

## DETERMINATION OF SUCCINATE DEHYDROGENASE ACTIVITY OF RUMEN MICROORGANISMS: AN APPROACH TO PARTITIONING OF SUCCINATE METABOLISM IN THE RUMEN

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### Introduction

Since Blackburn and Hungate (1963) have estimated high velocity constants for conversion of added succinate to propionate in the rumen mixed culture, it has been accepted that the major pathway of succinate metabolism in the rumen is the propionate formation. On the other hand, there are interesting findings that cell-free extracts of mixed rumen microorganisms possess succinate dehydrogenase activity (Palmquist and Baldwin, 1966) and reduce succinate to succinic semialdehyde as the first step of metabolic conversion leading from succinate to glutamate (Emmanuel and Milligan, 1972). These findings imply that a partitioning of pathways involved in succinate metabolism takes place in the rumen.

For proving this assumption, the succinate dehydrogenase (SDH) activity of rumen microorganisms, actually in the mixed culture, has to be estimated. The present study was initiated to investigate the possibility that changes of oxidation-reduction (redox) potential (Eh) connected with the reduction of thionine, a redox indicator dye, can be utilized effectively in determining the SDH activity of rumen microorganisms in the mixed culture.

### Materials and Methods

Rumen fluid was obtained from a rumen-fistulated wether fed an 80% roughage diet at 5 hr after feeding. One hundred ml of strained rumen fluid was incubated at 39 °C in a glass vessel with a rubber stopper which contained four holes. Oxygen-free CO<sub>2</sub> was bubbled at a constant rate of one bubble per second from a glass tube inserted through a hole of rubber stopper. Through two holes, a platinum electrode for Eh measurement and a glass electrode for pH measurement were placed in the fluid. At about 3 hr after the commencement of incubation, the Eh of rumen

fluid reached to an equilibrated level of approximately -200 mV. Then 4 ml of solution containing a certain quantity of thionine, succinic acid or malonic acid, and/or of their combination, was introduced into the rumen fluid.

The Eh change was detected with a Horiba model H7 pH meter equipped with a recording apparatus. The Eh detected between platinum and standard calomel electrodes was adjusted to the Eh between platinum and standard hydrogen electrodes. The solutions tested were prepared freshly before each incubation trial.

The quantities of thionine (thionine acetate, Merck), succinic acid and malonic acid (Wako Pure Chemical) used in the incubation trials were described in the text. Methylene blue (Merck) was used in one trial.

### Results and Discussion

A preliminary experiment, conducted with the incubation method described above, found out that not only thionine (potential of half-reduced thionine, E<sub>0</sub> + 63 mV at pH 7.0) but also other redox indicator dyes, methylene blue (E<sub>0</sub> +11 mV), indigo tetrasulphonate (E<sub>0</sub> -46 mV), indigo trisulphonate (E<sub>0</sub> - 81 mV), and indigo disulphate (E<sub>0</sub> -125 mV), are easily reduced in the rumen mixed culture and give respective change in the electrode potential. The results indicated that the use of redox indicator dyes in biological systems is not necessarily limited to the range of redox reactions catalized by enzyme preparations and enzymes in cell-free extracts but is applicable in determining the activity of redox enzymes in living organisms.

Among the dyes reduced, thionine seemed to accept electrons from succinate-fumarate redox reaction (E<sub>0</sub> +31 mV) taking place in the rumen mixed culture. The present study tried to demonstrate that thionine reduction is coupled with SDH system. The results obtained were described

below.

Twenty  $\mu$  moles of thionine dissolved in 4 ml of water was introduced into the incubated rumen fluid in which Eh has been already at steady state. The Eh changed immediately and the potential change indicated the reduction of thionine since the fluid Eh fluctuated up to proximate level of thionine  $E'_0$  (+61 mV). In order to give an explanation that SDH system takes part in the reduction of thionine, 100  $\mu$  moles of malonic acid, a competitive inhibitor of SDH, together with 20  $\mu$  moles of thionine were introduced into the incubated rumen fluid. In this case little potential change was observed. Malonic acid diminished much the reduction of thionine. When the mixture of thionine 20  $\mu$  moles, malonic acid 100  $\mu$  moles and succinic acid 100  $\mu$  moles was introduced, the potential change was almost the same as that by the mixture of thionine and malonic acid. On the other hand, the reduction of methylene blue (20  $\mu$  moles) was not affected by malonic acid. These facts demonstrated the specificity of thionine reduction which is coupled with SDH system, and led to an understanding that thionine can be utilized in determining SDH activity in the rumen mixed culture.

The determination of SDH activity using thionine and Eh meter is based on a theoretical explanation that the potential difference between 50 %-reduced dye and 99 %-reduced dye is 60 mV. The time course change of fluid Eh is charted by the recorder. The time required for complete reduction of thionine and the rate of thionine reduction in the incubated rumen fluid were shown in table 1. The estimated average rate of thionine reduction was 7.4  $\mu$  moles/dl/min. This rate is equivalent to 3.7  $\mu$  moles/dl/min of SDH activity because SDH catalyzes formation of fumarate by removal of two hydrogen atoms from succinate and then thionine molecule accepts one hydrogen atom for its reduction. The estimated SDH activity was almost equal to the rate of succinate turnover to propionate in the rumen, in which the latter was calculated by Blackburn and Hungate (1963). Such a coincidence must be discussed. Supposed that the present experiment is dealing with the rate of reverse reaction of fumarate reduction catalized by fumarate reductase, the agreement is reasonable because fumarate reductase is considered as an enzyme involved in

TABLE 1. REDUCTION OF THIONINE IN WETHER RUMEN FLUID

Thionine added	Time required for reduction	Rate of reduction
$\mu$ moles/dl	second	$\mu$ moles/dl/min
20	174	6.9
40	320	7.5
60	462	7.8

succinate formation system. However, Hopgood and Walker (1969) mentioned that fumarate reductase is not identical with SDH in *Ruminococcus flavefaciens*.

There have been still remained the problem of identity of the two enzymes and of the direction of reaction catalized by the two enzymes. Notwithstanding, results of the present experiment have provided the information on the possibility that the determination of SDH activity in the rumen mixed culture can clarify not only changes of SDH activity itself in changes of the rumen fermentation caused by alteration of diet but also enables to reveal an obscurity on partitioning of pathways involved succinate metabolism in the rumen.

(Key Words: Oxidation-Reduction Potential, Succinate Dehydrogenase, Rumen Microorganisms)

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