tonin concentration was observed in spite of a several-fold increase in the rate of turnover (*12*). At a constant concentration of serotonin, the rates of biosynthesis and utilization of this substance must be equal. Therefore, a change in the concentration of tryptophan hydroxylase might indicate a corresponding change in the rate of serotonin turnover. Data in support of this relationship have been obtained by Fuxe (*14*), who has found by histofluorescence a decrease in serotonin turnover as a result of adrenalectomy in rats. One can envision a homeostatic mechanism in the brain whereby a stressful situation leads to a high concentration of corticosterone in the blood, and this in turn increases the activity of tryptophan hydroxylase in the midbrain. The increased activity of this enzyme permits the increased synthesis of serotonin, which is then available in increased quantity to supply the increased demand at the synaptic endings which results from continuing stress. It is particularly important that the relevance of these findings to the severe emotional instability often associated with adrenal cortical hormone excess or insufficiency (*15*) be explored.

*Note added in proof*: In recent experiments we have obtained smaller differences in midbrain tryptophan hydroxylase activities between normal and adrenalectomized rats than are reported here. They range from 16 to 40 percent, with a mean of 24 percent ($N = 29$). This may be due to a recent change in housing conditions for the rats, from a stressful to a nonstressful environment, for we have also observed that subjecting normal but not adrenalectomized rats to various types of stress leads to increased tryptophan hydroxylase activity in the midbrain.

EFRAIN C. AZMITIA, JR.
BRUCE S. MCEWEN
*Rockefeller University,*
*New York 10021*

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## Acetyl Coenzyme A Carboxylase: Filamentous Nature of the Animal Enzymes

Abstract. *Acetyl coenzyme A carboxylases purified from several animal tissues exist as enzymatically active polymeric filaments of high molecular weight and have similar electron microscopic, hydrodynamic, and catalytic properties. These filaments reversibly dissociate into inactive protomers of uniform size. Their reassembly into catalytically active filaments is promoted by the presence of an allosteric activator.*

Acetyl coenzyme A carboxylase (E.C. 6.4.1.2) catalyzes the regulatory and committed step in fatty acid biosynthesis *de novo* in animal tissues (*1*, *2*). Citrate acts as a "feed forward" allosteric activator of the acetyl CoA carboxylases from these tissues (*2*) but has no activating effect on those from plant (*3*) or microbial sources (*4*, *5*); activators of the latter enzymes are still unknown. Under conditions of carboxylase assay, the activation of the avian liver enzyme by citrate or isocitrate occurs concomitantly with ag-

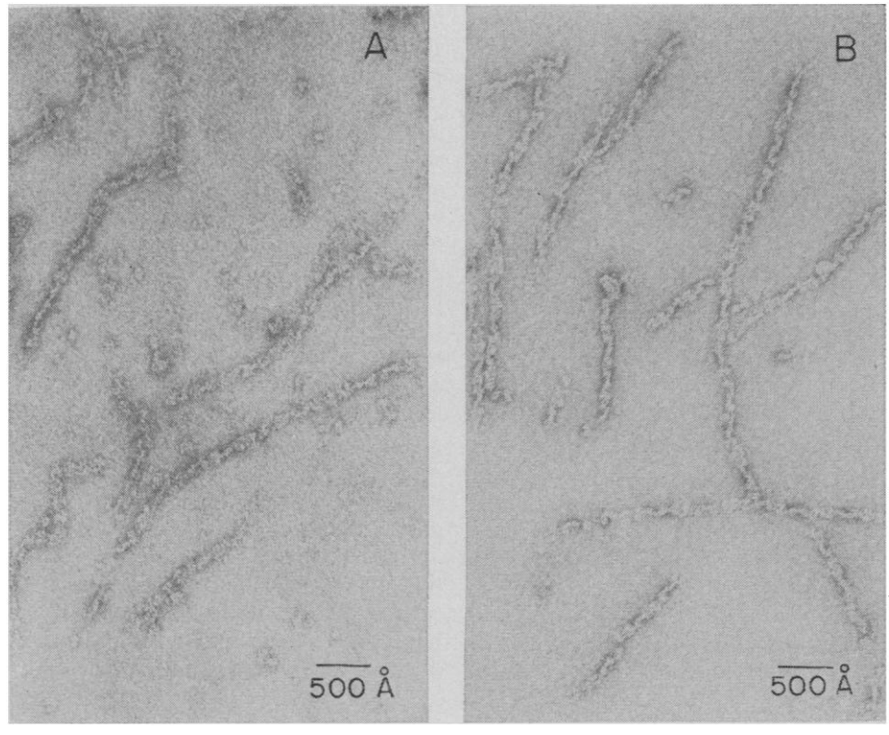


Fig. 1. Filamentous forms of acetyl CoA carboxylases from avian liver (A) and bovine adipose tissue (B), in the presence of citrate. Dilute solutions (20 μg/ml) of chicken liver (*8*) or bovine perirenal adipose tissue (*12*) carboxylase in 50 m*M* tris ($Cl^-$) buffer containing 10 m*M* potassium citrate, 5 m*M* 2-mercaptoethanol, and 0.1*M* ethylenediaminetetraacetate at *p*H 7.5 were applied as droplets to carbon-collodion support films. After the preparations were stained with 4 percent aqueous uranyl acetate and the collodion was removed by heating at 180°C for 10 minutes (*16*), they were photographed through a Siemens Elmiskop IA electron microscope.

gregation of the protomeric form and gives rise to filamentous polymeric species (*6*, *7*). It is possible that these unique filamentous structures are characteristic of all acetyl CoA carboxylases from animal tissues. The availability in our laboratory of homogeneous preparations of the enzymes from bovine perirenal adipose tissue and avian liver has permitted the comparison of the structures and molecular properties of two carboxylases from rather diverse animal species and organ systems.

The acetyl CoA carboxylase from bovine adipose tissue has an unbranched filamentous structure in the presence of citrate (Fig. 1B). These stained filaments are nearly indistinguishable from those of the avian liver enzyme (Fig. 1A). They have a definite twisted appearance with indentations along the filament axis, and these are suggestive of a helical structure. Filament lengths varied from 500 to 5000 Å. As pointed out earlier (*7*, *8*), the degree of polymerization, hence filament length, of the avian liver carboxylase depends upon *p*H, ionic strength, and other factors. The length variation of filaments of both the bovine and avian enzymes is similar and approximates a Poisson distribution (Fig. 2). The widths of individual filaments ranged from 70 to 100 Å. The electron micrographs of the avian enzyme revealed a period of 130 to 140 Å along the longitudinal axis of the filament. We believe that this period corresponds to the length of the protomeric unit because the long dimensions of protomers observed in preparations of disaggregated filaments (in assay reaction mixture without activator) falls within this range. Furthermore, the same periodicity is present in paracrystalline fibers of the avian enzyme (*9*). The filamentous forms of both carboxylases were also observed in preparations shadowed with uranium or stained with phosphotungstate and in unstained preparations on exceptionally thin carbon support films.

The hydrodynamic properties of the bovine and avian enzymes are consistent with their appearance by electron microscopy. Both carboxylases are unusually large protein structures (Table 1) having sedimentation coefficients of 50 to 68*S* and molecular weights (by sedimentation equilibrium) of several million daltons. A high degree of asymmetry is indicated by the marked dependence of sedimentation velocity upon the concentration of the polymeric form and is responsible for the

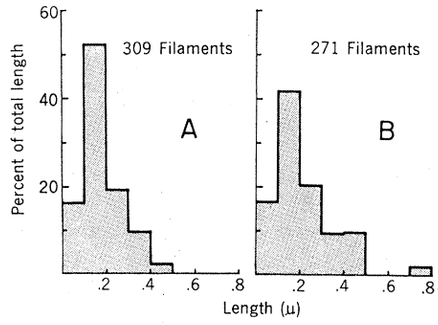


Fig. 2. Weight frequency distribution of lengths (*17*) of acetyl CoA carboxylase filaments from chicken liver (histogram A) and bovine adipose tissue (histogram B). Filament lengths were measured from electron micrographs prepared as described in Fig. 1.

characteristic hypersharp gradients observed (*6*) in analytical sedimentation velocity patterns of both enzymes. It has been possible to dissociate the polymeric form of each carboxylase into protomers of the same molecular weight with 0.5*M* NaCl under slightly alkaline conditions (*p*H 8 to 9). The protomer preparations of the avian and bovine enzymes appear monodisperse in the analytical ultracentrifuge and have sedimentation coefficients of 13.1*S* and 14.4*S*, respectively (Table 1). The molecular weight of the protomer of the liver carboxylase which is composed of four nonidentical subunits, each of about 100,000 daltons, was found to be 410,000 daltons (*7*, *10*). Initial studies (*11*) on the molecular weight of the bovine adipose tissue carboxylase protomer by the sedimentation equilibrium method indicate a weight of approximately 550,000 daltons. Thus, from sedimentation studies and electron microscopy the carboxylase filaments are linear polymers composed of an indeterminate number of identical protomers.

The catalytic activities of the acetyl CoA carboxylases are intimately associated with their state of aggregation. Activation by citrate and isocitrate produces a marked increase in catalytic activity of both enzymes (see Table 1). The principal kinetic effect is on the maximum velocity and not on the $K_m$ (Michaelis constant) values for the substrates of the reaction (*8*, *12*). The sedimentation velocities of the two enzymes, determined by centrifugation in a sucrose density gradient, in the carboxylase assay reaction mixture, with and without tricarboxylate activator (citrate and isocitrate), are compared in Table 1. Under assay conditions, in the absence of tricarboxylate activator, both carboxylases sediment as 13 to 15*S* protomeric species. The presence of activator in the density gradients containing reaction mixture promotes the transition of the protomeric to the 47 to 50*S* polymer. That there are protomeric and polymeric filamentous forms under these conditions is most readily visualized by electron microscopy.

Our study shows that acetyl CoA carboxylases from bovine adipose tissue and avian liver have similar filamentous structures in the catalytically active state. Moreover, the partially purified carboxylase from human liver, obtained in our laboratory, also appears to have filamentous structure (*11*). The

Table 1. Comparison of the catalytic and molecular properties of the bovine adipose tissue and avian liver acetyl CoA carboxylases. The acetyl CoA carboxylases were isolated from bovine perirenal adipose tissues (*12*) and chicken liver (*8*). Microscopic form was assessed after dilution of the carboxylase (5 to 10 μg) in 1 ml of the carboxylase assay reaction mixture with or without tricarboxylic activator. Electron micrographs of these mixtures were then prepared by the droplet technique as described in Fig. 1. The rate of acetyl CoA carboxylation was determined as described (*8*). Sedimentation velocities were measured either in sucrose density gradients containing assay reaction mixture in the presence or absence of activator (*6*) or by analytical ultracentrifugation (*7*). Molecular weight was determined by sedimentation equilibrium (*7*). Both carboxylases were maintained in the protomeric form for molecular weight and sedimentation velocity (analytical ultracentrifuge) determination with 0.5*M* NaCl and 50 m*M* tris(hydroxymethyl)aminomethane ($Cl^-$), *p*H 8 to 9 (*7*).

| Presence of activator | Microscopic form | Carboxylation rate (μmole $min^{-1}$ $mg^{-1}$)* | Sedimentation velocity | | Molecular weight |
|---|---|---|---|---|---|
| | | | Density gradient (*S*) | Analytical ultracentrif. (*S*) | |
| | | *Bovine adipose tissue* | | | |
| None | Protomeric | 0.16 | 13–15 | 14.4 | 550,000 |
| 10 m*M* Citrate | Filamentous | 12 | 47–50 | 68 | Several million |
| | | *Chicken liver*† | | | |
| None | Protomeric | 0.30 | 13–15 | 13.1 | 410,000 |
| 20 m*M* DL-Isocitrate | Filamentous | 11 | 47–50 | 59 | 4–5 million |

* Micromoles of acetyl CoA carboxylated per minute per milligram of enzyme protein (*8*). † Some properties of the chicken liver carboxylase were reported earlier (*6*, *7*).

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.

ALBRECHT K. KLEINSCHMIDT
JOEL MOSS
M. DANIEL LANE
*Department of Biochemistry, New York University School of Medicine, New York 10016*

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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 ($\mu^2$) |
|---|---|---|---|---|---|
| | *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.