Biliverdin reductase-like activity in buffalo mammary tissues

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Summary. Lactating buffalo mammary tissues exhibited biliverdin reductase activity. The supernatant fraction obtained from the tissue possessed the enzyme activity. Enzyme activity could not be detected in non-lactating mammary tissues, and was absent in lactating and non-lactating mammary tissues from cow and goat.

Biliverdin has been reported to be present as a normal constituent of fresh drawn buffaloes' milk[2], the average concentration being 0.622 µg/ml[3]. Recently, Rao[4] has observed the binding of biliverdin with casein. Biliverdin extracted from buffaloes' milk has been shown to be the IXα isomer[5]. As biliverdin is absent from the blood serum of normal healthy buffaloes, it seems probable that the bilirubin present in the blood serum must be undergoing oxidation in the mammary tissues to give rise to biliverdin in milk. When ^{14}C bilirubin was administered into a buffalo blood system through the jugular vein, ^{14}C biliverdin was detected in the milk[6]. Therefore buffalo mammary tissue was examined for biliverdin reductase activity.

Materials and methods. Lactating and non-lactating buffalo mammary tissues were collected from an abattoir. The tissues were weighed, chilled and processed according to Singleton and Laster[7]. The supernatant of the mammary homogenate was then assayed for biliverdin reductase activity[7].

The reversibility of the reaction was tested at the end of the forward reaction by the addition of NAD^+ (Sigma Chemical Company) to the reaction mixture. The assay mixture contained (in µmoles): potassium phosphate buffer, pH 7.4, 50; NADH, 1.0; biliverdin 0.06, and supernatant fraction of the tissue homogenate in a total volume of 1.0 ml. The reversibility of the reaction was tested by the addition of 1.0 µmole NAD^+ to the reaction mixture at the end of the forward reaction. The concentration of NAD^+ may not be a decisive factor in the reversibility of biliverdin reductase activity, as Singleton and Laster[7] failed to regenerate biliverdin from enzymatically formed bilirubin at the end, of the forward reaction even when excess NAD^+ was added. The absorption spectra of bilirubin and biliverdin formed in the forward and reverse reactions were recorded in the assay mixture.

Heat-denatured supernatant fraction was tested for reductase activity. The supernatant fractions from lactating and non-lactating cow and goat mammary tissues were also examined for reductase activity.

Results and discussion. Lactating buffalo mammary tissues have been observed to be capable of reducing biliverdin, while this reductase activity could not be demonstrated in the non-lactating tissues. The decline in A_{670} was a linear function of time for 15 min (fig. 1). This reaction has an absolute requirement for reduced NAD. Incubation of biliverdin with supernatant fraction produced no reaction, A_{670} remaining constant until the addition of reduced coenzyme. Further, this activity was absent from cow and goat mammary tissues.

Heat-denatured supernatant fraction did not possess the reductase activity.

Biliverdin reductase activity has been reported to be absent from the liver of ruminants but not of other mammals[8]. The increase in A_{670} during the oxidation of bilirubin is also a linear function of time (fig. 1). The absorption spectra of bilirubin and biliverdin formed during the forward and reverse reactions are shown in figure 2. Oxidation of bilirubin in buffalo mammary tissue, involving the reverse reaction of biliverdin reductase, may explain the occurrence of biliverdin in buffaloes' milk. This phenomenon is in accordance with the observation of Rao et al.[6], who recovered ^{14}C biliverdin in buffaloes' milk after the i.v. administration of ^{14}C bilirubin. Colleran and O'Carra[8] attribute the apparent irreversibility of biliverdin reductase to the poor binding of bilirubin to the active site of the enzyme, owing to its different conformation from that of biliverdin. However, in our studies it was observed that the reaction catalyzed by biliverdin reductase was readily reversible. Further, the concentration of NAD^+ appears to have no bearing on the reversibility of the reaction, as observed by Singleton and Laster[7].

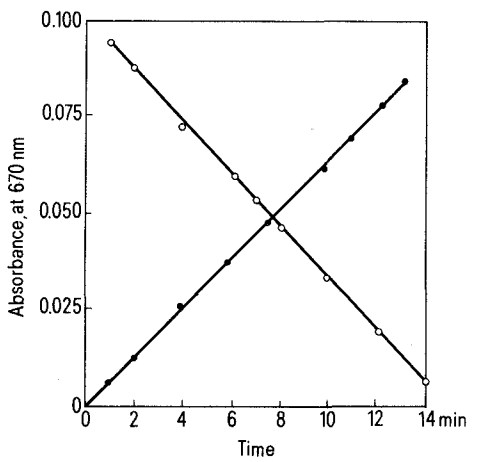

Figure 1. Buffalo mammary tissue associated biliverdin reductase activity (○) and the reverse reaction (●) observed in the supernatant.

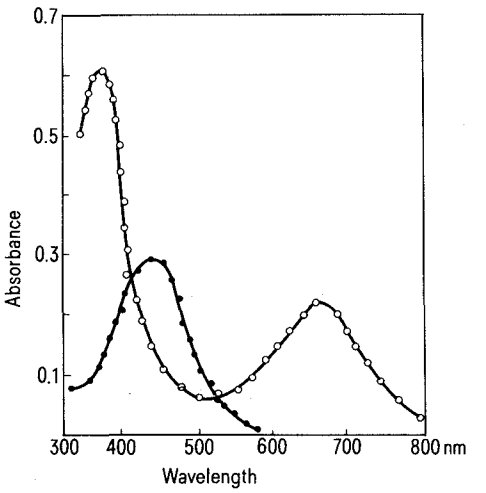

Figure 2. Absorption spectrum of bilirubin (●) formed in forward reaction. Absorption spectrum of biliverdin (○) formed in reverse reaction.

The sequence of bile pigment formation heme → biliverdin → bilirubin occurs in human beings, most of the mammals and also in reptiles and fishes[9,10]. However, in some vertebrates like chickens and possibly other birds, the sequence seems to stop at the biliverdin level due to lack of biliverdin reductase[11] both in liver and spleen, and consequently those species excrete mostly unconjugated biliverdin in bile[10,12]. A similar explanation cannot be suggested for the origin of biliverdin in buffaloes' milk, since biliverdin reductase activity is present in spleen, though this enzyme has been reported to be absent from the liver of ruminants[8]. To explain the conversion of ^{14}C bilirubin of blood serum to ^{14}C biliverdin, which was recovered in milk[6], the oxidation of bilirubin in buffalo mammary tissues appears to be a possible mechanism. Since biliverdin does not occur in normal sera[13], the presence of bilirubin in buffaloes' milk is attributed to the oxidation of bilirubin in the mammary tissues as shown in the present study. In the light of the above mentioned facts, it may be surmised that the biliverdin reductase like activity from buffalo mammary tissue is different from that observed in liver, spleen and placenta. To explain the occurrence of biliverdin in buffalo milk we suggest that the oxidation of bilirubin in buffalo mammary tissue is catalyzed by an oxidase. It is of interest to note that recently bilirubin oxidase has been characterized for the first time in the microorganism *Myrothecium verrucaria*[14-16].

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Changes of the cell components of *Escherichia coli* and *Pseudomonas fluorescens* in deficient medium

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Summary. Strains of *Escherichia coli* and/or *Pseudomonas fluorescens* prelabeled with 14-C-glutamic acid show differences in the marker distribution in the cell components. Moreover, there are essential distinctions in the trend and rate of degradation of macromolecular fractions under starvation conditions.

The physiological changes occurring in bacterial cells exposed to environments without essential exogenous substrates are usually studied with strains of *Escherichia coli*[1-3]. We used, for comparison, *Pseudomonas fluorescens* and examined the cell component changes during exposure of bacteria to a medium without a carbon source. The main reason for performing this work is that the phenomenon of microbial survival in nutrient-limited natural waters occurs frequently[4,5], but the processes are understood very poorly. The results presented here on the distribution of radioactive label originating from 14-C-precursors into different macromolecular fractions of bacteria before and after starvation supplement our previous observations[6,7].

Materials and methods. Escherichia coli strain CCM 2260 and *Pseudomonas fluorescens* strain CCM 2115 from the Czechoslovak Collection of Microorganisms of the J.E. Purkyně University Brno were used for experiments. The overnight inoculum (12 h) was prepared with bacteria taken from agar slants.

For preparation of labeled culture, 14-C-glutamic acid or 14-C-valine (4 kBq per 1 ml of media) was added to 3000 ml of Proteose peptone medium (Oxoid). During

Table 1. The 14-C label remaining in 14-C-prelabeled cells of *E.coli* and/or *P.fluorescens* after starvation in Davis mineral medium without carbon source

Strain	Without TCA precipitation (cpm)			TCA precipitate (cpm)		
	0 h	72 h	168 h	0 h	72 h	168 h
E.coli	32880 (100)*	22070 (67.1)	16520 (50.2)	30460 (92.6)	18930 (57.6)	10570 (32.1)
P.fluorescens	1560 (100)	1120 (71.8)	764 (49.1)	1510 (100.6)	840 (53.8)	600 (38.5)

*In brackets the counts are expressed as percentage of label which remains in cells or TCA precipitates when compared to the total cellular label before starvation.