may recognize different multiples of the same chemical determinant; either of these possibilities is supported by the fact that an antigen in a preparation of pneumococcal type XIV polysaccharide is precipitated by 603 but not 167 (see above).

Finally, it is of considerable biologic interest that three proteins in our series of 64 and one in Cohn's series of 53 IgA myelomas (*6*) identify the same antigen. In addition, a relatively high and unexpected frequency of tumors producing myeloma proteins that react with DNP has also been found (*1*). The method of production of tumors used in these studies may selectively stimulate proliferation of clones of cells producing a restricted range of antibodies.

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References and Notes

1. H. N. Eisen, E. S. Simms, M. Potter, in preparation.
2. M. A. Leon and M. Potter, unpublished observations.
3. M. A. Leon, K. R. McIntire, M. Potter, in preparation.
4. H. N. Eisen, J. R. Little, C. K. Osterland, E. S. Simms, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 75 (1967).
5. H. Metzger, *Proc. Nat. Acad. Sci. U.S.* **57**, 1490 (1967).
6. M. Cohn, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 211 (1967).
7. M. Potter and C. R. Boyce, *Nature* **193**, 1086 (1962).
8. M. Potter and R. Lieberman, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 187 (1967).
9. E. C. Gotschlich and T.-Y. Liu, *J. Biol. Chem.* **242**, 463 (1967). We thank Dr. Gotschlich for this C polysaccharide and for testing the precipitability with the mucopeptide and polysaccharide fractions.
10. Tumor MOPC 167 was induced in a BALB/c mouse given three injections of 0.5 ml of Bayol F intraperitoneally when the mouse was 2, 4, and 6 months of age. This mouse was immunized with ovalbumin at the same time. Tumor Mc 603 was induced by Dr. K. R. McIntire in an ex-germfree, nonimmunized BALB/c mouse by the same method as above. Tumor MOPC 299 was similarly induced in a BALB/c mouse. This mouse was immunized concomitantly with red blood cells from sheep, pig, horse, cow, goat, and mule. Further characterization of 299 was omitted because this transplant line began producing little or no myeloma protein.
11. Streptococcal culture extracts and polysaccharides prepared by Dr. R. Lancefield, Rockefeller University.
12. *Salmonella* and *E. coli* lipopolysaccharides given by Dr. O. Lüderitz, Max-Plank-Institut.
13. Dextrans and levans given by Dr. A. Jeanes, Northern Regional Research Laboratory, USDA, Peoria, Illinois.
14. Mannans were given by Dr. M. Slodki, Northern Regional Research Laboratory, USDA, Peoria, Illinois.
15. Teichoic acids were provided by Dr. D. Tipper, Department of Pharmacology, University of Wisconsin.
16. Hemicelluloses were provided by Dr. R. L. Whistler, Purdue University.
17. Type-specific pneumococcal polysaccharides were supplied by Dr. M. Heidelberger, New York University, and Dr. R. Brown, New York State Department of Health Laboratories.
18. J. I. Fahey, *J. Exp. Med.* **114**, 399 (1961); M. Potter and E. L. Kuff, *J. Mol. Biol.* **9**, 537 (1964).
19. M. Potter, in *Meth. Cancer Res.* **2**, 105 (1967).
20. We thank Miss R. Lieberman for discussions and suggestions in the preparation of the manuscript.

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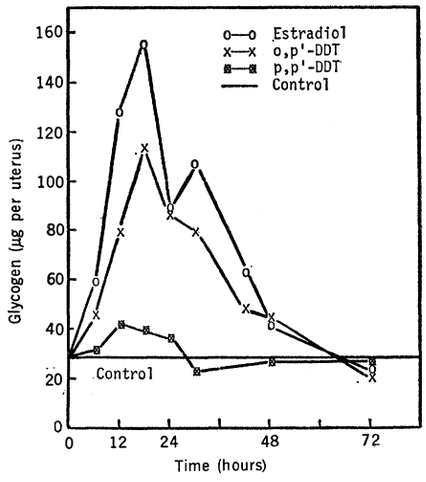

Fig. 1. Glycogenic response of immature rat uterus to DDT isomers and 17β-estradiol. Four milligrams of *o,p'*-DDT or *p,p'*-DDT, or 0.4 μg of 17β-estradiol, was injected subcutaneously at 0 time. Each point consists of determinations of 4 to 14 uteri of 22- to 25-day-old rats; the control level was established by use of 70 rat uteri. Glycogen was determined by the anthrone procedure (*12*).

Estrogenic Activity of o,p'-DDT in the Mammalian Uterus and Avian Oviduct

Abstract. *When rats and birds were treated with* o,p'*-DDT, their reproductive tissues exhibited the same response as when they were treated with estrogen. Weight, water content, glycogen, and RNA increased in uteri and oviducts of rats, chickens, and quail receiving* o,p'*-DDT;* p,p'*-DDT produced little if any response. The* o,p'*-DDT did not accumulate in the reproductive or adipose tissues to a greater extent than* p,p'*-DDT.*

The similarity in the configuration of DDT (*1*) to the synthetic estrogen, diethylstilbestrol, has prompted investigation of the estrogenic activity of DDT in mammals and birds (*2*). These studies have yielded conflicting results and both positive (*2*) and negative (*3*) evidence of estrogenicity was obtained. Recently, Levin, Welch, and Conney (*4*) reported that *o,p'*-DDT is estrogenic, while *p,p'*-DDT is only weakly active. Since commercial technical DDT contains approximately 80 percent *p,p'*-DDT and 15 to 20 percent *o,p'*-DDT, this finding offers a possible explanation for inconsistent reports in the literature.

We investigated the estrogenic activity of *o,p'*-DDT and *p,p'*-DDT, comparing the time course of effects of these DDT isomers with 17β-estradiol on several biochemical constituents of the immature rat uterus. Glycogen, an extremely sensitive indicator of estrogen action (*5*), increased after *o,p'*-DDT administration in a manner very similar to 17β-estradiol (Fig. 1), while *p,p'*-DDT exhibited only slight activity. The *o,p'*-DDT stimulated characteristic estrogenic responses in the uterus—increases in wet weight, water content, and RNA content 24 hours after administration being similar to those elicited by estradiol.

Woodwell (*6*) and Wurster and Wingate (*7*) have determined DDT residues in a number of species of bird and have implicated contamination by insecticides as a probable major cause of the decline in reproduction in several species. In view of the estrogenicity of *o,p'*-DDT in the uterus of a mammalian species, it was of interest to determine whether sublethal concentrations of this isomer of DDT would have an effect upon the oviduct, the reproductive tract of avian species. Five-week-old chickens (Cornish, weighing 700 to 900 g) and 25-day-old Japanese quail (Coturnix quail, weighing 70 to 90 g) received three daily intraperitoneal injections of *o,p'*-DDT, *p,p'*-DDT, or 17β-estradiol in olive oil. Twenty-four hours after the last injection (72 hours after the initial injection), the birds were killed and oviducts were removed for analysis. The results (Table 1) demonstrate that *o,p'*-DDT produced the same effects as estradiol in the oviducts of chickens and quail. A 100 percent increase in oviduct weight and 150 to 175 percent increases in glycogen content occurred. Little if any estrogenic activity was shown by *p,p'*-DDT.

In order to determine whether *o,p'*-

Table 1. Effect of DDT isomers on oviduct weight and glycogen content of chickens and quail. S.E., standard error.

Treatment	No. tested	Oviduct wt. (mg ± S.E.)	Glycogen (μg/oviduct ± S.E.)
	Chickens		
None (control)	9	65 ± 3	22 ± 2
p,p'-DDT, 3 × 50 mg	10	73 ± 3	29 ± 3
o,p'-DDT, 3 × 50 mg	8	122 ± 16	55 ± 8
Estradiol, 3 × 500 μg	10	110 ± 7	39 ± 5
	Japanese quail		
None (control)	6	14.9 ± 0.8	5.8 ± 0.2
p,p'-DDT, 3 × 5 mg	6	14.9 ± 2.3	5.4 ± 0.7
o,p'-DDT, 3 × 5 mg	6	32.0 ± 2.2	16.0 ± 2.1
Estradiol, 3 × 50 μg	6	42.8 ± 6.6	20.6 ± 5.0

Table 2. Concentrations of *o,p'*-DDT and *p,p'*-DDT in uteri and oviducts of rats, chickens, and quail after treatment with *o,p'*-DDT or *p,p'*-DDT. Rats (21 days old; body weight 43 g) received three daily injections of 2 mg of *o,p'*-DDT or *p,p'*-DDT subcutaneously; 14-week-old white Leghorn chickens (body weight 1100 g) received three daily injections of 50 mg of *o,p'*-DDT or *p,p'*-DDT subcutaneously; and 25-day-old Japanese quail (body weight 79 g) received three daily injections of 5 mg of *o,p'*-DDT or *p,p'*-DDT subcutaneously. All animals were killed 72 hours after the first injection.

Species	No. tested	Total dose (mg/kg)	Treatment with	Recovery in tissue		
				Uterus (μg/g)	Oviduct (μg per tissue)	Adipose (μg/g)
Rat	8	140	*o,p'*-DDT	0.26	0.012	36.7
	8	140	*p,p'*-DDT	2.57	.060	148.0
Chicken	2	136	*o,p'*-DDT	3.07	.570	33.4
	2	136	*p,p'*-DDT	4.34	.564	55.0
Quail	6	190	*o,p'*-DDT	7.23	.099	258.0
	12	190	*p,p'*-DDT	10.1	.128	331.0

DDT accumulated in the uterus and oviduct to a greater extent than *p,p'*-DDT, and exerted an estrogenic action because of a preferential uptake, uterus and oviduct of treated rats and chickens were analyzed for pesticide content (*8*) by gas-liquid chromatography with the use of an electron capture detector (Table 2). The *o,p'*- or *p,p'*-DDT isomers were given at the same total dosage, and the dosage rate administered to the two species was similar. In rats, *o,p'*-DDT was found to accumulate to a much lesser extent than *p,p'*-DDT. This finding is in accord with the accumulation factors given by Durham (*9*) for human subjects, in which *o,p'*-DDT has a value of 60, while *p,p'*-DDT is 300 (five times greater). In chickens, however, *o,p'*-DDT accumulated to about the same extent as *p,p'*-DDT when given at equivalent dosage rates. It seems likely that the estrogenic activity of *o,p'*-DDT is associated with the *o,p'*-DDT molecule, or a metabolite, and no apparent selective accumulation of this isomer takes place in the reproductive tissues. The presence of an active impurity in *o,p'*-DDT cannot be excluded at the present time, although we obtained it from two sources (*10*). The doses used to elicit the estrogenic effects in the rats, chickens, and quail are certainly not minimal, and preliminary work has indicated that quantities ten times less are active.

Woodwell (*6*) and other scientists engaged in investigation of pesticide problems (*11*) have estimated that over 1 billion pounds (453×10^6 kg) of DDT is presently available in the biosphere. Since *o,p'*-DDT is present in technical DDT to the extent of 15 to 20 percent, approximately 200 million pounds of an active estrogen may be present in the environment. The apparent lower accumulation of *o,p'*-DDT as compared to *p,p'*-DDT in rat and human tissues [Table 2 and (*9*)] raises the possibility that *o,p'*-DDT would be concentrated to a lesser extent in ecological food webs. If this were the case, *o,p'*-DDT might pose a less serious ecological hazard than would be expected from the amounts of technical DDT existing in the environment. A lack of information exists on the content of *o,p'*-DDT in feedstuffs, because surveys generally report total residues of DDT and its analogs and do not report the individual compounds. Usual survey methods do not distinguish between *o,p'*-DDT and *p,p'*-DDD (*8*). Adjustment of the physical parameters of a gas chromatograph will permit measurement of the separate isomers. A change in reporting of results by agencies currently making pesticide surveys might then make it possible to gauge the relative amounts of biologically active compounds more accurately.

We have demonstrated that *o,p'*-DDT is estrogenic in a mammalian species (rat) and in two avian species (chicken and quail). The physiological significance of this has not yet been assessed in either laboratory or farm animals, poultry, wild species of birds, fish, or man. Further studies are needed to determine the effects of this widely used pesticide upon fertility and reproductive parameters in all of these species. It is also essential to determine mechanisms of action and of metabolism of *o,p'*-DDT in plant and animal tissues. The estrogenicity of *o,p'*-DDT and its widespread occurrence in our environment form a possible basis for important ecological effects upon reproduction in mammals and birds.

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References and Notes

1. Abbreviations: *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane; *p,p'*-DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.
2. H. Burlington and V. F. Lindeman, *Proc. Soc. Exp. Biol. Med.* **74**, 48 (1950); W. B. Deichmann and M. L. Keplinger, *Toxicol. Appl. Pharmacol.* **8**, 337 (1966).
3. A. L. Fisher, H. H. Keasling, F. N. Schueler, *Proc. Soc. Exp. Biol. Med.* **81**, 439 (1952); J. F. Treon, J. Boyd, G. Berryman, J. Gosney, L. Hartman, D. Brown, J. Coomer, *Final Report* (Kettering Laboratory, Univ. of Cincinnati, College of Medicine, 30 June 1954); G. W. Ware and E. E. Good, *Toxicol. Appl. Pharmacol.* **10**, 54 (1967).
4. W. Levin, R. M. Welch, A. H. Conney, *Fed. Proc.* **27**, 649 (1968).
5. J. Bitman, H. C. Cecil, M. L. Mench, T. R. Wrenn, *Endocrinology* **76**, 63 (1965).
6. G. M. Woodwell, *Sci. Amer.* **216**, 24 (March 1967); G. M. Woodwell, C. F. Wurster, Jr., P. A. Isaacson, *Science* **156**, 821 (1967).
7. C. F. Wurster, Jr., and D. B. Wingate, *Science* **159**, 979 (1968).
8. *Pesticide Analytical Manual*, H. C. Barry, J. G. Hundley, L. Y. Johnson, Eds. (U.S. Dept. of Health, Education, and Welfare, Food and Drug Administration, 1963; revised 1964 and 1965), vol. 1.
9. W. F. Durham, *Residue Rev.* **18**, 21 (1967).
10. Aldrich Chemical Co., Milwaukee, Wis.; we thank Stanley A. Hall, Pesticide Chemicals Research Branch, ARS, USDA, for generously supplying samples of *o,p'*-DDT.
11. *Scientific Aspects of Pest Control*, Publication 1402 (National Academy of Sciences–National Research Council, Washington, D.C., 1966).
12. S. Seifter, S. Dayton, B. Novic, E. Muntwyler, *Arch. Biochem. Biophys.* **25**, 191 (1950).

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