We have purified propionyl-CoA carboxylase from normal, postmortem human liver to homogeneity. The isolation procedure, which provided an approximately 3000-fold purification and an overall yield of 26%, employed initial centrifugation of a cetyltrimethylammonium bromide-treated homogenate, followed by sequential chromatographic separations using DEAE-cellulose, Blue Sepharose, and Bio-Gel A-1.5m. The native enzyme has a molecular weight of ~540,000 and is composed of nonidentical subunits (α and β) of Mt = 72,000 and 56,000, respectively. When studied with analytical isoelectrofocusing techniques, it focuses as a single peak at pH 5.5. Each mole of native enzyme contains 4 mol of bound biotin, virtually all of which is monospecific by immunodiffusion and immunoelectrophoresis. Limited proteolysis with trypsin results in slow, time-dependent activation of the enzyme with preferential cleavage of the smaller subunit. Antiserum prepared against the native enzyme is shown to be monospecific by immunodiffusion and immunoelectrophoresis.

Propionyl-CoA carboxylase (EC 6.4.1.3), a biotin-dependent, mitochondrial enzyme, catalyzes the carboxylation of propionyl-CoA to d-methylmalonyl-CoA, a key step in the catabolic pathway for odd chain fatty acids and for isoleucine, threonine, methionine, and valine (1, 2). The enzyme was initially studied in crude extracts of animal tissues; it was later purified to homogeneity from pig heart mitochondrial fractions (3,4) and more recently, from bovine kidney mitochondria (5). The native enzyme from pig heart has a molecular weight of about 700,000 and contains 4 mol of biotin/mol of enzyme (4). Propionyl-CoA carboxylase from bovine kidney mitochondria is composed of two nonidentical subunits of molecular weights 74,000 and 56,000; biotin was found bound to the larger subunit (5).

The important role of propionyl-CoA carboxylase in human intermediary metabolism is evident from the often life-threatening recessively inherited propionic acidemias caused by deficiency of this enzyme (6). A molecular understanding of such propionyl-CoA carboxylase deficiency depends on the analysis of the normal and mutant enzymes. Although propionyl-CoA carboxylase activity has been measured in human skin fibroblasts (6), leukocytes (7), liver (8, 9), and amniotic fluid cells (11), very little is known about the structure of the human enzyme. Until now the enzyme has been only partially purified from human liver mitochondria (9) and from cultured fibroblasts of both controls and several propionyl-CoA carboxylase-deficient patients (12). Mutant enzymes had pH optima, ionic requirements, and substrate affinities similar to those of the normal enzyme, but were more labile to both cold and heat (12). Further studies of biochemical parameters of mutant enzymes from two major genetic complementation groups (pcc A and pcc C) were consistent with the notion that propionyl-CoA carboxylase consists of two nonidentical subunits (13).

In this communication we report the purification to homogeneity of propionyl-CoA carboxylase from normal human liver and describe some characteristics of the pure enzyme.

EXPERIMENTAL PROCEDURES

Materials—Avidin, catalase, thryoglobulin, aladose, pronase, biotin, ATP, EDC, and glutathione were purchased from Sigma. Chymotrypsinogen and tosylphenylalanlycloromethane-treated trypsin were purchased from Worthington; DEAE-cellulose (DE-52) from Whatman; Blue Sepharose from Pharmacia; coenzyme A, butyryl-CoA, acetyl-CoA, and crotonyl-CoA from P-L Biochemicals; Bio-Gel A-1.5m from Bio-Rad. Cetyltrimethylammonium bromide (practical), acrylamide, N,N'-methylenebisacrylamide, and N,N,N',N'-tetramethylethylenediamine (TEMED) were obtained from Eastman; fluorescein from Pierce; XM-100 membranes from Amicon; "H-serine and "HNaHCO3 from New England Nuclear. Amphotrye carrier buffers were purchased both from Bio-Rad and LKB. Serum from a patient with increased circulating immunoglobulin concentrations was used as a source of IgG. Goat anti-rabbit γ-globulin precipitating antibodies were obtained from Antibodies Inc. Seryl-tRNA synthetase (14) was isolated by one of us (F.K.). Propionyl-CoA was prepared from propionyl anhydride and cozymase A by the method of Flavin and Ochoa (15).

Assay of Enzyme Activity—Propionyl-CoA carboxylase was assayed by a modification of a procedure described previously (7). The enzyme was diluted in 10 mM phosphate buffer (pH 7.0) containing 1 mM 2-mercaptoethanol and 0.1 mg/ml of bovine serum albumin. The standard reaction mixture containing 50 mM Tris-HCl pH 8.0, 5 mM glutathione, 2 mM ATP, 100 mM KC1, 10 mM MgCl2, 10 mM [14C]-bicarbonate (specific activity 12.4 mCi/mmol), 3 mM propionyl-CoA, and enzyme was incubated at 37°C for 15 min. The reaction was stopped by the addition of 10% trichloracetic acid. Following centrifugation at 200 X g, an aliquot of the supernatant was tried slowly under a heat lamp, dissolved in water, and counted in Aquasol. One unit of enzyme activity is defined as that amount of enzyme catalyzing the fixation of 1 pmol of bicarbonate/min at 37°C.

Protein Determination—Protein was measured by the method of Lowry et al. (16) or by a fluorometric method (17), using crystalline bovine serum albumin as a standard. Protein concentration in the
fractions after column chromatography was determined by using $A_{280}$ : $\bar{A}_{260}$ absorbance ratios as described by Warburg and Christian (18).

Preparation of Antibody—White New Zealand rabbits were kept on a standard diet. One volume of complete Freund adjuvant obtained from Difco was added to 1 volume of protein diluted in 0.9% NaCl to a final concentration of 0.3 mg/ml and emulsified by pumping the mixture repeatedly through an 18-gauge needle using a 1-ml syringe.

Initially, 1.5 ml of the emulsion was injected into the hind foot pads and abdominal dermis. After 14 days, a booster of 150 µg of antigen in complete Freund's adjuvant was injected into the hind thigh and back. Intracardiac bleeding was performed 14 days after the second immunization. Sera were prepared and stored at -15°C. Purity of the antibody was determined by both Ouchterlony double diffusion and immunoelectrophoretic analysis in agar gels.

Electrofocusing—A sucrose-stabilized linear pH gradient (pH 4 to 6) of 1.2% ampholites was prepared in an LKB (model 8101) isolectric focusing column. Either crude extract or enzyme in different stages of purification was added to the denser solution and distributed throughout the gradient. The isoelectrofocusing run was for 62 h at 4°C to a final voltage of 800 V. After completion of the run, 1.5-ml fractions were collected and assayed for activity.

Analytical Polyacrylamide Gel Electrophoresis—Discontinuous polyacrylamide gel electrophoresis was carried out in 5 or 7.5% gels, pH 8.9 (19). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in 1.5-mm vertical slab gels which accommodated 10 samples (20). The gels, containing 7.5% acrylamide and 0.2% methylenebisacrylamide in the buffer described by Fairbanks et al. (21), were run for 3 h at 100 mA.

Preparation of Subunits—Separation of the subunits was accomplished by sodium dodecyl sulfate gel electrophoresis on 3-mm-thick vertical slabs (17 × 17 cm), using the buffer system described by Fairbanks et al. (21). Usually, 500 µg of pure enzyme was loaded on one gel. After the run, 4-mm strips were cut from the slab and stained with 0.1% Coomassie blue, 25% 2-propanol, and 10% acetic acid. After destaining, these reference strips were used to localize the position of the subunits in the nonstained remainder of the gel. The subunits were subsequently eluted from the latter with the same Sample Concentrator, model 1750, and concentrated by lyophilization.

Biotin Assay—Qualitative estimation of biotin content was made using the method of Swack et al. (22). The enzyme was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by incubation with fluorescent avidin. Quantitative spectrophotometric determination of biotin was performed as described by Green (23) using digestion with pronase at room temperature for 16 h (22).

RESULTS

Purification of Enzyme

Human liver, obtained at autopsy 5 to 8 h after death, was frozen and stored at -70°C. All operations were carried out at 0-5°C unless otherwise stated. All buffers contained 1 mM 2-mercaptoethanol.

Step I—Three hundred grams of frozen human liver were usually used for the purification of the enzyme. The liver was homogenized in 1500 ml of 10 mM potassium phosphate (pH 7.0) and $10^{-4}$ M NaEDTA (Fraction I, Table I). The degree of purity of propionyl-CoA carboxylase preparations was assessed by analytical polyacrylamide gel electrophoresis, using either 7.5% or 4% acrylamide. In both cases the protein migrated as a single band even when more than 20 µg was applied (Fig. 1, A and B).

Properties of the Purified Enzyme

Molecular Weight and Subunit Structure—The molecular weight of pure, native propionyl-CoA carboxylase was estimated on a Bio-Gel A-1.5m column to be $\sim$540,000, with a range of 500,000 to 575,000 from four separate determinations (Fig. 2). When the enzyme was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions, two nonidentical subunits were noted with mobilities corresponding to molecular weights of 72,000 and 56,000 (Figs. 1 and 3).

Biotin Determination—The biotin content of pure enzyme was determined spectrophotometrically on three different enzyme preparations using a method based on stoichiometric titration of avidin with biotin. About 4 mol of biotin were

| Step | Total activity | Specific activity | Purification Yield |
|------|----------------|------------------|--------------------|
|      | units x 10^5 | units/mg | -fold | % |
| 1. 10,000 x g supernatant | 16 | 6.5 x 10^4 | 1.0 | 100 |
| 2. CTB* extract | 17 | 8.8 x 10^4 | 1.4 | 110 |
| 3. DEAE-cellulose | 11 | 3.3 x 10^4 | 51 | 68 |
| 4. Blue Sepharose | 7.3 | 7.3 x 10^3 | 1120 | 46 |
| 5. Bio-Gel A-1.5m | 4.0 | 2.0 x 10^3 | 3076 | 25 |

* CTB = cetyltrimethylammonium bromide.
Human Hepatic Propionyl-CoA Carboxylase

FIG. 1. Polyacrylamide gel electrophoresis of pure propionyl-CoA carboxylase. The electrophoretic mobility of the enzyme in 7.5% polyacrylamide gel (20 pg of enzyme), in 5% polyacrylamide gel (10 pg), and in 7.5% sodium dodecyl sulfate (SDS) polyacrylamide gel under reducing conditions (30 pg) are shown.

FIG. 2. Estimation of the molecular weight of native propionyl-CoA carboxylase (PCC) on a Bio-Gel A-1.5m column. Calibration proteins were: 1, thyroglobulin; 2, catalase; 3, IgG; 4, E. coli seryl-tRNA synthetase.

bound to 1 mol of native enzyme (Table II). To identify the biotin-carrying subunit, the enzyme was run in 7.5% sodium dodecyl sulfate-polyacrylamide gel under reducing conditions. The gel was then exposed to fluorescent avidin which will bind to any biotin in the gel. The fluorescence generally appeared only in the position of the larger subunit. In some preparations, however, slight fluorescence appeared in the region of the smaller subunit as well (data not shown). In control experiments, no fluorescence was observed in the bands of bovine serum albumin, ovalbumin, chymotrypsinogen, and lysozyme. To estimate biotin content of each subunit, the subunits were isolated from a sodium dodecyl sulfate-polyacrylamide gel and assayed for biotin by the same method used for the native enzyme. The result presented in Table II shows that the large subunit contains biotin in a 1:1 biotin/protein molar ratio whereas the smaller subunit contains less than 0.1 mol of biotin/mol of subunit.

Limited Proteolysis—To examine the possibility that the smaller subunit might be a degradation product of the larger one, the effect of trypsin on pure propionyl-CoA carboxylase was studied. Both enzyme activity and gel electrophoresis were followed as functions of trypsin concentration and of time. At a trypsin/enzyme ratio of 1:500, no decrease in enzymatic activity was observed. Even at a ratio 1:100, about 84% of the original activity was still present after 3 h of incubation (Fig. 4). Only at a trypsin/enzyme ratio of 1:20 did the activity rapidly decrease with a half-life of about 60 min (Fig. 4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions did not suggest any conversion of a to β subunits (Fig. 5). Moreover, the β subunit was more sensitive to trypsin; only a slight amount was left after 3 h of incubation with trypsin, whereas much of the α subunit was still present (Fig. 5). The amino acid composition of both subunits also precludes the possibility of conversion of α to β forms (data not shown).

Kinetic Analyses—Pure human propionyl-CoA carboxylase demonstrated Michaelis-Menten kinetics for all three substrates. In these experiments, K_m values were estimated by simple extrapolation of the linear double reciprocal plots of

TABLE II

| Sample     | Molecular weight | Amount of biotin/mol protein |
|------------|------------------|-----------------------------|
| Native enzyme | 540,000          | 3.79                        |
| α Subunit  | 72,000           | 0.92                        |
| β Subunit  | 56,000           | 0.08                        |
substrate versus velocity to the negative intercept of the x axis. The $K_m$ values derived are the mean of at least two determinations at each substrate concentration. From a saturation curve for ATP at pH 8.0 with 3 mM propionyl-CoA and 10 mM bicarbonate, the apparent $K_m$ for ATP was found to be 0.08 mM (Fig. 6A). Similarly, at saturating concentrations of the other two substrates (with 2 mM ATP), the $K_m$ values for propionyl-CoA and sodium bicarbonate were calculated to be 0.29 mM (Fig. 6B) and 3.0 mM (Fig. 6C), respectively. These kinetic parameters are similar to those described for pure pig heart propionyl-CoA carboxylase (4) and for crude enzyme from human fibroblasts (12) except that, in the latter, the $K_m$ for ATP was an order of magnitude higher. The enzyme had a $K_m$ for butyryl-CoA of 1.2 mM. Moreover, it carboxylated acetyl-CoA, but only at a rate ~1.5% that for propionyl-CoA.

It did not carboxylate crotonyl-CoA. As noted previously for the pure enzyme from pig heart (24) and bovine liver (25), activity of the human enzyme was stimulated 6- to 7-fold by potassium ions. The estimated $K_m$ for this $K^+$ stimulation was 9.4 mM, a value nearly 7-fold higher than that reported for the enzyme from pig heart (24).

**pH Optimum and pH Stability**—The pH optimum for activity of the pure enzyme was very broad, between pH 7.2 and 8.8. Even at a pH as high as 9.6, the enzyme had about half of the maximal activity. No substantial differences in activity were found in two different buffers, potassium phosphate or Tris-HCl, over a pH range 7.4 to 8.2. However, when the enzyme was incubated without substrate at 37°C for 20 min with buffers of different pH values, and then assayed at pH 8.0, it retained more than 60% of activity at pH 6.4, but only 10% of activity at pH 9.2 and 3% of activity at pH 9.6.

**Isoelectrofocusing**—Pure propionyl-CoA carboxylase was electofocused as described under "Experimental Procedures." The major peak of activity was found at pH 5.5 (Fig. 7). However, in all preparations of pure enzyme, there was an additional peak consisting of about 5 to 10% of the total enzyme activity found, at a pH around 5.0. Thinking that these two peaks represented different isoenzymes, we focused crude extracts of liver homogenate; all of the enzyme activity focused at pH 5.0 (Fig. 7). The same pH value was also found for the fraction focused after DEAE-cellulose chromatography (Fig. 7). Only in focusing fractions after Blue Sepharose chromatography did we observe the shift of the major portion of activity to a pH value of 5.5, with a small amount of activity left at pH 5.0 (Fig. 7). It was not possible to focus the sample after treatment with cetyltrimethylammonium bromide because the enzyme became inactive under isoelectrofocusing conditions. In two out of six experiments, crude homogenates were heterogeneous with three main peaks at isoelectric points 4.6, 5.0, and 5.5; although the distribution of activity was uneven, at least 60% was always present at pH 5.0.

**Antibody Preparation and Immunotitration**—The purity of the antibody raised against pure human propionyl-CoA carboxylase was tested both by Ouchterlony double diffusion and by immunoelectrophoresis. In both cases only one precipitin line
Human Hepatic Propionyl-CoA Carboxylase

was observed even when crude homogenate was used as a source of antigen (Fig. 8). Immune titration curves, which were similar for both crude homogenate and pure enzyme, were linear over a wide range of enzyme activity (Fig. 9). Rabbit antisera against pure human propionyl-CoA carboxylase showed a low degree of species specificity, reacting readily with propionyl-CoA carboxylase in crude extracts prepared from monkey, bovine, rat, and mouse liver.

**DISCUSSION**

We have purified human hepatic propionyl-CoA carboxylase about 3000-fold from crude homogenates and have obtained a homogenous enzyme. Since the human livers available to us were obtained 5 to 10 h postmortem and had, therefore, undergone significant postmortem autolysis, we were forced to develop methods for purification of propionyl-CoA carboxylase from the crude homogenate rather than intact mitochondria. As with the purification of ornithine transcarbamylase from human liver (26), we observed that the addition of cetyltrimethyl ammonium bromide to the crude extract greatly increased the total recoverable enzyme activity. This step precipitated considerable amounts of protein from the homogenate which could then be removed by low speed centrifugation. The subsequent relatively simple purification scheme depended on affinity chromatography with Blue Sepharose. It was essential during this procedure to keep the ATP concentration at 3 mM. Lower concentrations caused the enzyme to be eluted very slowly and not quantitatively, and higher concentrations caused the release of at least five additional proteins, as shown by polyacrylamide gel electrophoresis. Furthermore, the descending portion of the propionyl-CoA carboxylase peak eluted from DEAE-cellulose contained another protein which was also bound on Blue Sepharose and was eluted with ATP. This contaminant had a native molecular weight of 470,000 and a subunit structure close to that of the β subunit of propionyl-CoA carboxylase. Removal of this impurity required either rechromatography on Bio-Gel A-1.5m or electrofocusing. To our knowledge, human propionyl-CoA carboxylase is the first biotin enzyme purified in this manner. It is likely that Blue Sepharose can be used for all biotin enzymes which require ATP for their activity.

Analysis of pure human propionyl-CoA carboxylase shows that the native enzyme has a molecular weight of 540,000 and is composed of two nonidentical subunits of molecular weight 72,000 and 56,000. Similar molecular weights have been reported for native propionyl-CoA carboxylase isolated from pig heart (4) and bovine kidney (5) mitochondria. In the latter, the subunit structure and molecular weights of the subunits were also studied and found to be comparable to those of human liver. This subunit composition is not restricted to eukaryotes. Propionyl-CoA carboxylase from Mycobacterium smegmatis also consists of two subunits of molecular weight 64,000 and 53,000 (27). Since nonidentical subunits have now been found for propionyl-CoA carboxylases from organisms as diverse as bacteria and man, it seems likely that such a structure is common to all prokaryotes and eukaryotes. It should be mentioned, however, that such a structure has not been reported for all biotin-dependent carboxylases, some of which have been shown to be composed of identical subunits (28, 29), others of nonidentical ones (30).

It could be argued that the smaller (β) subunit found in our studies is produced by *in vitro* proteolysis of the larger (α) one, and, therefore, that our demonstration of nonidentical subunits is artifactual. This seems most unlikely on several grounds. Our experiments with *in vitro* treatment of pure carboxylase with trypsin failed to demonstrate any conversion...
of the larger subunit to the smaller one, showing rather that the human enzyme is quite resistant to trypsin. Since trypsin is by no means the only possible protease, this finding is inconclusive. More convincing are the following: first, the markedly different amino acid compositions of the two putative subunits found in our studies and to be reported subsequently; and second, the demonstration by Lau et al. (5) that the molecular weight of the pure bovine kidney enzyme was the same when purified in the presence of protease inhibitors in their absence.

All available information concerning molecular weight and subunit structure suggests the existence of subunit stoichiometry in both human and bovine propionyl-CoA carboxylases. This thesis is strongly supported by our nearly quantitative elution of the subunits in a molar ratio from sodium dodecyl sulfate-polyacrylamide gels. Furthermore, based on amino acid composition of subunits (data not presented here), which were eluted quantitatively from the gels with formic acid followed by hydrolysis, an approximate molar ratio was observed.

Our analysis shows that 1 molecule of biotin is bound/molecule of the larger (α) subunit. A small amount of biotin was also found occasionally with the smaller subunit, as shown both by the fluorescent avidin technique and by quantitative assay. Perhaps, as has been proposed for β-methylcrotonyl-CoA carboxylase (31), the smaller subunit may contain a second binding site for free biotin. Alternatively, the minimal binding to the smaller subunit may be artificial.

Pure propionyl-CoA carboxylase is remarkably sensitive to pI. Another biotin enzyme, 3-methylcrotonyl-CoA carboxylase from *Achromobacter*, has been shown to dissociate into two nonidentical subunits above pH 9.0, thus losing enzymatic activity (32). The dissociation is reversible, reassociation can be stimulated by the substrate, 3-methylcrotonyl-CoA (32). It can be anticipated that our enzyme undergoes similar dissociation. We have already shown that, in the presence of substrate, enzymatic activity is preserved well beyond pH 9.0.

We found a significant difference in isoelectric points between the pure enzyme and the crude enzyme fractions. This change in pI occurred during chromatography on Blue Sepharose. We suggest three possible explanations for this finding: first, that during that step we remove a nucleotide or nucleic acid loosely bound to the enzyme molecule; second, that we break a complex between the enzyme and some acidic protein; and third, that this step selectively changes the conformation of the molecule in such a way as to expose more positive charges. We consider the first two possibilities more likely since, in some crude extracts, the enzyme focused in more than one peak, all between pH 4.6 and pH 5.5. Such variation in extracts could be due to various degrees of formation of some protein complex, or to various amounts of some complexing factor present. These situations in turn could reflect posttranslational or freezing effects in the livers.

Recently McKeon et al. reported that the isoelectric point of propionyl-CoA carboxylase measured in crude extracts of normal liver differed from that in liver of a patient with propionyl-CoA carboxylase deficiency (the pcc C complementation group) (33). The authors concluded that these differences in the isoelectric point reflected a structural alteration in the mutant enzyme. In view of our observation, it is unlikely that measurement of isoelectric points in crude extracts is a valid criterion for the comparison of normal and mutant enzyme structure.

At least four distinct genetic complementation groups of human propionyl-CoA carboxylase mutants have recently been reported (34, 35). Biochemical studies in our laboratory of two major complementation groups, pcc C and pcc A, suggested different structural gene mutations (13). Experiments with antisera prepared against pure human propionyl-CoA carboxylase are now in progress to characterize these mutants further.

REFERENCES

1. Halen, D. R., Fung, J. Y., Hughey, C. S., and Lane, M. D. (1962) *J. Biol. Chem.* 237, 2140–2147
2. Tanaka, K., Armanage, I. M., Ramsdell, H. S., Hsia, Y. E., Lapasky, S. R., and Rosenberg, L. E. (1975) *Proc. Natl. Acad. Sci. U. S. A.* 72, 3892–3896
3. Tietz, A., and Ochoa, S. (1959) *J. Biol. Chem.* 234, 1394–1400
4. Kaziro, Y., Ochoa, S., Warner, R. C., and Chen, J.-Y. (1961) *J. Biol. Chem.* 236, 1917–1923
5. Lau, E. P., Cochran, B. C., Munson, L., and Fall, R. R. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 214–218
6. Hsia, Y. E., Scully, K., and Rosenberg, L. E. (1971) *J. Clin. Invest.* 50, 127–130
7. Hsia, Y. E., and Scully, K. J. (1973) *J. Pediatr.* 83, 625–628
8. Frenkel, E. P., and Kitchens, R. L. (1975) *Br. J. Haematol.* 31, 501–513
9. Giorgio, A. J., and Whitaker, W. R. (1973) *Biochem. Med.* 5, 473–478
10. Morrow, G., Lebowitz, J., and Gües, H. (1975) *Am. J. Obstet. Gynecol.* 121, 269–272
11. Gompertz, D., Gooley, P. A., Thom, H., Russell, G., Johnston, A. W., Mejor, D. H., MacLean, M. W., Ferguson-Smith, M. E., and Ferguson-Smith, M. A. (1975) *Clin. Genet.* 8, 244–250
12. Hsia, Y. E., Scully, K. J., and Rosenberg, L. E. (1979) *Pediatr. Res.* 13, 746–751
13. Wolf, B., Hsia, Y. E., and Rosenberg, L. E. (1978) *Am. J. Hum. Genet.* 30, 455–464
14. Keizze, J. R., and Konigsberg, W. (1970) *J. Biol. Chem.* 245, 922–930
15. Flavin, M., and Ochoa, S. (1957) *J. Biol. Chem.* 229, 965–979
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
17. Böhle, P., Steia, S., Dairman, W., and Udenfriend, S. (1973) *Arch. Biochem. Biophys.* 155, 213–220
18. Warburg, O., and Christian, W. (1942) *Biochem. Z.* 431, 258–291
19. Davis, B. J. (1964) *Ann. N. Y. Acad. Sci.* 121, 404–427
20. Reid, M. S., and Bieleski, R. L. (1968) *Anal. Biochem.* 22, 374–385
21. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) *Biochemistry* 10, 2606–2617
22. Swack, J. A., Zander, G. L., and Utter, M. F. (1978) *Anal. Biochem.* 87, 114–126
23. Green, N. M. (1965) *Biochem. J.* 94, 23c–24c
24. Edwards, J. B., and Keech, E. O. (1968) *Biochim. Biophys. Acta* 159, 167–175
25. Giorgio, A. J., and Plaut, W. F. (1967) *Biochim. Biophys. Acta* 139, 487–501
26. Kalousek, F., Francois, B., and Roseberg, L. E. (1978) *J. Biol. Chem.* 253, 3939–3944
27. Henzkinson, K. F., and Allen, G. S. H. (1978) *Fed. Proc.* 37, 1525 (Abstr.)
28. Utter, M. F., Barden, R. E., and Taylor, B. L. (1975) *Adv. Enzymol.* 42, 1–72
29. Inos, H., and Lowenstein, J. M. (1972) *J. Biol. Chem.* 247, 4825–4832
30. Wood, H. G., and Barden, R. E. (1977) *Annu. Rev. Biochem.* 46, 385–413
31. Lynen, F. (1975) in *Energy, Regulation and Biosynthesis in Molecular Biology* (Ebner, K., ed.) pp. 671–698, W. de Gruyter, New York
32. Schiele, U., Niedermeir, R., Sturzer, M., and Lynen, F. (1975) *Eur. J. Biochem.* 60, 259–266
33. McKeon, C., Wolf, B., and Fanes, R. Z. (1979) *Fed. Proc.* 38, 497–498
34. Gravel, R. A., Fong, K., Scully, K. J., and Hsia, V. E. (1977) *Am. J. Hum. Genet.* 29, 378–388
35. Wolf, B., Willard, H. F., and Rosenberg, L. E. *Am. J. Hum. Genet.*, in press.
Isolation and characterization of propionyl-CoA carboxylase from normal human liver. Evidence for a protomeric tetramer of nonidentical subunits.

F Kalousek, M D Darigo and L E Rosenberg

J. Biol. Chem. 1980, 255:60-65.