dinoacetate methyltransferase (EC 2.1.1.2) activity. These enzymes, which have a restricted distribution, may well serve as markers for future studies on the problem of the origin of decidual tissue[3]. As for related tissues, we have observed transamidinase activity for the first time in commercial beef-placenta preparations and chick-egg yolk sac, but not in human term placenta.

The presence of significant levels of transamidinase in each of three tissues of a single animal offers the possibility of comparing the relative repressibility of transamidinase in these tissues. Table I shows that dietary creatine represses transamidinase in rat kidney and pancreas to the same relative extent, whereas decidual transamidinase is repressed to a significant, but lesser, extent. Whether this difference in repressibility reflects a difference in permeability to creatine, or other factors, is not known.

It would appear that the creatine-transamidinase and deciduoma model systems can indeed be combined, affording an opportunity to study enzyme repression, hormone effects[3,5], and differentiation[3,6] in the same non-embryonic mammalian tissue.

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A soluble c-type cytochrome from anaerobically grown *Escherichia coli* and various Enterobacteriaceae

It is generally accepted that *Escherichia coli* and related facultative anaerobes lack cytochromes *c* and *b*, but contain substantial amounts of cytochrome b_1[1]. It is the purpose of this note to show that a soluble cytochrome *c* consistently is formed when bacteria of this group are cultivated anaerobically on a synthetic medium containing glucose as the principal carbon source. This finding is an outgrowth of comprehensive studies being carried out in this laboratory on the enzymic and chemical constitution of *E. coli* cells grown on complex and synthetic media under both aerobic and anaerobic conditions. An important feature of these studies is the characterization of enzymes as either membrane bound or soluble. In order to make

this distinction we have processed cells in the manner described by HUNT, ROGERS AND HUGHES[2] to obtain a cell-wall-membrane fraction and a soluble cytoplasmic fraction.

The cell-wall-membrane fraction of aerobically grown cells contains the bulk of the cytochrome b_1; the remainder of the b_1 is attached to particulate derivatives of the membrane in the crude cytoplasmic fraction. When the particles are removed from the latter fraction by centrifugation for several hours at 140 000 × *g*, the soluble cytoplasmic fraction remaining is cytochrome free. These results correspond in every respect to those obtained by TISSIERES[3] with *E. coli* "ghosts".

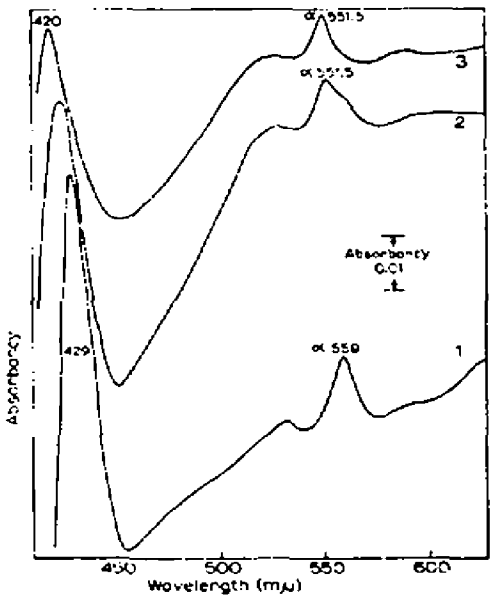

Fig. 1. Difference spectra of *E. coli*, strain K_{12}, fractions. Cells were grown anaerobically under N_2 on a synthetic medium containing 0.05 M NH_4Cl, 0.04 M potassium phosphate buffer (pH 7.2), 0.25% glucose, and 5 ml/l of a mineral salts stock solution (10 g $MgSO_4 \cdot 7\ H_2O$, 1 g $MnCl_2 \cdot 4\ H_2O$, 0.4 g $FeSO_4 \cdot 7\ H_2O$, and 0.1 g $CaCl_2$ per liter). The pH of the culture was maintained between 6.0 and 7.0 during growth by the addition of NaOH. Cells were harvested during the exponential phase of growth at an absorbancy of 0.5 (420 mμ), washed with 10 mM potassium phosphate buffer (pH 7.3), and treated to yield a cell-wall-membrane and a cytoplasmic fraction[4]. Difference spectra (oxidized with ferricyanide *versus* reduced with dithionite) were obtained in the Cary model-14 recording spectrophotometer using the 0.0–0.1–0.2 slide wire. Curve 1, the cell-wall-membrane fraction. Curve 2, the crude cytoplasmic fraction; Curve 3, the soluble cytoplasmic fraction obtained from the crude cytoplasm by centrifuging for 4 h in the Spinco preparative centrifuge at 140 000 × *g* to remove particulate matter containing cytochrome b_1.

However, when similar studies were carried out using anaerobic cells, and especially cells grown anaerobically in a mineral salts–glucose medium, an important difference was noted as is indicated in Fig. 1. The cell-wall-membrane fraction gives the same spectrum as the aerobic cell-wall-membrane fraction, although the cytochrome b_1 concentration is always lower in preparations from anaerobically grown cells. However, the crude cytoplasmic fraction shows broader overlapping peaks shifted toward shorter wavelengths than the comparable aerobic preparations. Upon centrifugation at high speed a particle fraction containing cytochrome b_1 is

obtained as it was before with crude cytoplasm from aerobic cells, but the soluble cytoplasmic fraction remaining contains a *c*-type cytochrome not previously observed in the aerobic-cell cytoplasmic fraction. This cytochrome seems to be truly soluble since it is not sedimented by centrifugation at 200 000 × *g* for 4 h.

Inasmuch as our observations on this cytochrome *c* were limited to the K_{12} strain of *E. coli*, we proceeded to examine other species or strains of Enterobacteriaceae. All of the microorganisms listed in Table I contain soluble cytochrome *c*

TABLE I

DISTRIBUTION AND RELATIVE CONCENTRATION OF *c*-TYPE CYTOCHROME IN THE ENTEROBACTERIACEAE

Organism*	Medium	A_{551} mμ/mg protein	*E. aurescens* (%)
E. coli, K_{12}	Synthetic	1.4	24**
E. coli, B	Synthetic	0.4	7
E. coli, W	Synthetic	0.6	10
E. aurescens, ATCC 12814	Synthetic	5.9	100
E. freundii, ATCC 8454	Synthetic	0.4	7
E. intermedia, ATCC 6730	Synthetic	0.3	5
Aerobacter cloacae, ATCC 961	Synthetic	1.0	17
Paracolobactrum aerogenoides, ATCC 11604	Complete***	0.2	3

* Cells were grown and processed as indicated in Fig. 1 to yield a clarified soluble cytoplasmic fraction.

** Concentration relative to *E. aurescens* at 100.

*** Because of the limited growth anaerobically in the synthetic medium, 1.4% casamino acids (Difco) replaced the NH_4Cl in the synthetic medium.

when grown anaerobically on a synthetic mineral salts glucose medium. *Escherichia aurescens* produces the greatest amount of *c*-type cytochrome and is the only species which produces detectable traces of cytochrome *c* aerobically. Because of this we cannot rule out the possibility that the other strains may produce traces of this cytochrome aerobically which are too small to be detected by our instruments. The high content of cytochrome *c* in the *E. aurescens* soluble cytoplasmic fraction makes it possible to obtain sharper and more symmetrical peaks in the spectra (Fig. 2). In addition, *E. aurescens* cells are providing an excellent source of cytochrome *c* for purification work and for studies on the function of this cytochrome in anaerobic metabolism.

In summary, *E. coli* and related facultative anaerobes contain a soluble *c* type cytochrome when cultivated anaerobically in a mineral salts-glucose medium. It is easily detected if care is taken to remove the membrane-bound cytochrome b_1 which might otherwise obscure it. It has an α band at 551.5 mμ, a β band at 523 mμ, and a Soret band at 420 mμ when reduced or at 408 mμ when oxidized. Its spectrum is similar to the *c*-type cytochrome recently reported[4] for glutamate-grown *Salmonella typhimurium* (α, 551 mμ; β, 525 mμ; reduced 416 mμ, oxidized 409 mμ). In retro-

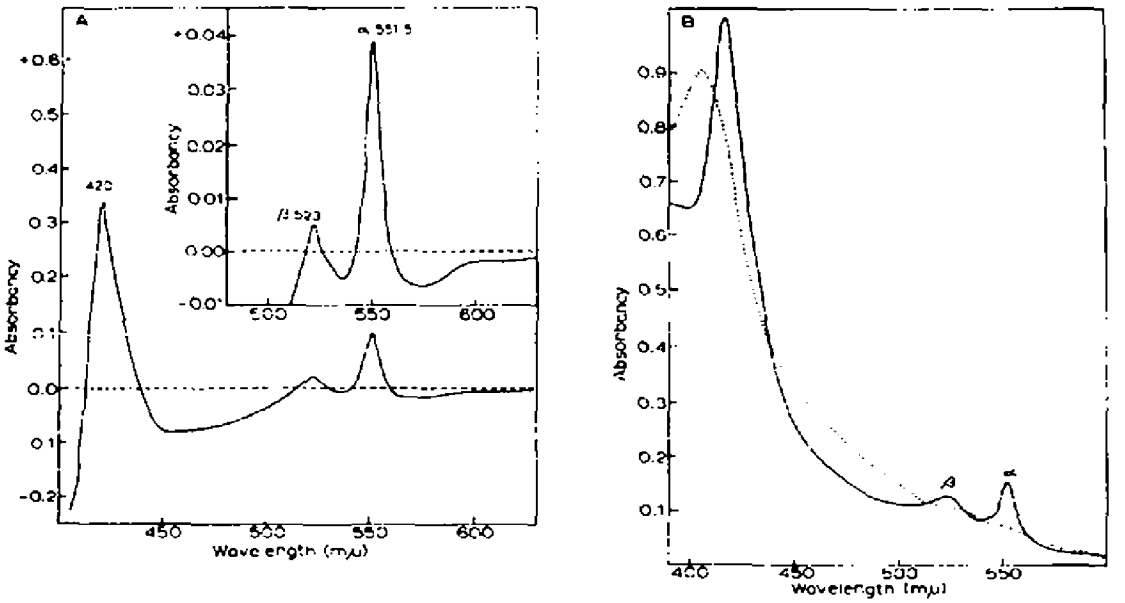

Fig. 2. Absorption spectra of soluble cytoplasmic fraction from *E. aurescens* prepared as described in Fig. 1. A, difference spectrum obtained with the 0.0–1.0 2.0 slide wire. Insert: difference spectrum of another preparation showing more detail in the α and β band region using the 0.0–0.1–0.2 slide wire. B, ... , oxidized spectrum; - - -—, reduced spectrum.

spect, the failure to observe a *c*-type cytochrome in the facultative Enterobacteriaceae with a cytochrome-b_1 pattern in the past may have been due to masking by large amounts of cytochrome *b*, or to observations on cells grown under conditions where cytochrome *c* production is low.

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