DDT Residues in an East Coast Estuary: A Case of Biological Concentration of a Persistent Insecticide

Abstract. *DDT residues in the soil of an extensive salt marsh on the south shore of Long Island averaged more than 13 pounds per acre (15 kilograms per hectare); the maximum was 32 pounds per acre (36 kilograms per hectare). A systematic sampling of various organisms from the vicinity showed concentrations of DDT increasing with trophic level through more than three orders of magnitude from 0.04 part per million in plankton to 75 parts per million in a ring-billed gull. Highest concentrations occurred in scavenging and carnivorous fish and birds, although birds had 10 to 100 times more than fish. These concentrations approach those in animals dying from DDT poisoning, which suggests that many natural populations in this area are now being affected, possibly limited, by DDT residues. Similar concentrations have been reported elsewhere in North America.*

DDT residues (*1*) have become an intrinsic part of the biological, geological, and chemical cycles of the earth (*2*) and are measurable in air (*3*), water (*4*), soil (*5*), man (*6*), and even in animals from the Antarctic, many hundreds of miles from places where DDT has been applied (*7, 8*). While the presence of residues does not prove an effect on living systems, the world-wide distribution of a substance as persistent and broadly toxic as DDT is itself reason to question whether residues are accumulating to toxic levels in certain populations. Accumulation occurs either through direct absorption from the environment or by concentration along food chains, and this latter phenomenon has been documented in several aquatic situations (*9*). Such accumulations have been correlated with recent declines in populations of carnivorous birds (*8, 10*) and other organisms. Residues of persistent pesticides are now so widespread that they must be considered as potentially aggravating the problems of eutrophication by degrading populations of consumers.

We measured residues of DDT in the soils of a brackish marsh on the south shore of Long Island, New York, and in various organisms from the area. Results showed a high concentration of residues in the marsh and a systematic increase in DDT residues with increase in trophic level, thus providing an especially clear example of what has been called "biological magnification." In many cases the concentrations approached those in organisms known to have died of DDT poisoning, which suggests that DDT residues are currently reducing certain animal populations within the estuary.

The marsh from which soil samples were taken was at the mouth of Carmans River at the eastern end of Great South Bay, Long Island. The area was selected as representative of relatively undisturbed marsh. The marsh along the western side of the river was sampled by a modification of the technique described by Woodwell and Martin (*5*). A "sample" consisted of six subsamples, each subsample being a core 4.8 cm in diameter and either 20 or 40 cm long, taken with a sharpened aluminum tube pressed into the soil. In each area of interest, subsamples were collected systematically about 10 m apart. Seven such samples were taken, four in the *Spartina patens* marsh, two along the margins of drainage ditches dug for mosquito control, and one from the bottom of the estuarine bay a few meters from the edge of the marsh.

For DDT analyses, 2-cm increments from equal depths among the six subsamples were pooled. Analyses were performed on the increments from 0 to 2, 4 to 6, 8 to 10, and 18 to 20 cm, and where deeper samples were taken, 38 to 40 cm. Total residues were calculated on a weight-per-area basis by integrating the area under the curve expressing residues per square meter at the various depths.

Plankton were collected in a No. 6 (0.239-mm mesh) plankton net. All organisms were living when taken except as indicated; fish were netted; birds were shot. Samples were stored frozen until analyzed, and mud samples were oven-dried before analysis. In most cases, whole organisms were analyzed, but feathers, beaks, feet, and wing tips of birds were discarded. Analyses were on 1-g samples of the homogenized organism. Extraction was from Florisil with petroleum ether–diethyl ether as described by Cummings *et al.* (*11*). Analyses of samples to which measured quantities of DDT, DDE, and DDD, individually, had been added prior to extraction indicated recoveries averaging 96 percent. Analyses for DDT, DDE, and DDD were by electron-capture gas chromatography; certain identifications were confirmed by thin-layer chromatography (*11*).

Residues of DDT in the *Spartina* marsh varied widely from less than 3 to more than 32 lb/acre (Table 1). The mean concentration of the four samples from this marsh (each a composite of 6 cores) was 13.1 lb/acre. Slightly lower quantities occurred along the ditches, but total residues were still 1 to 5 1b/acre; submerged bay bottom contained 0.28 lb/acre. In all of these samples most of the residues (approximately 90 percent) occurred in the upper 4 cm of the profile. Residues were highly variable in relative proportions of DDT, DDE, and DDD. In general there was an increase in the proportion of DDE, and a decrease in DDT, with increase in depth. In the 0- to 2-cm samples, the mean DDE content was about 25 percent; in the 18- to 20-cm samples it was about 60 percent. Residues in the bottom of the bay contained only traces of DDT and DDD, the principal residue being DDE.

Thirty-nine samples of plants and animals from the vicinity were analyzed. Arrangement of the samples in sequence according to increasing concentration of DDT residues (Table 2) shows a progression according to both size and trophic level, larger organisms and higher carnivores having greater concentrations than smaller organisms

Table 1. DDT residues (DDT + DDE + DDD) (*1*) in Carmans River marsh and in the bottom mud of Great South Bay, N.Y., August 1966. Each sample was a composite of six subsamples, taken to the depths indicated.

Zone	Sample No.	Depth (cm)		Total residues Lb/acre	Total residues Kg/ha
Spartina mat	1	0–20		2.69	3.01
	2	0–40		9.23	10.3
	3	0–20		7.86	8.81
	4	0–40		32.6	36.5
			Mean	13.1	14.7
Drainage ditch	1	0–20		4.63	5.19
	2	0–40		1.10	1.23
			Mean	2.87	3.21
Bay bottom (submerged)		0–40		0.28	0.31

and organisms at lower trophic levels. Total residues ranged through three orders of magnitude from 0.04 part per million (ppm) in plankton to 75 ppm in a ring-billed gull. Shrimp contained 0.16 ppm; eels, 0.28; insects from the marsh, 0.30; and mummichogs (*Fundulus*), 1.24 ppm. Among fish, the needlefish, a carnivore, had the highest content, 2.07 ppm, about twice that of *Fundulus*, which forms part of its food. In general, the concentrations of DDT residues in carnivorous birds were 10 to 100 times those in the fish on which they feed. Concentrations of DDT in the waters of Great South Bay must be assumed to be less than the 0.0012-ppm saturation limit, a reasonable estimate probably being closer to 0.00005 ppm (*4, 12*). Based on this estimate, birds near the top of these food chains have concentrations of DDT residues about a million times greater than the concentration in the water.

The shift in relative proportions of DDT, DDE, and DDD with progression in trophic level is also conspicuous. Organisms containing high proportions of DDT, as opposed to its metabolites, are common at lower trophic levels; at upper levels, most of the residue is DDE. In most organisms, DDD and DDE are somewhat less toxic than DDT.

The secondary effects of applications of DDT to marshes, streams, and forests are the subject of an extensive literature. Single applications in the range of 0.1 to 0.3 lb/acre have repeatedly caused drastic reductions in populations of crayfish, shrimp, amphipods, isopods, annelids, fish, fiddler crabs, blue crabs, and others, sometimes with no recovery for years (*13*). Aerial spraying with 0.5 lb of DDT per acre in New Brunswick, Canada, caused extremely high mortality of young salmon, reduced salmon food organisms, and was correlated with reduced reproductive success in woodcock (*14, 15*). Applications of 1 to 5 lb/acre are known to have serious long-term effects on amphibians, fish, and birds (*16–18*).

While it is not true that residues distributed through 4 cm of highly organic soil are continuously available to the biota in the same degree as immediately after spraying, there is little question that residues in soil are leached by water, moved by erosion, and absorbed by mud-dwelling and mud-scavenging organisms. As a result of such processes, DDT residues in a marsh inevitably enter environmental cycles. Deleterious effects on wildlife from 13 lb/acre on the Carmans River marsh might therefore be expected. Detailed long-term observations have shown substantial reductions during the past decade in local populations of shrimp, amphipods, summer flounder, blue crab (*Callinectes sapidus*), spring peeper (*Hyla crucifer*), Fowler's toad (*Bufo woodhousei fowleri*), woodcock (*Philohela minor*), and various other species, known to be sensitive to DDT, that are indigenous to this area (*13–19*). Other aspects of human disturbance unquestionably contribute to degradation of estuaries, but do not offer adequate explanations for all of these declines. This is especially true for declines in populations indigenous to marshes that have been remote from other disturbances (*19*).

The concentration of DDT residues that affect animals in nature is difficult to appraise. Analyses of whole organisms, rather than of specific organs, are most representative of the degree of exposure to DDT, although correlation of such measurements with death is not precise. Nevertheless, broad correlations exist between whole-body concentrations and mortality (*20*) and are useful in appraising the hazards of residues in the estuary we sampled. For instance, fish of several species, known to have been killed by DDT, contained whole-body concentrations of 1 to 26 ppm, commonly averaging 4 to 7 ppm (*18, 21*); the concentrations reported for living fish in Table 2 lie between 0.17 and 2.07 ppm, within and somewhat below this apparently lethal region. Birds known to have died of DDT poisoning contained 30 to 295 ppm of DDT residues when

Table 2. DDT residues (DDT+DDE+DDD) (*1*) in samples from Carmans River estuary and vicinity, Long Island, N.Y., in parts per million wet weight of the whole organism, with the proportions of DDT, DDE, and DDD expressed as a percentage of the total. Letters in parentheses designate replicate samples.

Sample	DDT residues (ppm)	Percent of residue as		
		DDT	DDE	DDD
Water*	0.00005			
Plankton, mostly zooplankton	.040	25	75	Trace
Cladophora gracilis	.083	56	28	16
Shrimp†	.16	16	58	26
Opsanus tau, oyster toadfish (immature)†	.17	None	100	Trace
Menidia menidia, Atlantic silverside†	.23	17	48	35
Crickets†	.23	62	19	19
Nassarius obsoletus, mud snail†	.26	18	39	43
Gasterosteus aculeatus, threespine stickleback†	.26	24	51	25
Anguilla rostrata, American eel (immature)†	.28	29	43	28
Flying insects, mostly Diptera†	.30	16	44	40
Spartina patens, shoots	.33	58	26	16
Mercenaria mercenaria, hard clam†	.42	71	17	12
Cyprinodon variegatus, sheepshead minnow†	.94	12	20	68
Anas rubripes, black duck	1.07	43	46	11
Fundulus heteroclitus, mummichog†	1.24	58	18	24
Paralichthys dentatus, summer flounder‡	1.28	28	44	28
Esox niger, chain pickerel	1.33	34	26	40
Larus argentatus, herring gull, brain (d)	1.48	24	61	15
Strongylura marina, Atlantic needlefish	2.07	21	28	51
Spartina patens, roots	2.80	31	57	12
Sterna hirundo, common tern (a)	3.15	17	67	16
Sterna hirundo, common tern (b)	3.42	21	58	21
Butorides virescens, green heron (a) (immature, found dead)	3.51	20	57	23
Larus argentatus, herring gull (immature) (a)	3.52	18	73	9
Butorides virescens, green heron (b)	3.57	8	70	22
Larus argentatus, herring gull, brain§ (e)	4.56	22	67	11
Sterna albifrons, least tern (a)	4.75	14	71	15
Sterna hirundo, common tern (c)	5.17	17	55	28
Larus argentatus, herring gull (immature) (b)	5.43	18	71	11
Larus argentatus, herring gull (immature) (c)	5.53	25	62	13
Sterna albifrons, least tern (b)	6.40	17	68	15
Sterna hirundo, common tern (five abandoned eggs)	7.13	23	50	27
Larus argentatus, herring gull (d)	7.53	19	70	11
Larus argentatus, herring gull§ (e)	9.60	22	71	7
Pandion haliaetus, osprey (one abandoned egg)‖	13.8	15	64	21
Larus argentatus, herring gull (f)	18.5	30	56	14
Mergus serrator, red-breasted merganser (1964)‡	22.8	28	65	7
Phalacrocorax auritus, double-crested cormorant (immature)	26.4	12	75	13
Larus delawarensis, ring-billed gull (immature)	75.5	15	71	14

* Estimated from Weaver *et al.* (*4*). † Composite sample of more than one individual. ‡ From Captree Island, 20 miles (32 km) WSW of study area. § Found moribund and emaciated, north shore of Long Island. ‖ From Gardiners Island, Long Island.

analyzed as whole birds, the average for several species being about 112 ppm (*17*); the birds in our sample contained residues ranging from 1 to 75 ppm.

Living birds and fish that were analyzed in this study contained DDT residues that exceed one tenth of the mean concentrations in organisms known to have died of acute exposures to DDT. This in itself implies that concentrations of DDT in this food web are approaching the maximum levels observable in living organisms and now occasionally reach acutely lethal levels in both birds and fish. Two observations lead to this conclusion: (i) the trophic-level effect has been shown to produce concentrations in carnivorous birds and fish that are 10 to 1000 times the concentrations lower in the food web. We must assume that, although we have sampled fish-eating carnivorous birds such as the merganser, carnivorous or scavenging birds feeding on birds would have even higher levels, probably by as much as another 10-fold. Such birds probably would not survive. (ii) Perhaps even more important because it occurs at all trophic levels, great variability characterizes the total amounts of residues in animals from the wild. Differences in the range of 5- to 10-fold can be expected between the minimum and maximum concentrations in birds of a single species killed under similar conditions by DDT (*17*). While the causes of such variability are not clear, its existence implies that mortality from DDT residues is now occurring in these populations through elimination of individuals at the upper end of the range of concentrations. This constant attrition or "cropping" process would be extremely difficult to detect, since it does not produce spectacular "kills," and dead individuals, widely scattered, tend to disappear rapidly (*17, 22*). The probability appears high that not only are the populations of many of the organisms of this sampling now being affected by accumulation of DDT residues, but that other species in the area have already been depleted to the point where study is difficult or impossible.

Acute mortality, however, is but one effect of DDT. Sublethal concentrations, although studied less, may actually have more important effects on populations in nature. In the laboratory, sublethal amounts of DDT reduce reproductive success in bobwhite, ring-necked pheasants, and mice (*23*). Evidence has linked chlorinated hydrocarbon residues with reduced reproduction in field populations of trout (*24*), osprey, woodcock, bald (*25*) and golden eagles, peregrine falcon, and others (*8, 10, 15*). Serious population declines have occurred for some of these species. Minute quantities of chlorinated hydrocarbons affect the patterns of behavior of goldfish and upset temperature-regulating mechanisms in salmon (*26*). Such sublethal effects might be expected within the populations represented in Table 2.

One important conclusion is that analyses of water have limited meaning when evaluating the effects of DDT residues on animal populations. Water can be expected to contain a lower concentration of DDT than other components of an ecosystem—quantities that are usually vanishingly small or "nondetectable" (*4*). Even these very low concentrations may be important because natural mechanisms can concentrate residues many thousands of times. A better criterion of hazard from DDT pollution would be analyses of carnivores or other organisms that concentrate the residues.

Concentrations of DDT residues reported here are not unique to this marsh or even to Long Island. Observations from widely scattered fish and bird populations in North America show concentrations approximating those reported here (*8, 9*), which suggests that DDT residues are moving through the biological, geological, and chemical cycles of the earth at concentrations that are having far-reaching and little-known effects on ecological systems.

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References and Notes

1. DDT residues include DDT and its decay products (metabolites), DDE and DDD; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD, also known as TDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.
2. G. M. Woodwell, *Sci. Amer.* **216**, 24 (March 1967).
3. P. Antommaria, M. Corn, L. DeMaio, *Science* **150**, 1476 (1965); G. M. Woodwell, *Forest Sci.* **7**, 194 (1961).
4. L. Weaver, C. G. Gunnerson, A. W. Breidenbach, J. J. Lichtenberg, *Public Health Rep.* **80**, 481 (1965).
5. C. A. Edwards, *Residue Rev.* **13**, 83 (1966); G. M. Woodwell and F. T. Martin, *Science* **145**, 481 (1964).
6. G. E. Quinby, W. J. Hayes, Jr., J. F. Armstrong, W. F. Durham, *J. Amer. Med. Ass.* **191**, 175 (1965).
7. W. J. L. Sladen, C. M. Menzie, W. L. Reichel, *Nature* **210**, 670 (1966); J. L. George and D. E. H. Frear, *J. Appl. Ecol.* **3** (suppl.), 155 (1966).
8. "The effects of pesticides on fish and wildlife," *U.S. Fish and Wildlife Service Circ. 226* (1965); "Pesticides in the environment and their effects on wildlife," *J. Appl. Ecol.* **3** (suppl.) (1966).
9. E. G. Hunt and A. I. Bischoff, *Calif. Fish and Game* **46**, 91 (1960); J. J. Hickey, J. A. Keith, F. B. Coon, *J. Appl. Ecol.* **3** (suppl.), 141 (1966); J. O. Keith, *ibid.*, p. 71; E. G. Hunt, *Scientific Aspects of Pest Control* (Nat. Acad. Sci.-Nat. Res. Council, Publ. No. 1402, 1966), p. 251.
10. P. L. Ames, *J. Appl. Ecol.* **3** (suppl.), 87 (1966); I. Prestt, *ibid.*, p. 107; S. Cramp, *Brit. Birds* **56**, 124 (1963); J. D. Lockie and D. A. Ratcliffe, *ibid.* **57**, 89 (1964); D. A. Ratcliffe, *ibid.* **58**, 65 (1965); D. A. Ratcliffe, *Bird Study* **12**, 66 (1965); A. Sprunt, *Audubon Mag.* **65**, 32 (1963).
11. J. G. Cummings, K. T. Zee, V. Turner, F. Quinn, R. E. Cook, *J. Ass. Offic. Anal. Chemists* **49**, 354 (1966); M. F. Kovacs, Jr., *ibid.*, p. 365.
12. M. C. Bowman, F. Acree, Jr., M. K. Corbett, *J. Agr. Food Chem.* **8**, 406 (1960).
13. H. H. Ross and W. Tietz, Jr., *Illinois Nat. Hist. Surv.* (1949) (mimeographed); P. F. Springer, *Proc. 50th Annu. Mtg. N.J. Mosquito Extermination Ass.* (1963), pp. 194–203; P. A. Butler and P. F. Springer, *Trans. 28th North American Wildlife and Natural Resources Conf.* (1963), pp. 378–390; P. F. Springer, *Diss. Abstr.* **22**, 1777 (1961); P. F. Springer and J. R. Webster, *Mosquito News* **11**, 67 (1951); R. A. Croker and A. J. Wilson, *Trans. Amer. Fish. Soc.* **94**, 152 (1965); A. D. Hess and G. G. Keener, Jr., *J. Wildlife Management* **11**, 1 (1947); J. L. George, R. F. Darsie, Jr., P. F. Springer, *ibid.* **21**, 42 (1957).
14. M. H. A. Keenleyside, *Can. Fish Cult. No. 24*, 17 (1959); F. P. Ide, *Trans. Amer. Fish. Soc.* **86**, 208 (1956).
15. B. S. Wright, *J. Wildlife Management* **29**, 172 (1965).
16. B. A. Fashingbauer, *Flicker* **29**, 160 (1957); R. J. Barker, *J. Wildlife Management* **22**, 269 (1958); P. Goodrum, W. P. Baldwin, J. W. Aldrich, *ibid.* **13**, 1 (1949); C. S. Robbins, P. F. Springer, C. G. Webster, *ibid.* **15**, 213 (1951); L. B. Hunt, *ibid.* **24**, 139 (1960); G. J. Wallace and E. A. Boykins, *Jack-Pine Warbler* **43**, 13 (1965); R. B. Anderson and W. H. Everhart, *Trans. Amer. Fish. Soc.* **95**, 160 (1966); R. L. Rudd, *Pesticides and the Living Landscape* (Univ. of Wisconsin Press, Madison, 1964).
17. C. F. Wurster, Jr., D. H. Wurster, W. N. Strickland, *Science* **148**, 90 (1965); D. H. Wurster, C. F. Wurster, Jr., W. N. Strickland, *Ecology* **46**, 488 (1965).
18. K. Warner and O. C. Fenderson, *J. Wildlife Management* **26**, 86 (1962).
19. Long-term field studies by D. Puleston and A. P. Cooley, many of them specifically covering the Carmans River marsh and documented in personal notes kept over more than 20 years, show the decline or disappearance of these and many other populations, including American bittern, *Botaurus lentiginosus*, least bittern, *Ixobrychus exilis*, green heron, and marsh hawk, *Circus cyaneus*. During this period the physical characteristics of the marsh have remained largely unchanged.
20. DDT and its residues are nerve toxins; when DDT is suspected as a cause of death in vertebrates, the best appraisal is by analyses of residue concentrations in the brain [W. E. Dale, T. B. Gaines, W. J. Hayes, Jr., G. W. Pearce, *Science* **142**, 1474 (1963); L. F. Stickel, W. H. Stickel, R. Christensen, *ibid.* **151**, 1549 (1966)]. However, DDT residues can be stored in adipose tissues for long periods without conspicuous effects; symptoms occur when fat reserves are utilized, redistributing the toxin [R. F. Bernard, *Mich. State Univ. Mus. Publ. Biol. Ser.* **2**(3), 155 (1963)]. In many species of birds, for example, fat reserves are utilized during reproduction and migration. Because of the accumulation of residues in other tissues, analyses of whole bodies appears to be a better criterion of exposure to DDT and of its hazard to the organism than analyses of brain tissues alone.

21. A. V. Holden, *Ann. Appl. Biol.* **50**, 467 (1962); F. H. Premdas and J. M. Anderson, *J. Fish. Res. Board Can.* **20**, 827 (1963); D. Allison, B. J. Kallman, O. B. Cope, C. C. Van Valin, *Science* **142**, 958 (1963).
22. W. Rosene, Jr., and D. W. Lay, *J. Wildlife Management* **27**, 139 (1963).
23. J. B. DeWitt, *J. Agr. Food Chem.* **3**, 672 (1955); *ibid.* **4**, 863 (1956); R. E. Genelly and R. L. Rudd, *Auk* **73**, 529 (1956); R. F. Bernard and R. A. Gaertner, *J. Mammal.* **45**, 272 (1964).
24. G. E. Burdick *et al., Trans. Amer. Fish. Soc.* **93**, 127 (1964).
25. On the basis of 61 specimens taken from many parts of the United States, the bald eagle averages 11 ppm of DDT residues in breast muscle, a quantity probably approaching lethal concentrations (*8*).
26. R. E. Warner, K. K. Peterson, L. Borgman, *J. Appl. Ecol.* **3** (suppl.), 223 (1966); D. M. Ogilvie and J. M. Anderson, *J. Fish. Res. Board Can.* **22**, 503 (1965).
27. Supported in part by a grant from the Research Foundation of the State University of New York and in part by Brookhaven National Laboratory under the auspices of AEC.

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Bacteriophage S13: A Seventh Gene

Abstract. *Genetic complementation tests of ochre-type suppressible mutants of bacteriophage S13 have revealed a seventh gene. A mutation of this gene has no significant effect on synthesis of the phage replicative form DNA, but like five other phage genes it prevents the appearance of single-stranded DNA. With the new gene, it seems likely that approximately 70 to 100 percent of the phage genes are accounted for.*

An exhaustive analysis was initiated by E. S. Tessman (*1*) to identify all the genetic functional units of phage S13, a functional unit (or gene) being defined as equivalent to a genetic complementation group. With the new gene described here, we estimate that 70 to 100 percent of the functional units are accounted for.

As a result of isolating many temperature-sensitive (*t*) and amber-type suppressible (*su*) mutants, six complementation groups have been found and designated I, II, IIIa, IIIb, IV, V. Groups I through IV were identified in the original experiments on S13 complementation groups (*1*). At that time IIIa and IIIb were recognized as separate categories by complementation tests between *su* mutants, but those tests gave low burst sizes, suggesting that there was intragenic complementation. However, the low burst sizes could be due to a polarity effect (*2*) because subsequent complementation tests with *t* mutants showed unambiguously that IIIa and IIIb mutants strongly complement and therefore represent separate complementation groups (*3*). Mutants of group V affect lysis and show properties similar to those of the gene identified in the closely related bacteriophage ϕX174 (*4*).

The new group was found by isolating ochre-type *su* mutants that are suppressible by the ochre suppressor *Escherichia coli* CA165 (*5*). The ochre mutants were induced by treatment with hydroxylamine (*6*) to a survival of 0.45. The treated phage were plated on a derivative of strain CA165. This strain is normally not sensitive to S13, presumably because of failure to adsorb the phage, so the sensitive derivative CA165.2 was isolated by a method previously applied to other suppressing hosts (*7*, *8*).

We used ochre-type *su* mutants that grew on CA165.2 but not on the *Escherichia coli* strain C600.1 (an S13-sensitive derivative of the amber suppressor C600) nor on C122 (nonsuppressing).

Complementation tests with C122 as the host were performed by the method of E. S. Tessman (*1*). The ochre mutants were tested against amber mutants from each of the known complementation groups. In unmixed infection, none of the bacteriophage used can multiply in C122 except for group V mutants. The group V mutants not only multiply intracellularly but are also difficult to use in complementation tests because they frequently produce large bursts when infected cells are diluted for plating. Therefore, decisive complementation tests could be done only with groups I through IV. Among 13 ochre mutants, only *su*HT205 (Table 1), complemented amber mutants in each of five previously known groups tested (omitting group V).

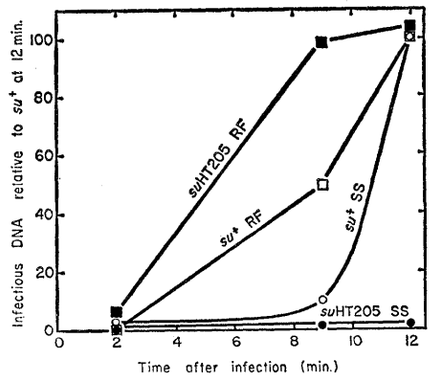

Fig. 1. Synthesis of double-stranded replicative form (RF) and single-stranded (SS) DNA as a function of the time after infection of the nonpermissive host C122 with the group VI ochre mutant *su*HT205, compared to infection with wild type phage. Data on RF and SS are relative to the respective su^+ values at 12 minutes, which are normalized to 100. Assays of infectious DNA were made with spheroplasts of CA165.2.

When assayed on the indicator strains C600.1 and CA165.2, the ochre mutant *su*HT205 plates only on CA165.2 while all the ambers plate with a much higher efficiency on C600.1. Thus, on each indicator strain essentially only one of the two parent types was assayed, and so the true burst sizes are larger than those shown (except for tests involving group IV mutants for which there is a phenomenon of poor rescue, discussed below).

Although tests with group V mutants

Table 1. Burst sizes in complementation tests of *su*HT205.

Mutant tested against *su*HT205	Complementation group	Burst size			
		Assayed on amber suppressor C600.1		Assayed on ochre suppressor CA165.2	
		Mutant alone	Mixed infection	Mutant alone*	Mixed infection
*su*HT205	?			.28	
*su*HS 52	I	0.9	30		
*su*HS129	II	< 0.1	50		
*su*HS 94	IIIa	0.3	35		
*su*HS132	IIIb	0.2	35		
*su*HS113	IV	0.6	1.6		20
*su*HT205	?	< 0.01		0.7	
*su*HS 52	I	2.2	19	0.2	90
*su*HS 86	II	1.2	54	0.4	114
*su*HS 62	IIIa	2.9	37	< 0.01	99
*su*HS 66	IIIb	3.7	16	< 0.01	72
*su*HS100	IV	2.5	1.8	0.3	49

* The discrepancy between the values for the burst sizes of amber mutants assayed on strain C600.1 versus CA165.2 is due to a low efficiency of plating for these amber mutants on the ochre suppressor, CA165.2.