comparison, a sample of muscle nuclear DNA with marker was also banded. Both the I-DNA and nuclear DNA were less dense than the marker (Fig. 2, C and D). The EB shift (*14*) with circular mitochondrial DNA was not observed. In the absence of EB, chick mitochondrial DNA has a buoyant density of 1.708 g/cm^3 (*15*), that for the marker DNA is 1.707 g/cm^3, and that for the chick nuclear DNA is 1.701 g/cm^3.

The acid-insoluble radioactivity which results from labeling with [^{3}H]thymidine in the experiments reported herein is in DNA: it is ribonuclease insensitive, it resists base hydrolysis, is digestible by deoxyribonuclease (Table 4), and bands as DNA in CsCl (Fig. 2). The ratios of the absorbancies at 260 and 280 nm of purified DNA is 1.95 after deproteinization; treatment with amylase, ribonuclease, and pronase; banding in CsCl; and elution from hydroxyapatite. On denaturation and banding the buoyant density increases (*5*).

Although we have reported that I-DNA and nuclear DNA have the same buoyant densities (*3*), here they differ slightly (Fig. 2, A and B). This result has been observed in three separate experiments and may reflect a difference in base composition between I-DNA and nuclear DNA.

In conclusion, the difference in sensitivity of nonmitochondrial cytoplasmic DNA, which we have called I-DNA, as compared with nuclear DNA, to various inhibitors of DNA synthesis makes it entirely unlikely that I-DNA arises as an artifact of tissue fractionation.

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DDT Residues: Distribution of Concentrations in Emerita analoga (Stimpson) along Coastal California

Abstract. *The total concentrations (tDDT) of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] in* Emerita analoga *from 19 California beaches reflect tDDT contamination nearby. Animals near the Los Angeles County sewer outfall contain over 45 times as much tDDT as animals near major agricultural drainage areas. Sediments near the outfall probably contain over 100 metric tons of tDDT—a reservoir for input into marine organisms. The effluent from a plant that manufactures DDT is a probable source.*

Marine organisms from the waters off southern California consistently contain more DDT, DDD, and DDE (tDDT) (*1*) than those taken from near Monterey and San Francisco bays, despite the fact that these latter areas receive drainage from extensive agricultural areas (*2*). This observation has been attributed to a general southerly drift of DDT-laden aerial particles coupled with an input from sewer systems (*2, 3*). I here report on a determination of the relative importance of the various inputs of tDDT into the California coastal waters and provide a profile of the extent of tDDT contamination along the coast of California.

Emerita analoga (Stimpson), the common surf-zone sand crab, was selected as an indicator organism because it is a widely distributed particulate filter feeder in which individual range is confined to at most a few kilometers (*4, 5*). Most oceanic tDDT not found in living organisms is adsorbed onto particles in the size range from 4 to 2000 μm selected by *Emerita* (*5, 6*). *Emerita* will also rapidly take up DDT not associated with particulate material. This ability was demonstrated when 25 animals were placed in 25 liters of seawater (filtered through glass fibers) containing 7.8 parts per trillion (ppt) of ring-labeled [^{14}C]DDT and left there for 24 hours. No sand was provided, and therefore the animals were not expected to feed; feeding was not observed during intermittent observations. The DDT was extracted with hexane from an acid digest (*7*) and counted with a scintillation counter (Nuclear-Chicago Unilux II). Each of the animals contained an average of 1016 ppt of [^{14}C]DDT or 325 times the final seawater concentration. This uptake was largely active. In a similar experiment 58 live animals took up an average of 50 times as much [^{14}C]DDT as did ten animals killed by exposure to Dry Ice; the dorsal carapace and telson, major surfaces for passive adsorption, analyzed separately from the rest of the animals in two cases, accounted for less than one-fifth of the label.

Female animals, each weighing over 2 g, with a mean weight of 3.4 g, were collected from 19 beaches between the Golden Gate Bridge in San Francisco and a point 5 km south of Ensenada, Mexico. Since 36 animals, each over 1 g in weight, collected from a single location in October 1970 showed no correlation between the size of animal and the amount of tDDT, no correction factor for size was deemed appropriate.

I blotted the animals dry, removing the eggs when present, and placed them on Dry Ice within 30 minutes of the time of capture. Within 50 hours the samples were weighed and placed in individual DDT-free shell vials for acid digestion, hexane extraction, and subsequent cleanup on a silica-gel column (*7*). The extracts contained compounds with retention times and partition coefficients matching (within 2 to 3 percent) standards of the DDT series. No interfering chromatographic peaks were present, the large aggregate of peaks characteristic of polychlorinated biphenyls was not present, and the base line was always clearly defined (*8*). The results of the gas-liquid chromatograph-

ic analyses are summarized in Table 1 (*9*). Since egg-laying and the large resultant loss of tDDT from the adult to the eggs is not a synchronous event in the population (*4*), the standard error is large; some animals had recently spawned and lost tDDT, while others harbored undeveloped lipid-rich and therefore tDDT-rich eggs.

Figure 1 is a logarithmic plot of the mean values of the concentrations of tDDT in *Emerita* at various positions along the California coast. Peak values occur at the mouth of San Francisco Bay, in the vicinity of Monterey Bay, and, most dramatically, off the Palos Verdes Peninsula. The first two peaks can be attributed to drainage from major agricultural areas: the San Joaquin Valley and the Sacramento Valley drain into San Francisco Bay, the Salinas Valley into Monterey Bay. The third peak, over 45 times the size of the other peaks, corresponds to the location of the Los Angeles County sewer outfall. The effluent from the Montrose Chemical Corporation of California, the sole manufacturer of DDT in the United States, enters this sewer system. Settleable solids from the primary sewer system (1.44×10^6 m^3 per day) are processed and sold as fertilizer; the liquid effluent and sludge are discharged between 1500 and 3600 m offshore at the White's Point outfall. On 30 March 1970, the Los Angeles County Sanitation District monitored the amount of tDDT found in the sewer system just downstream of the Montrose plant and compared this value with the amount immediately upstream. The results showed a difference of 290 kg/day (*10*). The flow for the next published monitoring date, 27 July 1970, was listed as 19.2 kg/day. Flows of between 11 and 34 kg/day were measured in January 1971 (*11*).

No measurement of tDDT input into the ocean from the White's Point outfall was made prior to March 1970. However, there are indications that the input has been considerable for a long period of time. Sediments taken 15 cm below the surface of the bottom and over 6 km from the outfall contained 310 parts per billion of chlorinated hydrocarbon pesticides (CHP), almost entirely tDDT (*10*). This value is probably much less than the amount originally laid down at that stratum, since anaerobic bacteria can break these pesticides down to undetectable water-soluble products (*12*). Of five samples taken off the top 2.5 cm of sediment within 8 km of the outfall, the lowest amount of

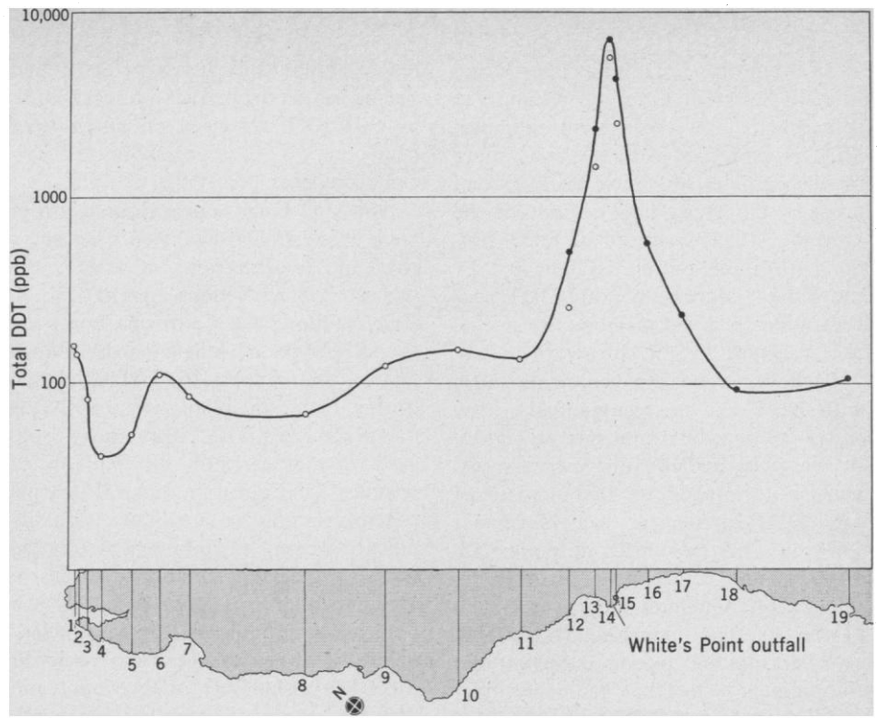

Fig. 1. Plot of mean concentrations in parts ber billion (ppb) (wet weight) of tDDT in *Emerita analoga* at various locations along the California coast. (Open circles) Samples collected in November 1970; (solid circles) samples collected in February 1971. The curve is a freehand interpolation between points.

CHP found was 16 parts per million. Using this value as a low estimate for the concentration in sediments within a rectangle extending along the shore 8 km in both directions and out to sea 8 km from shore, one can calculate the amount of DDT in the top 2.5 cm. If we assume a sediment density of 2 g/cm^3, the amount of CHP is 102 metric tons. A year of input at the highest measured rate of 290 kg/day would be required to account for these surface sediments. In addition, it seems likely that most of the residues are carried

Table 1. Mean concentrations ($\bar{X}$) and standard error (S.E.) in parts per billion (wet weight) of DDT, DDD, and DDE in *Emerita analoga* for given latitudes along the California coast. N, animals collected in November 1970; F, animals collected in February 1971.

Station	Latitude	DDT		DDE		DDD	
		No.	$\bar{X}$ ± S.E.	No.	$\bar{X}$ ± S.E.	No.	$\bar{X}$ ± S.E.
1 N	37°48′	8	69. ± 13.	7	69. ± 13.	7	19. ± 4.
2 N	37°46′	8	74. ± 8.	6	51. ± 7.	7	17. ± 1.
3 N	37°38′	8	35. ± 3.	8	37. ± 5.	7	11. ± 1.
4 N	37°30′	7	11. ± 1.	7	24. ± 1.	7	4.7 ± 0.2
5 N	37°13′	8	7.0 ± 1.2	8	43. ± 10.	8	2.9 ± 0.6
6 N	37°00′	8	26. ± 3.	8	70. ± 5.	8	16. ± 2.
7 N	36°50′	8	24. ± 2.	7	52. ± 7.	7	9.8 ± 1.1
8 N	35°42′	8	4.2 ± 0.5	8	60. ± 7.	7	3.8 ± 0.6
9 N	35°10′	8	14. ± 2.	8	100. ± 8.	8	11. ± 1.
10 N	34°28′	8	14. ± 1.	7	122. ± 8.	8	17. ± 3.
11 N	34°16′	7	36. ± 3.	7	84. ± 5.	7	14. ± 1.
12 N	34°02′	7	20. ± 2.	7	210. ± 12.	7	21. ± 3.
12 F	34°02′	7	23. ± 4.	8	460. ± 50.	8	30. ± 9.
13 N	33°53′	6	580. ± 90.	5	680. ± 100.	5	210. ± 40.
13 F	33°53′	8	340. ± 30.	8	1590. ± 60.	8	410. ± 30.
14 N	33°42′	7	150. ± 20.	7	4900. ± 480.	6	590. ± 80.
14 F	33°42′	8	78. ± 11.	8	6900. ± 900.	8	270. ± 60.
15 N	33°42′	8	88. ± 4.	7	2200. ± 200.	8	240. ± 20.
15 F	33°42′	7	48. ± 8.	7	4200. ± 400.	7	160. ± 40.
16 F	33°34′	7	45. ± 8.	7	470. ± 50.	7	56. ± 7.
17 F	33°22′	8	18. ± 4.	8	190. ± 20.	8	25. ± 6.
18 F	32°48′	7	13. ± 2.	8	71. ± 12.	8	9.1 ± 2.6
19 F	31°50′	6	26. ± 3.	6	68. ± 5.	6	12. ± 1.

away from this area, since the outfall is equipped with diffusers designed to spread and dilute the effluent and since DDT is associated with the finer, more mobile particles which are easily carried away by the strong tidal currents in the area (*6, 13*). This spread of DDT residues from the outfall is indicated by the general decrease of total DDT concentration in *Emerita* from the outfall area to point 3, 573 km away.

Particles in bottom sediments laden with DDT can be resuspended in the water column during storms or periods of upwelling and mixing. Such a resuspension is implied by the increase of total DDT in *Emerita* from November 1970 to February 1971 at points 13, 14, 15, and 16, despite a reported reduction of input into the sewer system (Table 1). The increased DDE/DDT ratio during this period indicates that the animals have taken relatively older residues such as those that have resided in bottom sediments and have been lifted by rough winter seas. Thus this reservoir of well over 100 metric tons is of potential biological importance and should be considered, along with Cox's estimate of 46 metric tons suspended in the photic zone of the California coastal waters, in estimating the total local burden of DDT (*14*).

The maximum DDT value listed in Table 1 does not correspond geographically to the maximum DDE value. Two explanations are plausible. (i) There is a major DDT input, distinct from the DDE input, which is closer to point 14 than to point 15—the Los Angeles City sewer system, which is totally separate from the Los Angeles County system, has an outfall almost directly offshore from point 14. There is no known major input of DDT into the Los Angeles City sewer system; moreover, since both systems have primary treatment, they should degrade approximately the same fraction of DDT to DDE—yet the DDE/DDT ratios differ dramatically. (ii) Currents that sweep materials north and west from the outfall, present in November 1970, swept the bulk of "fresh" sewage beyond the Palos Verdes Peninsula into Santa Monica Bay (*13, 15*). This "fresh" sewage would have a lower DDE/DDT ratio than older sewage so that animals exposed to it would contain relatively higher amounts of DDT. Low DDE/DDT ratios can be found near other locations where DDT entered the marine environment comparatively recently, such as mouths of rivers which drain major agricultural areas (points 1 and 7) and runoff from sites of local DDT usage (DDT was used on citrus trees near point 11 and presumably on Mexican crops near point 19).

Although *Emerita* near drainage from areas where DDT has been used show elevated concentrations of tDDT, the highest concentrations of tDDT in *Emerita* along the California coast are found near the effluent from the sewage system that accepts the industrial discharge from the plant where DDT is manufactured. This observation suggests that historically the buildup of residues in California coastal marine organisms could be attributed, to a significant degree, to industrial waste discharge rather than merely to extensive agricultural usage. Data taken at two points in time indicate that bottom sediments serve as an important reservoir for DDT residues from this input and that this reservoir may become available for biological uptake when these sediments are stirred up.

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1. In this study only *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], *p,p'*-DDD [1,-1-dichloro-2,2-bis(*p*-chorophenyl)ethane], *p,p'*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] were measured. The terms DDT, DDD, and DDE refer, respectively, to these compounds. The term tDDT used in this report refers to the total of these three compounds.
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Alkyl Isocyanates as Active Site-Specific Inhibitors of Chymotrypsin and Elastase

Abstract. *Alkyl isocyanates react specifically with the two serine proteinases, chymotrypsin and elastase, to yield inactive enzyme derivatives containing 1 mole of reagent per mole of enzyme. Octyl isocyanate inactivates chymotrypsin only, while butyl isocyanate inactivates both enzymes but shows greater efficiency toward elastase than toward chymotrypsin. These reagents may thus represent unique chemical "yardsticks" for the measurement of the relative dimensions of the active sites of the two very similar enzymes.*

Chymotrypsin and elastase are pancreatic proteinases with similar structure and catalytic function. The structural properties were first established by the complete elucidation of the primary amino acid sequences (*1*) and subsequently by high-resolution x-ray crystallography which has led to elegant three-dimensional models for both enzymes (*2*). The two molecules show extensive homology in their primary amino acid sequences and their folded structures are also remarkably similar (*3*). The active site serine (*4*), which is common to both enzymes (and which is acylated and deacylated in the amide and ester hydrolysis catalyzed by the enzymes), is located near the edge of a pronounced cavity or pocket on the surface of the two globular proteins. Since it has now been established in the case of chymotrypsin (*5*) that this cavity is responsible for the binding of the substrate component which contributes the acyl group to the amide or ester bond cleaved by the enzyme, the cavity has been referred to as the "binding pocket" (*3*). The only significant difference in the function of chymotrypsin and elastase is expressed by their sub-