Dohme, Inc., contained the potassium salt of gibberellic acid (Gibrel) and about 80 percent apparently inactive materials.
15. S. Zalik, G. A. Hobbs, A. C. Leopold, *Proc. Am. Soc. Hort. Sci.* 58, 201 (1951).
16. P. W. Brian *et al.*, *J. Sci. Food Agr.* 5, 602 (1954).

16 December 1957