

# PROOF OF EXISTENCE BY STAINING OF ICDH, SDH, MDH, LDH, ALDOLASE, ALC DH AND $\alpha$ -KETO-DH IN *TRICHOMONAS VAGINALIS*

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## INTRODUCTION

It has been already proved by the present author using biochemical methods that the Krebs cycle exists within trichomonas vaginalis (hereafter abbreviated as T.V.) body<sup>1,2</sup>. But it might be possible that this cycle, unlike those in higher animal cells, is an incomplete one with a part missing, or one in which some kinds of enzymes are abundant but other kinds of enzymes are present in a lesser amount. Especially in T. V. with mainly anaerobic metabolism it is doubtful that the Krebs cycle is working in a regular way and it might be possible that they show only the potentiality<sup>3</sup>.

We report here the results of a study whether these related enzymes exist in T.V. or not, and also of their localization at the optical microscope level.

## METHOD

We used T.V. strain separated from a male patient with some urological disease and maintained by continuous subculture method for more than 30 generations. T.V. cells which had been subcultured two days ago and proliferated were collected by centrifugation and further washed and centrifuged twice by normal saline.

Staining was done by a variant of the Barka-Anderson staining method<sup>4</sup>. Staining fluid was applied for approximately 20 minutes.

## RESULTS

In order to discriminate from the so-

called nothing-dehydrogenase, we used as a control a staining fluid which included only normal saline without any basic substance, among and let it act for the same time period under the same condition.

Among three enzymes, ICDH, MDH, and SDH, which are on the Krebs cycles, the activity of ICDH and SDH is low and that of MDH is high, as shown on the photographs. With these kinds of staining method the active parts of the enzymes are shown by the blue color of NBT granules and are black on monochrome photographs.

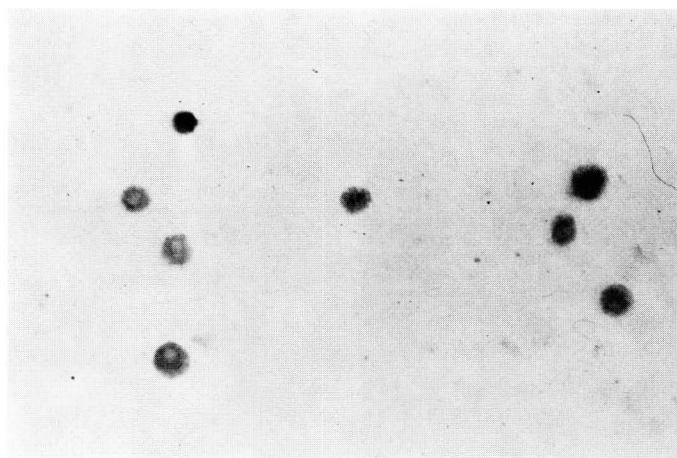
As to ICDH, some enhancement of activity is observed, compared to the control, but its localization is not clear.

As to SDH, the activity is even less significant so we can hardly observe a clear difference from the control. It is known from biochemical measurements that the activity of these two enzymes does exist, but not in large amounts, as confirmed by this result.

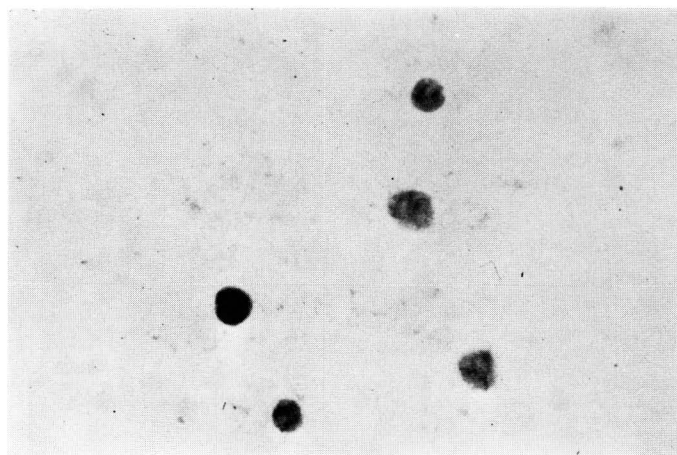
On the other hand MDH is clearly stained in large amounts compared with the above two enzymes. This activity can be observed over the whole cytoplasmic region.

For the enzymes LDH and aldolase, which are related with glycolysis outside the Krebs cycle, comparatively high activity is observed and LDH is seen to localize, as it is the case with MDH, on the cytoplasm, and for aldolase, in the region around the cell membrane and the nuclear membrane.

For Alc DH, the activity is of moderate



control



experiment

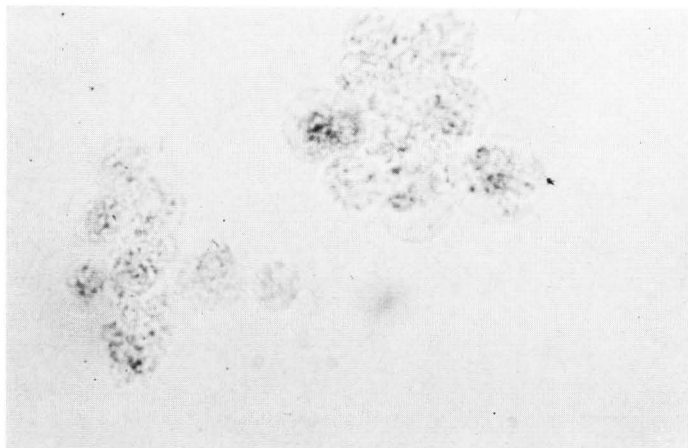
Fig. 1. Staining of ICDH. No significant activity observed.

strength compared with the other enzymes and its localization is not clear. For  $\alpha$ -Keto DH, the activity was not significant.

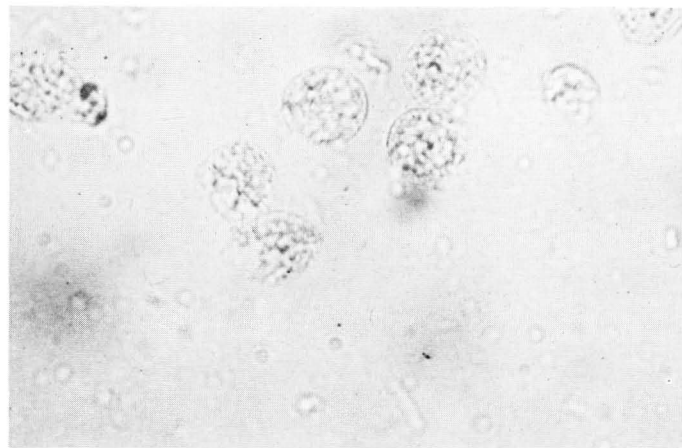
#### DISCUSSION

We have already proved biochemically that the activity of SDH, ICDH and  $\alpha$ -Keto DH in T.V. cell is low. We have also shown at the same time that their activity is not zero. The present proof by staining method confirms this result. But Ozaki<sup>5)</sup>, Tanabe<sup>6)</sup> et al. have suggested that there is a possibility that SDH does not exist in T.V.. The present author's opinion is that this enzyme does not have much activity in T.V. and exists only as a potentiality.

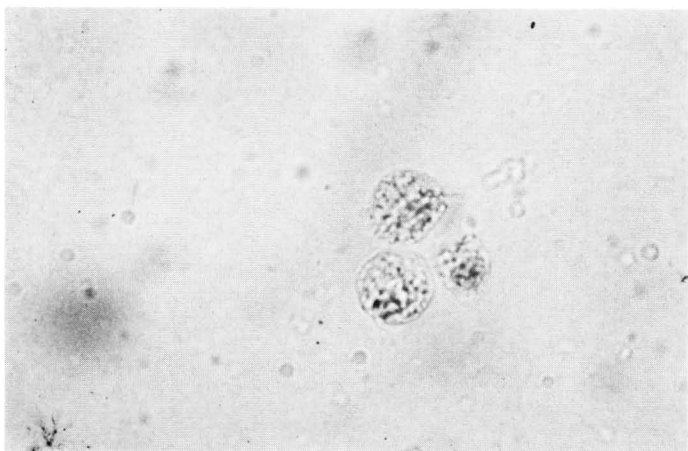
The fact that LDH takes nutrition mainly from sugar, suggests us that LDH activity is high, but it is not well known what kind of meaning the activity of Alc DH has under natural conditions. For aldolase it is peculiar that its localization differs from dehydrogenases, but it must be necessary in T.V. body. The meaning of this localization is unknown, To the authors' knowledge there is no report on the proof of the existence of these enzymes in protozoa except the present authors' biochemical proof and Tanaka's<sup>7)</sup> report on ICDH, SDH, MDH, and their isozymes. There is also recent unpublished report by Tanabe. Summarizing these works it may be concluded that all glycolytic enzymes do exist



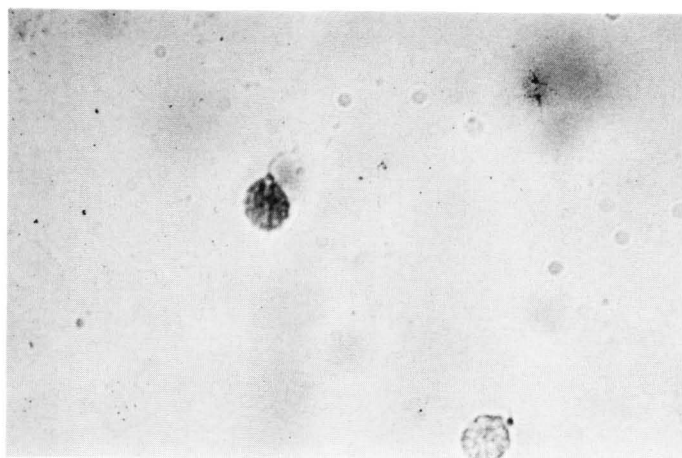
control 1



control 2

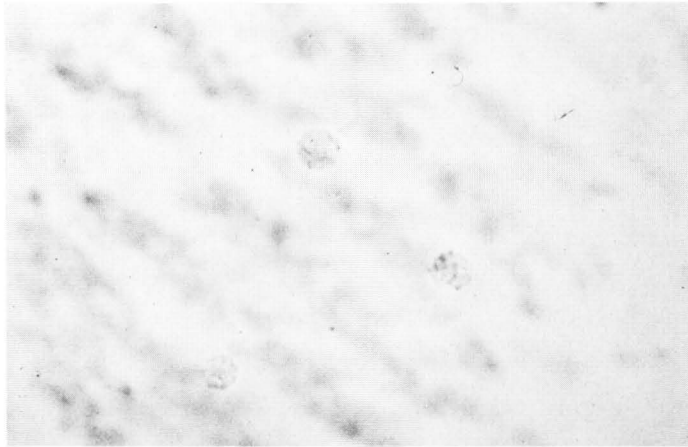


experiment 1

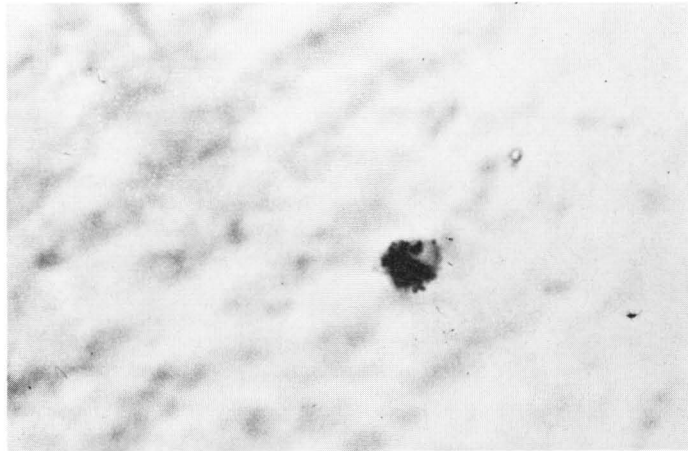


experiment 2

Fig. 2. Staining of SDH. Slight activity in cytoplasm

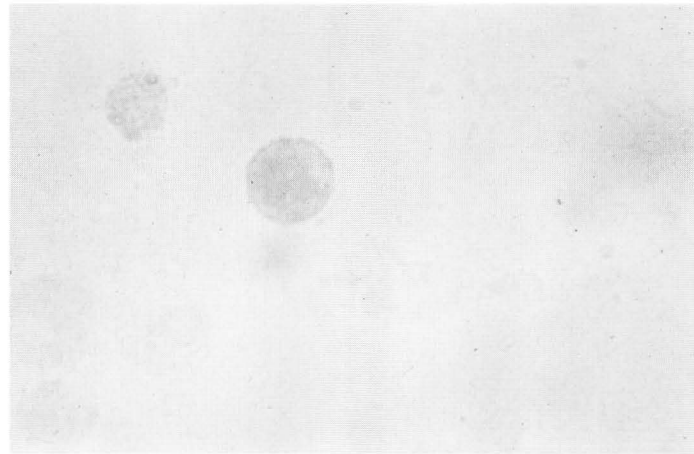


control

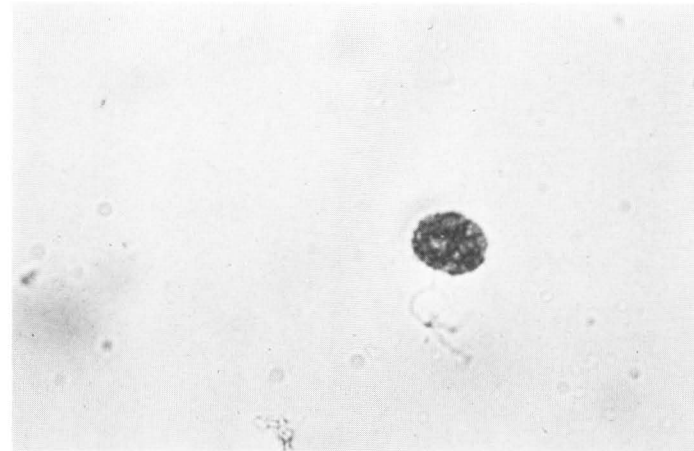


experiment

Fig. 3. Staining of MDH. High activity observed throughout.

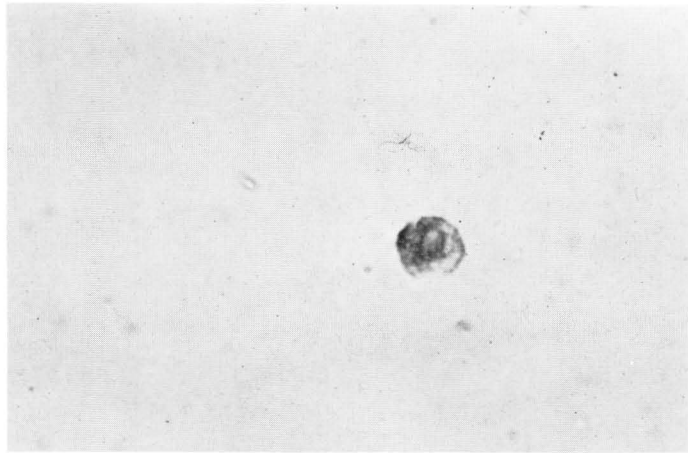


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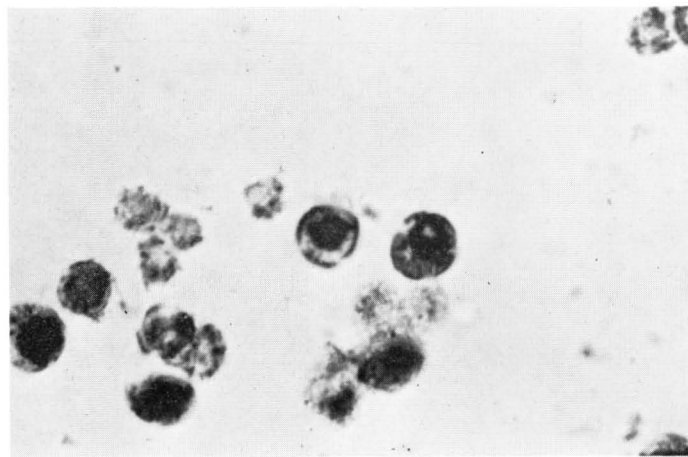


experiment

Fig. 4. Staining of LDH. Granules within cytoplasm are well stained and the flagellas are also stained, but nucleus remains unstained.

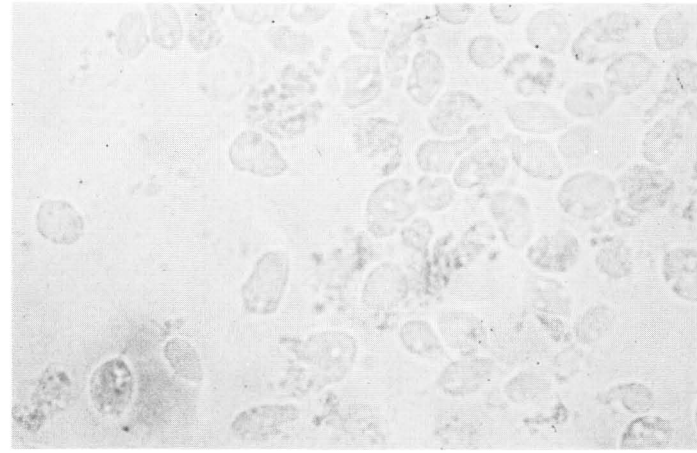


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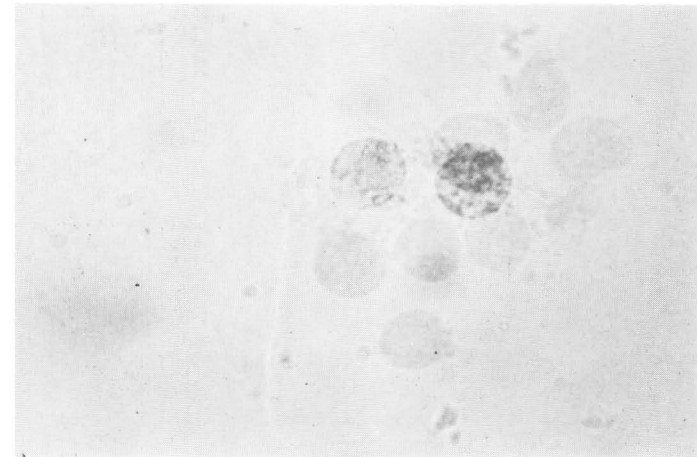


experiment

Fig. 5. Staining of aldolase. Activity observed around the nucleus and the T.V. cell membrane.

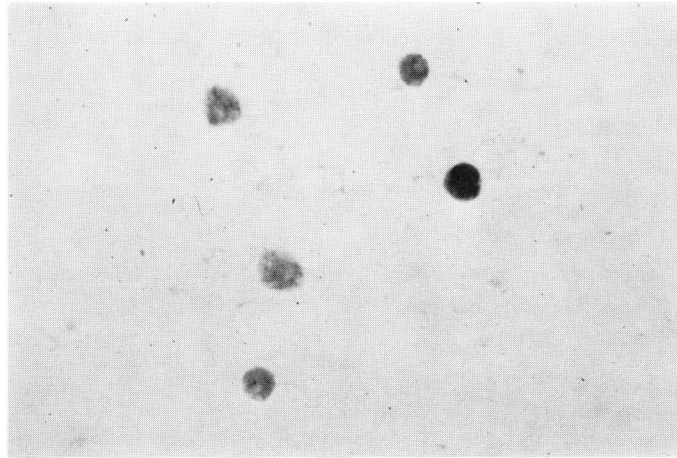


control

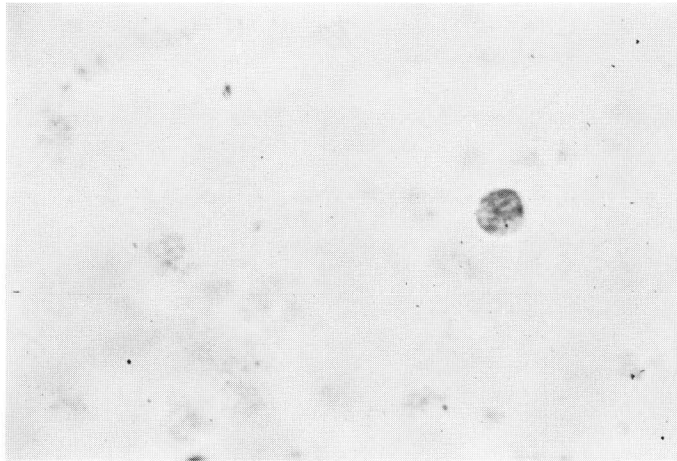


experiment

Fig. 6. Staining of AlcDH. Difference in activity is observed according to the T.V. condition. Distribution on the cytoplasm.



control



experiment

Fig. 7. Staining of  $\alpha$ -Keto DH. No significant activity observed.

but there is a large difference in their amount. It might be possible that the glycolysis system does not have a complete form in T.V.

There have been many studies on metabolism of the T.V. and though there are reports on materials consumed and produced by culture T.V., only few are concerned with metabolism within the T.V. The present study complements the authors' biochemical study on crushed T.V. fluid and confirms the result of the latter.

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和文抄録

膾トリコモナス虫体内の ICDH,  $\alpha$ -Keto DH, LDH, MDH,  
SDH, aldolase, AlcDH の染色による証明

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木 下 英 親

膾トリコモナス虫体内の dehydrogenase 各種を染色法で検討してみた。LDH, Aldolase, AlcDH, MDH,

の活性は虫体内にみとめられたが,  $\alpha$ Keto DH, SDH, ICDH にはあまり活性がみとめられなかった。