and inactive fractions, with $R_p$ values of 0.15 and 0.34. Sliced gels eluted into NaHPO$_4$/KH$_2$PO$_4$ buffer, pH7.0, over 24h at 4°C showed the latter band to be inactive and the former to contain fumarase activity. Addition of the inactive protein to the active enzyme caused complete inhibition of activity. Dialysis of the initial pooled inactive protein over 36h resulted in high fumarase activity in the dialysis residue, and the dialysate after concentrated by ultrafiltration caused marked inhibition of both seminal-plasma and pig heart fumarase activity. The pH optimum of seminal-plasma fumarase was 7.0 with malate as substrate and 6.7 with fumarate as substrate.

We have simultaneously purified four enzymes from a single pooled sample of human seminal plasma, and found that they have similar properties to enzymes purified from other human and animal sources. This is the first time that such a preparation has been achieved in this way, and may be of considerable use in investigating enzymes from small samples of biological material. In view of the possible importance of fumarase in spermatozoal-motility assay (Crabbe, 1976), a seminal-plasma fumarase inhibitor could be involved in a control of semen viability in vivo, particularly as we have found that part-two seminal-plasma ejaculates contain an inhibitor of fumarase activity in part-one ejaculated sperm cells and seminal plasma (J. Kavanagh & M. J. C. Crabbe, unpublished work).

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Partial Purification and Some Properties of Acetyl-Coenzyme A Carboxylase from Bovine Mammary Tissue

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Acetyl-CoA carboxylase [acetyl-CoA–CO$_2$ ligase (ADP forming), EC 6.4.1.2], which catalyses the formation of malonyl-CoA from acetyl-CoA, plays a critical part in the control of fatty acid biosynthesis, both in acute and long-term regulation [see Volpe & Vagelos (1976) for review]. The enzyme has been purified from several mammalian tissues and its activity shown to be modulated in vitro by citrate (and some closely related tricarboxylic acids) and by long-chain acyl-CoA thioesters. A mechanism for regulation, involving phosphorylation and dephosphorylation of the enzyme with consequent inactivation and re-activation has been proposed by Carlson & Kim (1974a, b), working with a preparation from rat liver. However, Denton (1975) was not able to show the presence of such a phosphorylation–dephosphorylation cycle in rat epididymal fat-pad.

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The experiments discussed in the present paper were carried out as part of an investigation into factors involved in the regulation of milk-fat production during lactation. Acetyl-CoA carboxylase has been partially purified from bovine mammary gland. Observations made during its characterization suggest that regulation of enzyme activity by phosphorylation might take place in that organ.

Ox mammary tissue was obtained within minutes of death in a slaughterhouse and placed on ice. All subsequent stages of purification were carried out at 0–4°C. Alveolar tissue was dissected out and passed twice through a heavy-duty mincer before homogenization in the Tris/HCl buffer described by Manning et al. (1976) with an MSE Atomix with Polytron assembly. The homogenate was centrifuged for 45 min at 12,000 g, and the supernatant decanted through glass wool to remove gross fat. Solid (NH₄)₂SO₄ was added to 25% saturation and the resulting precipitate collected by centrifugation at 12,000 g, for 45 min. The pellet was dissolved in 10 mM-potassium phosphate buffer, pH 7.0, containing, as did all other buffers used, 0.5 mM-EDTA and 0.2 mM-dithiothreitol and adsorbed on to Ca₃(PO₄)₂ gel (gel : protein ratio, 2 : 1, w/w). Protein containing enzyme activity was eluted with 0.2 M-potassium phosphate buffer, pH 7.0, and the eluate was brought to 25% saturation with (NH₄)₂SO₄. The resulting precipitate was stored at −20°C. In this form the enzyme preparation was stable for several weeks and had a specific activity, when assayed as a solution in 10 mM-potassium phosphate buffer, pH 7.0, of 60 nmol of acetyl-CoA carboxylated/min per mg of protein, a purification of approx. 20-fold over the initial 12,000 g, supernatant.

Optimal substrate concentrations were found to be similar to those reported by Miller & Levy (1969) for rat mammary gland acetyl-CoA carboxylase. Maximal rates of reaction were obtained with 0.125 mM-acetyl-CoA (K_m 40 μM) and 15 mM-NaHCO₃.

![Fig. 1. Effect of ATP on acetyl-CoA carboxylase activity](image_url)

Partially purified acetyl-CoA carboxylase at a final concentration of 3.5 mg/ml was incubated at 37°C with 50 mM-Tes (2-[(hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)ethanesulphonic acid), pH 7.0, 8 mM-MgCl₂, 10 mM-sodium citrate, 2.5 mM-dithiothreitol and, where indicated, 2 mM-ATP or labelled ATP. Samples were removed at intervals and assayed for acetyl-CoA carboxylase activity or acid-precipitable radioactivity as appropriate. Open symbols represent enzyme activity determined on incubations in the absence (○) or presence of ATP (□). Closed symbols represent radioactivity measurements from incubations containing: [γ-³²P]ATP (10 μCi/μmol) (●); [γ-³²P]ATP (10 μCi/μmol) and enzyme inactivated by heating for 3 min at 100°C (▲); [U-¹⁴C]-ATP (1 μCi/μmol) (■).
Both ATP and Mg\(^{2+}\) were inhibitory above their respective optimal concentrations of 2 and 10 mM. Citrate activated the enzyme 10-fold at a concentration of 10 mM; preincubation was not necessary for full activation.

During the determination of optimal substrate concentrations it was observed that preincubation of the enzyme with ATP/Mg\(^{2+}\) in the absence of acetyl-CoA resulted in a marked inactivation of the enzyme. Incubation with 2 mM-ATP for 1 h brought about a loss of 80% of the initial activity. No loss of activity was observed on incubation in the absence of ATP. Similar results were reported by Carlson & Kim (1974a) using a partially purified acetyl-CoA carboxylase from rat liver. A series of experiments, essentially similar to those described by these workers, was performed to investigate the inactivation phenomenon further. Samples of the enzyme preparation were incubated at 37°C in the presence and absence of ATP, as described in the legend to Fig. 1. Over a 1 h period, enzyme activity in the incubation containing ATP decreased to less than 20% of the original. In the absence of ATP no inactivation was observed. Binding of radioactivity from \([\gamma-\text{P}]\)ATP increased throughout incubation, whereas very little \(^{32}\text{P}\) was acid-precipitable when the incubation mixture contained heat-inactivated enzyme. Very little binding of radioactivity from \([\text{U-}^{14}\text{C}]\)ATP was noted. It would appear that inactivation of the enzyme is associated with the phosphorylation of one or more components of the enzyme preparation.

In further experiments, \(^{32}\text{P}\)-labelled proteins were isolated by \((\text{NH}_4)_2\text{SO}_4\) precipitation after incubation of the enzyme preparation with \([\gamma-\text{P}]\)ATP. The labelled proteins were redissolved in appropriate buffers and subjected to chromatography on columns of Sephadex G-25, DEAE-cellulose and Sepharose 6B. In each case, \(^{32}\text{P}\) radioactivity was eluted coincident with acetyl-CoA carboxylase activity.

These results strongly imply the presence in our partially purified enzyme preparation of a protein kinase that catalyses the phosphorylation, and consequent inactivation, of acetyl-CoA carboxylase. Although the occurrence of such a kinase remains to be more exhaustively confirmed, and the existence of an appropriately specific protein phosphatases in extracts of bovine mammary tissue has not yet been demonstrated, our findings are consistent with the phosphorylation–dephosphorylation cycle proposed by Carlson & Kim (1974b) for the regulation of rat liver acetyl-CoA carboxylase activity in vivo. Such mechanisms are well established for other enzyme systems (Cohen, 1976) and serve to render the enzyme open to control by hormone-sensitive kinases and phosphatases.

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Calf Thymus Ribonucleotide Reductase: Purification and Properties

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Ribonucleotide reductase, the enzyme responsible for the production of the deoxyribonucleotides, has been isolated and carefully studied in Escherichia coli and Lactobacillus leichmannii (Hogenkomp & Sando, 1974). Ribonucleotide reductase from regenerating rat liver (Larsson, 1969), Novikoff hepatoma cells (Moore & Hurlebert, 1966) and rabbit bone marrow (Hopper, 1972) has also been studied, but only after partial purification.