ADDENDUM

Quinones from other microorganisms have been examined; however, not with respect to other electron carriers. LESTER AND CRANE[11] have found both a benzoquinone (Q_8) and a naphthoquinone in *Escherichia coli*; the benzoquinone was present in a high concentration only in cells grown aerobically. In the present studies three quinones have been crystallized from *E. coli*: a vitamin K_2-like napthoquinone and two benzoquinones, one with an absorption maximum at 260 mμ and the other at 270–275 mμ. The three quinones were distinguishable by chromatography and were present in cells grown aerobically. Under anaerobic conditions the concentrations of the various quinones were altered. It is difficult to quantitate the quinones because of losses during fractionation and overlapping spectra; however, it appears that under anaerobic conditions the napthoquinone and the 270 benzoquinone are diminished in concentration, while the other benzoquinone is increased. The heterogeneity of these compounds in this facultative anaerobe may provide information concerning the biological role of the various benzo- and naphthoquinones, and the effects of these compounds on oxidative phosphorylation in *E. coli* are being investigated.

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[1] A. F. BRODIE, B. R. DAVIS AND L. F. FIESER, *J. Am. Chem. Soc.*, 80 (1958) 6454.
[2] A. F. BRODIE, M. M. WEBER AND C. T. GRAY, *Biochim. Biophys. Acta*, 25 (1957) 448.
[3] A. F. BRODIE AND J. B. BALLANTINE, *J. Biol. Chem.*, 235 (1960) 232.
[4] A. F. BRODIE AND C. T. GRAY, *Science*, 125 (1957) 534.
[5] A. F. BRODIE, *J. Biol. Chem.*, 234 (1959) 398.
[6] H. B. BURCH, O. A. BESSEY AND O. H. LOWRY, *J. Biol. Chem.*, 175 (1948) 457.
[7] J. L. PEEL, *Biochem. J.*, 69 (1958) 403.
[8] K. SCHILLING AND H. DAM, *Acta Chim. Scand.*, 12 (1958) 347.
[9] A. M. PAPPENHEIMER, JR. AND E. D. HENDIE, *J. Biol. Chem.*, 180 (1949) 597.
[10] K. S. AMBE AND F. L. CRANE, *Science*, 129 (1959) 98.
[11] R. L. LESTER AND F. L. CRANE, *J. Biol. Chem.*, 234 (1959) 2169.
[12] M. M. WEBER, A. F. BRODIE AND J. E. MERSELIS, *Science*, 128 (1958) 896.

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Preparation and properties of glutamic dehydrogenase from human placenta

The oxidation of L-glutamic acid by human placenta has been observed[1] but the properties of the placental enzyme were not studied. The key role of this enzyme in intermediary metabolism and the evidence that its rate is under hormonal control[2] led us to compare the properties of the placental and liver enzymes.

For each enzyme preparation, 2–4 fresh human placentas were trimmed free of cord and membranes, cut into sixths, washed free of adherent blood and immersed in ice-cold 0.9 % NaCl. The tissue was scraped free of gross connective-tissue strands and homogenized in 2 vol. of ice-cold 0.15 *M* KCl for 1 min in a Waring Blendor. The crude homogenate was centrifuged for 10 min at 700 × *g* at 0° in an angle-head centrifuge to remove fibrous tissue and debris. The resulting homogenate was strained through four layers of fine-mesh cheese-cloth and centrifuged at 8000 × *g* for 30 min at 0° to sediment the mitochondrial fraction. An acetone powder of the mitochondria was prepared in the usual way at — 15°, and extracted twice with 10 vol. 0.05 *M* potassium phosphate buffer, pH 7.4, for 30 min at roomtem perature with continuous gentle stirring. The aqueous extract was centrifuged at 8000 × *g* for 10 min at 0° to remove the sediment. 0.5 vol. of Na_2SO_4 saturated at 38° was added to the aqueous extract slowly with continuous gentle stirring, the solution was stirred for an additional 10 min, and the resulting precipitate was removed by centrifugation at 15° for 10 min at 8000 × *g*. 0.33 vol. Na_2SO_4 was added to the supernatant fluid and the fraction precipitating between 33 and 50 % saturation was similarly removed and dissolved in 0.05 *M* potassium phosphate buffer, pH 7.4, of approximately 0.1 the volume of the aqueous extract. The light straw-colored solution could be stored at — 18° for at least one week with less than 5 % loss in activity; thawing and re-freezing did not result in loss of activity.

The assay for glutamic dehydrogenase was similar to that of STRECKER[3] except that the increment in absorbancy between readings made at 30 and 60 sec after addition of the final reactant was taken as a measure of the enzyme activity. The values reported were determined in 0.05 *M* potassium phosphate buffer, pH 7.4. Protein was measured according to LAYNE's modification of the method of WARBURG AND CHRISTIAN[4]. When the original homogenate was assayed, KCN was added to a final concentration of 10^{-3} *M* and protein was determined by multiplying the nitrogen content by 6.25.

The results of a typical preparation are shown in Table I. Approximately 2000 units of activity in the aqueous extract were obtained from each placenta used in a preparation. Estimation of glutamic dehydrogenase activity of the whole homogenate from four different preparations gave an average specific activity of 0.08; the overall purification of the enzyme ranged between 600-and 1000-fold from the original homogenate and 5-fold from the aqueous extract of the acetone-dried mitochondria. The enzyme preparation was not homogenous in filter-paper electrophoresis in 0.02 *M* barbital buffer, pH 8.6.

TABLE I

PURIFICATION OF GLUTAMIC DEHYDROGENASE FROM HUMAN PLACENTA

Step	*Volume ml*	*Total activity units**	*Total protein mg*	*Specific activity units/mg protein*	*Yield %*
Aqueous extract of acetone powder (4 placentas)	235	9860	811	12.1	100
Na_2SO_4, 33–50% satn.	25	6000	99	60.3	61

* One unit of enzyme is the amount which causes a change in absorbancy of 0.001/min.

Abbreviations: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

TABLE II

MICHAELIS CONSTANTS OF REACTANTS IN GLUTAMATE OXIDATION

Reactant	K_m*	*S.E.*** K_m
Glutamate	$0.44 \cdot 10^{-3}$	$1.55 \cdot 10^{-7}$
DPN	$3.79 \cdot 10^{-5}$	$7.05 \cdot 10^{-8}$
TPN	$7.32 \cdot 10^{-6}$	$1.96 \cdot 10^{-10}$

* Estimated by method of least squares from the usual LINEWEAVER-BURK transformation in which $Y = 1/v$, $X = 1/[S]$.

** Standard error, calculated according to SNEDECOR[6] from the least-squares analyses.

Homogenates of liver and kidney from a human fetus of 19 weeks gestational age each contained about 10 times as much activity per mg protein as did term placenta; the placenta of this fetus contained the same amount of activity as term placenta.

Like the liver enzyme[3], human placental glutamic dehydrogenase will accept either DPN or TPN as coenzyme, although the activity with TPN is only about one-third that with equimolar DPN. The pH optimum for the forward reaction (glutamate oxidation) in 0.2 *M* phosphate buffer was 8.5–8.6. This value is identical with that reported by STRECKER[3] for the crystalline enzyme from beef liver. The enzyme prepared by OLSON AND ANFINSEN[5] showed a pH optimum at 7.7 under similar conditions. The Michaelis constants (Table II) of the forward reaction agree with those reported for the crystalline enzyme from beef liver. The preparation showed no alcohol dehydrogenase, corticosterone dehydrogenase, or hydrocortisone dehydrogenase activity. Neither L-aspartate nor DL-α-methylglutamate was oxidized by the enzyme under the conditions of the usual assay. Lactate was dehydrogenated at about one-third and L-glutamine at about one-half the rate of glutamate. When these substrates were each added together with glutamate the rates were additive. This suggests that lactic dehydrogenase and glutamine deaminase were present in these preparations rather than that these substances serve as substrates for glutamic dehydrogenase.

The characteristics of L-glutamic acid dehydrogenase of human placenta are very similar to those of the enzyme prepared from beef liver.

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1 G. GAULL AND C. A. VILLEE, *Biochim. Biophys. Acta*, 39 (1960) 560.
2 L. L. ENGEL AND J. SCOTT, *Recent Progr. in Hormone Research*, in the press.
3 H. J. STRECKER, *Arch. Biochem. Biophys.*, 46 (1953) 128.
4 E. LAYNE, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Acad. Press. N.Y., 1955, p. 454.
5 J. A. OLSON AND C. B. ANFINSEN, *J. Biol. Chem.*, 202 (1953) 841.
6 G. W. SNEDECOR, *Statistical Methods*, Iowa State College Press, Ames, Iowa, 4th edition, 1946.

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