fact that citrate-activated acetyl CoA carboxylases from adipose tissue, rat liver (*13*), and mammary gland (*14*) have high sedimentation coefficients suggests that they, too, exist as filamentous species. Thus, it may be significant that the acetyl CoA carboxylases, from animal tissues, which are citrate-activated and regulate fatty acid biosynthesis have this unique quaternary structure, while their counterparts in *Escherichia coli* (*5*), yeast (*4*), and plant tissues (*3*) do not. This high degree of structural organization exhibited by the animal carboxylases suggests a possible structural role in addition to their known catalytic and regulatory functions. Conceivably, the carboxylase filaments could serve as an organizing matrix for a loose supramolecular complex of other enzymes taking part in lipid biosynthesis. Cytofilaments with dimensions similar to those of the carboxylase filaments have been observed surrounding the triglyceride droplets in thin sections of developing and mature adipose tissue cells (*15*). Whether these intracellular filaments are identical to the acetyl CoA carboxylase filaments isolated from adipose tissue is still a matter of conjecture.

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References and Notes

1. J. Ganguly, *Biochim. Biophys. Acta* **40**, 110 (1960); S. Numa, M. Matsuhashi, F. Lynen, *Biochem. Z.* **334**, 203 (1961); D. B. Martin and P. R. Vagelos, *J. Biol. Chem.* **237**, 1787 (1962); H. C. Chang, I. Seidman, G. Teebor, M. D. Lane, *Biochem. Biophys. Res. Commun.* **28**, 682 (1967).
2. P. R. Vagelos, *Annu. Rev. Biochem.* **33**, 139 (1964).
3. P. F. Heinstein and P. K. Stumpf, *Fed. Proc.* **27**, 647 (1968).
4. M. Matsuhashi, S. Matsuhashi, F. Lynen, *Biochem. Z.* **340**, 263 (1964).
5. A. W. Alberts and P. R. Vagelos, *Proc. Nat. Acad. Sci. U.S.* **59**, 561 (1968).
6. C. Gregolin, E. Ryder, A. K. Kleinschmidt, R. C. Warner, M. D. Lane, *ibid.* **56**, 148 (1966).
7. C. Gregolin, E. Ryder, R. C. Warner, A. K. Kleinschmidt, M. D. Lane, *ibid.*, p. 1751.
8. C. Gregolin, E. Ryder, M. D. Lane, *J. Biol. Chem.* **243**, 4227 (1968).
9. A. K. Kleinschmidt and M. D. Lane, *Biophys. J.* **9**, A106 (1969).
10. C. Gregolin, E. Ryder, R. C. Warner, A. K. Kleinschmidt, H. C. Chang, M. D. Lane, *J. Biol. Chem.* **243**, 4236 (1968).
11. J. Moss, A. K. Kleinschmidt, M. Yamagishi, M. D. Lane, unpublished observations.
12. J. Moss, M. Yamagishi, A. K. Kleinschmidt, M. D. Lane, *Fed. Proc.* **28**, 1548 (1969).
13. S. Numa, E. Ringelmann, B. Riedel, *Biochem. Biophys. Res. Commun.* **24**, 750 (1966).
14. A. L. Miller and H. R. Levy, *J. Biol. Chem.* **244**, 2334 (1969).
15. W. Bloom and D. W. Fawcett, *A Textbook of Histology* (Saunders, Philadelphia, ed. 9, 1968), p. 169; M. C. Schotz, J. E. Stewart, A. S. Garfinkel, C. F. Whelan, N. Baker, in *Drugs Affecting Lipid Metabolism*, W. L. Holmes, L. A. Carlson, R. Paoletti, Eds. (Plenum, New York, 1969), p. 161; H. Sheldon, personal communication (1969).
16. C. N. Gordon and A. K. Kleinschmidt, *Biochim. Biophys. Acta* **155**, 305 (1968).
17. C. E. Hall and P. Doty, *J. Amer. Chem. Soc.* **80**, 1269 (1958).
18. Supported by grants from the John A. Hartford Foundation, Inc., New York; NIH grant (AM-09116), and American Heart Association. M.D.L. was supported by PHS research career program award K3-AM-18487 and J.M. by PHS medical scientists training grant 5TO5 GM-01668. We thank H. Annus, M. Pendergast, and F. Zaboretsky for assistance.

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Avian Thyroid: Effect of p,p'-DDT on Size and Activity

Abstract. *Feeding sublethal amounts of* p,p'-*DDT to pigeons caused an increase in thyroid weight and a reduction in colloid content of the follicles. This may reflect a hyper- or hypofunctioning gland and may be connected with recent reductions in egg shell weights in wild birds. The effect was accompanied by increased liver weight.*

The finding of symptoms suggesting hyperthyroidism in Bengalese finches (*Lonchura striata*) fed sublethal doses of *p,p'*-DDT (*1*, *2*) prompted further work on the effect of DDT on the avian thyroid. Three groups of four homing pigeons (*Columba livia*) were force-fed every second day for 42 days with gelatin capsules containing *p,p'*-DDT (*2*) dissolved in olive oil (1 mg of DDT in 10 mg of oil). The three doses were approximately 18, 36, and 72 mg of DDT per kilogram of body weight per capsule based on the body weight on the initial day of the experiment (mean, 408 ± 17 g) (Table 1). The mean total dosage of DDT given amounted to 381, 765, and 1517 mg/kg. Six birds (mean body weight, 400 ± 22 g) were maintained as controls. Four of these were given 200 mg of olive oil in a capsule every second day, while the other two were not given any oil in order to assess any possible effect of the solvent alone. The birds were fed on maize, wheat, and tick beans and maintained in individual cages (40 by 40 by 64 cm) at 20°C. No birds died, although one receiving 72 mg/kg every second day started to tremor on day 30. On day 42 they were all killed, and the bodies were weighed and dissected. Both thyroid glands and the liver and brain of each bird were weighed. The final body weights showed a similar mean weight loss in both control birds (2.46 percent) and birds fed DDT (2.78 percent). Sex was also determined at dissection. The low and medium treatments were given to three females and one male, while two females and two males received high treatment. Control consisted of three females and three males.

The liver and brain of each of the birds fed DDT and one of the controls were analyzed for *p,p'*-DDT and *p,p'*-DDE. A 1- to 2-g sample of each tissue was ground with sand and anhydrous sodium sulfate and extracted with redistilled hot *n*-hexane and acetone. The extract was subjected to cleanup (*3*) and then analyzed by gas-liquid chromatography (*4*) (Table 1).

The weights of the liver and both thyroids (Table 1) of each bird were calculated as a percentage of the brain weight (mean, 2.154 ± 0.029 g), because this is a better indicator of body size than the more variable body weight. The percentages were then cor-

Table 1. The DDT residues (mean ± S.E.) in the livers and brains of the control birds and of birds fed three levels of DDT. The thyroid weight (mean ± S.E.) and activities as measured by colloid areas are also given. The *p,p'*-DDT doses were administered every second day for 42 days. The numbers in parenthesis are the range; ND, none detected.

	Concentration (ppm, wet weight)		Total thyroid weight (mg)	Total thyroid weight as percentage of brain weight	Colloid area per field (μ^2)
	p,p'-DDT	*p,p'*-DDE			
			Control		
Liver	ND*		39 ± 5	1.75 ± 0.23	86,120 ± 14,430
Brain	ND*		(28 to 55)	(1.30 to 2.57)	(42,480 to 145,800)
			18.2 mg/kg		
Liver	29.7 ± 9.0	30.2 ± 8.0	45 ± 7	2.17 ± 0.38	18,570 ± 10,940
Brain	9.0 ± 1.8	10.7 ± 2.3	(26 to 58)	(1.23 to 2.85)	(2,700 to 49,080)
			36.4 mg/kg		
Liver	56.5 ± 17.1	62.1 ± 18.9	80 ± 15	3.67 ± 0.65	19,620 ± 11,900
Brain	6.6 ± 0.6	12.0 ± 4.4	(48 to 112)	(2.47 to 5.09)	(0 to 48,360)
			72.2 mg/kg		
Liver	149.5 ± 41.9	288.6 ± 43.1	79 ± 7	3.69 ± 0.32	6,060 ± 5,940
Brain	12.4 ± 1.6	40.6 ± 5.8	(59 to 95)	(2.99 to 4.49)	(0 to 23,880)

* Concentrations below 0.1 ppm.

related with the concentration of DDT in the liver in parts per million. The latter was used because it is an indicator of the amount of DDT circulating in the body rather than the amount stored.

Regression analysis showed that, as the DDT content increased, the liver weight also increased (Fig. 1) ($r = 0.8378$; d.f. 16; $P < .1$ percent; equation: $y = 335 + 44.21\ x$, where $y =$ liver weight as a percentage of brain weight and $x =$ square root of concentration of DDT in the liver in parts per million). Although the mean of the liver to brain weight percentages was lower in the two control birds not receiving olive oil (249 percent) than in the four receiving 100 mg of oil each day (354 percent), there are similar percentages in each group. Also, the mean percentages of liver to brain weight of the three treated groups (556, 628, and 916 percent) are very much higher than the control groups, although their oil consumption is lower in two instances (37, 75, and 146 mg/day, respectively). Therefore the increase in liver size was apparently a reaction to the DDT rather than to the olive oil in which it was fed.

There was a similar significant increase in total thyroid weight with in crease in concentration of DDT in the liver (Table 1) ($r = 0.6252$; d.f. 16; $P < 1$ percent; equation: $y = 1.898 + 0.149\ x$, where $y =$ thyroid weight as a percentage of brain weight and $x =$ square root of concentration of DDT in the liver in parts per million). The thyroid weights of the two control groups were similar (no oil, 1.83 percent; olive oil, 1.71 percent of the brain weights).

After the thyroids were weighed, they were fixed in 10 percent buffered formalin solution and sectioned; they were then stained with hematoxylin and eosin and visually examined. All the control birds showed follicles containing colloid quantities within normal limits which were reasonably evenly distributed throughout the gland (Fig. 2a). Lymphocytic foci were noted in two birds and three showed lymphoid follicles.

Of the four birds fed 18 mg/kg every second day, two showed evidence of some reduction in follicular size and colloid amount associated with a minimum degree of hyperplasia of the epithelium. The other two showed marked hyperplasia together with very low colloid amounts and prominent blood capillaries. An occasional lymphoid

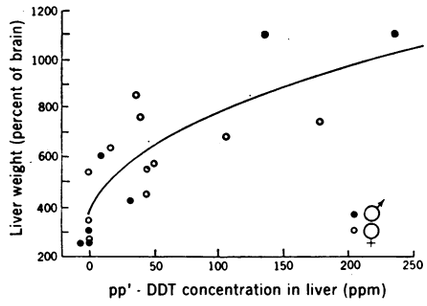

Fig. 1. Correlation of liver weight, expressed as a percentage of brain weight, and the concentration of *p,p'*-DDT in the liver in parts per million (wet weight). The calculated regression line is also shown.

follicle was noted in one of the former birds. A similar situation existed in the four birds fed 36 mg/kg every second day, except that the loss of colloid in the two most affected birds appeared to be absolute. Occasional lymphoid follicles were noted in two birds.

One bird fed 72 mg/kg every second day showed an occasional thyroid follicle containing colloid, particularly at the periphery of the gland, but all the central areas showed almost complete colloid loss associated with hyperplastic epithelium. The other three birds fed 72 mg/kg every second day showed almost complete to absolute loss of colloid material with marked hyperplasia (Fig. 2b). There was also evidence of vascular congestion with dilated capillaries. One bird showed a thyroid cyst.

To make a quantitative estimation of the effect of DDT on thyroid activity, the material was analyzed on the Quantimet image analyzer computer (*5, 6*). The Quantimet couples a television system and a computer with the microscope and operates on the principle of line scanning for counting and measuring (area) any particle of sufficient contrast in a microscope image. Additional sections of the thyroids (5 μ thick) were cut and stained with periodic acid–Schiff reagent and placed in the Quantimet for measurement of colloid area within the thyroid follicles. A $\times$ 5 objective was used and the area of field investigated was 608,000 μ^2. The colloid areas of 50 different fields from the left and right thyroids of each bird were measured and the means calculated (the means of the four means for each treatment are given in Table 1). The mean colloid area per field for each bird was also correlated with the concentration of DDT in the liver. Regression analysis showed that as DDT content increased the colloid area decreased ($r = -0.6771$; d.f. 16; $P < 1$ percent; equation: $y = 69{,}770 - 5{,}790\ x$, where $y =$ colloid area per field in μ^2 and $x =$ square root of DDT concentration in the liver in parts per million). The use of colloid areas gives a more sensitive measurement than thyroid weight of any effect of low concentrations of DDT on the thyroid. Although the difference in the thyroid weights of the group receiving low treatment and the controls was too small to be significant, the difference in activity is significantly large ($P < 1$ percent). With regard to individual birds, one bird in the low treatment group had a mean colloid area of 2700 μ^2. This is 3 percent of the mean control area. Because the liver content of this bird was only 11.4 ppm of *p,p'*-DDT, a concentration at least 1/20 of the lowest lethal content (239.0 ppm was

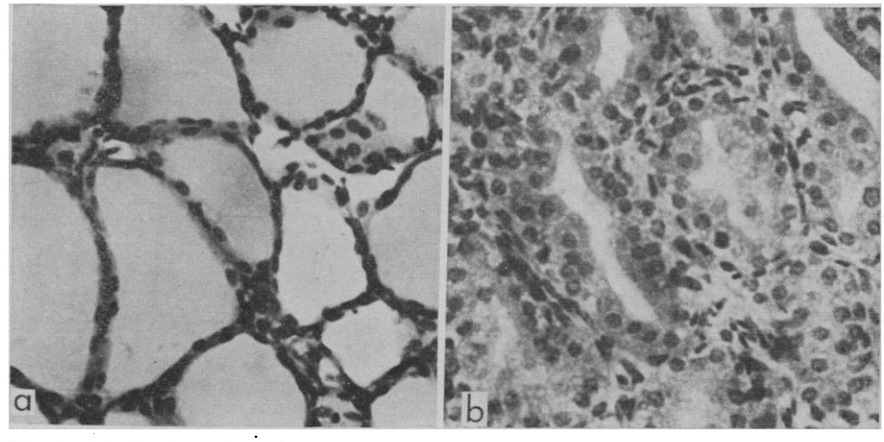

Fig. 2. (a) Section of thyroid from control bird showing follicles containing normal quantities of colloid. (b) Section of thyroid from bird receiving 72 mg of *p,p'*-DDT per kilogram every second day, showing complete loss of colloid material associated with hyperplastic epithelium.

found in a living bird receiving the high treatment), it seems likely that even the low "background" quantities found in wild birds (*7*) could have an effect on thyroid activity.

An increase in thyroid size with hyperplasia associated with a reduction in colloid content of the follicles may reflect either a hyper- or hypofunctioning gland. A hyperfunctioning gland may be the result of (i) stimulation of the pituitary or thyroid to produce excess thyroid-stimulating hormone (TSH) or thyroxine or (ii) a reduction in the concentration of circulating thyroxine causing the production of TSH by the pituitary. This in turn would then stimulate the thyroid to accelerate the formation and secretion of thyroxine. A reduction in the amount of circulating thyroxine would be brought about by increased hepatic activity, as indicated by the increase in liver size, causing increased hepatic metabolism of thyroxine. A second possibility for the reduction of thyroxine is that isomers of DDT may have an estrogenic activity (*8*), as estrogen increases the destruction of the circulating hormone (*9*). A hypofunctioning gland may result if (iii) DDT acts as a goitrogen. Goitrogens, such as thiouracil, produce the same histological picture as above, and the gland is hypofunctioning as the formation of thyroxine is suppressed within the gland (*10*).

With cause (i) the animal concerned would be in a hyperthyroidal state, and with cause (iii) in a hypothyroidal state. With cause (ii), as long as a sufficient supply of thyroxine remained available, signs of hypothyroidism would not develop. If the thyroid could not achieve a balance with the breakdown, symptoms of hypothyroidism, and, possibly with overstimulation, even hyperthyroidism would develop. Fregly *et al.* (*11*) concluded that the increased thyroid weights in rats fed *o,p'*-DDD were producing symptoms of hypothyroidism. In Bengalese finches the symptoms suggested hyperthyroidism (*1*), though some reactions such as decreased egg weight can be produced by both hyper- and hypothyroidism (*12, 13*). The reduction in eggshell weight in birds of prey (*14*) and its correlation with DDT and its metabolites (*15*) suggests that many wild birds are in a hypothyroidal state (*13*). The decision on whether experimental birds are in a hyper- or hypothyroidal state awaits metabolic rate examinations because chemical analysis of protein-bound iodine in the blood as a measure of changes in iodine-containing hormones appears to be insensitive in birds (*16*). Marked differences in species responses to changes in thyroxine concentrations are likely, as opposite results are obtained with different strains of hens (*17*).

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References and Notes

1. Abbreviations are: *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; *o,p'*-DDD: 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.
2. D. J. Jefferies, *Nature* **222**, 578 (1969). The *p,p'*-DDT used in this and the present work was supplied by R. N. Emanuel Ltd., England, at a purity of greater than 99 percent. No other compounds were detected on analysis.
3. M. J. de Faubert Maunder, H. Egan, E. W. Godly, E. W. Hammond, J. Roburn, J. Thomson, *Analyst* **89**, 168 (1964).
4. A Perkin-Elmer 452 gas-liquid chromatograph equipped with electron capture detector and all-glass injection system was used. The 76-cm glass column was packed with Diatomite CQ coated with Silicone Epikote (0.25 percent Epikote). The nitrogen flow rate was 120 ml/min at 188°C oven temperature, and injection sample size was 5 μl. Quantitative estimation was by comparison of peak heights to standards. Sensitivity was 0.001 ppm.
5. The "Quantimet" Image Analyser Computer is manufactured by Metals Research Limited, Cambridge, England.
6. L. E. Mawdesley-Thomas and P. Healey, *Science* **163**, 1200 (1969); *New Sci.* **41**, 286 (1969).
7. C. H. Walker, G. A. Hamilton, R. B. Harrison, *J. Sci. Food Agr.* **18**, 123 (1967).
8. J. Bitman, H. C. Cecil, S. J. Harris, G. F. Fries, *Science* **162**, 371 (1968).
9. R. E. Burger, F. W. Lorenz, M. T. Clegg, *Poultry Sci.* **41**, 1703 (1962).
10. R. A. Larson, F. R. Keating, W. Peacock, R. W. Rawson, *Endocrinology* **36**, 149 (1945).
11. M. J. Fregly, I. W. Waters, J. A. Straw, *Can. J. Physiol. Pharmacol.* **46**, 59 (1968).
12. M. M. Oloufa, *Poultry Sci.* **32**, 391 (1953).
13. L. W. Taylor and B. R. Burmester, *ibid.* **19**, 326 (1940).
14. D. A. Ratcliffe, *Nature* **215**, 208 (1967).
15. J. J. Hickey and D. W. Anderson, *Science* **162**, 271 (1968).
16. W. J. Mellen and L. B. Hardy, *Endocrinology* **60**, 547 (1957).
17. E. W. Glazener, C. S. Shaffner, M. A. Jull, *Poultry Sci.* **28**, 834 (1949).
18. We thank Dr. L. E. Mawdesley-Thomas for carrying out the Quantimet examinations at Huntingdon Research Centre, Huntingdonshire, England, and Miss J. Relton for taking the photographs.

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Plus and Minus Single-Stranded DNA Separately Encapsidated in Adeno-Associated Satellite Virions

Abstract. *Based on physical and chemical determinations, the molecular weight of the type 4 adeno-satellite virus is 5.4×10^6 daltons, and the virion contains 1.4×10^6 daltons of DNA. Denaturation and renaturation studies indicate that the viral genome is a single-stranded DNA molecule and that each virion contains either a minus or a plus strand. Upon extraction, the minus and plus strands unite to form double-stranded DNA molecules with no obvious excess of unpaired strands.*

Adeno-associated satellite viruses are biologically defective and replicate only in the presence of competent adenovirions. When isolated from the virion, the DNA of satellite virus had been shown previously to be double-stranded by hydrodynamic and biochemical analyses (*1, 2*).

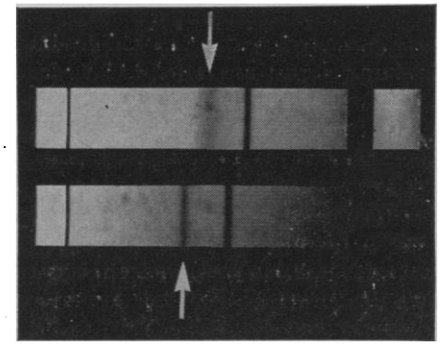

Fig. 1. Ultraviolet photograph of satellite virus DNA (white arrows) at equilibrium in CsCl (44,770 rev/min, 25°C, 23 hours). (Upper) Photo after osmotic release in presence of subtilis-phage 2C DNA. (Lower) Photo after 15 minutes at 67°C in $2 \times$ SSC. Internal reference (2C DNA) is on the right of the satellite band.

However, satellite virions gave staining patterns with acridine orange (*3*) and ultraviolet reaction patterns with dilute formaldehyde (*4*) consistent with patterns of a single-stranded DNA structure, whereas patterns on purified extracted satellite DNA confirm a double-stranded helix. Recent studies using agar diffusion techniques (*5*) yielded a strong reaction between satellite virus DNA *in situ* and rabbit antiserums prepared specifically against single-stranded DNA. Adenovirions (double-stranded DNA) gave no reaction to the test, whereas X14 rat virus and bacteriophage ΦX174 (single-stranded DNA's) gave strong positive reactions. Purified DNA extracted from satellite virions did not react (*5*). Apparently, "quasi" single-stranded regions exist in the virion which are "renatured" to form