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Effects of Dithiocarbamate on the Respiration of Sea-Urchin Spermatozoa in Relation to Copper and Zinc

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It is well known that the rate of respiration of sea-urchin spermatozoa is considerably low in the dense sperm suspension and it increases as the density of spermatozoa decreases. The increase of the respiration rate by diluting the sperm suspension was estblished to be ascribed to the catalytic action of certain heavy metal ions in sea water (ROTHSCHILD and TUFT, 1950; ROTHSCHILD, 1950, 1956; ROTHSCHILD and TYLER, 1954; TYLER, 1950, 1953; TYLER and ATKINSON, 1950; TYLER and ROTHSCHILD, 1951; etc.). In the preceding papers, the present author reported that dithiocarbamate, one of the metal chelating agents having a high affinity for copper and zinc, markedly augments the respiration of the spermatozoa in dense suspension (MURAMATSU, 1963 a, b). The augmentation of sperm respiration by dithiocarbamate was assumed to be due to the removal or unmasking of a certain blocking system containing heavy metals in the respiratory system of the sperm cell.

To elucidate the relation between these two different ways mentioned above for stimulating the sperm respiration, the effects of dithiocarbamate on the respiration augmented by the addition of copper or zinc ions were investigated. The results are shown below and a tentative interpretation is presented as to the mechanism of the activation of the respiration of sea-urchin spermatozoa.

Material and Methods

The sea-urchin, *Hemicentrotus pulcherrimus* was used as material. Artificial sea water, buffered at pH 8.2, scarcely containing heavy metal ions was employed for the suspending medium of spermatozoa, natural sea water being inadequate for observing the accurate effect of heavy metal ions. Procedures for preparing the dense sperm sample including 3×10^{10} spermatozoa per ml and the constituents of the artificial sea water were the same as in the previous paper (MURAMATSU, 1963 a). Oxygen uptake was measured at 20°C

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with WARBURG manometers run in duplicate or more. The dense sperm was diluted 1:6, 0.2 ml of the sperm in the main compartment being diluted with the medium in the side arm, at the initiation of measurement. Sodium dimethyldithiocarbamate (DMDTC) was used as an efficient reagent to augment the sperm respiration (see MURAMATSU, 1963 a). DMDTC was washed several times with carbon tetrachloride and dried in air a few days before use.

Results

The effect of $CuSO_4$ and that of $ZnSO_4$ are shown in Fig. 1 and Fig. 3. Both Cu^{++} and Zn^{++} were observed to enhance the sperm respiration. These results essentially coincided with the findings of ROTHSCHILD and TUFT (1950) and UTIDA and NANAO (1956).

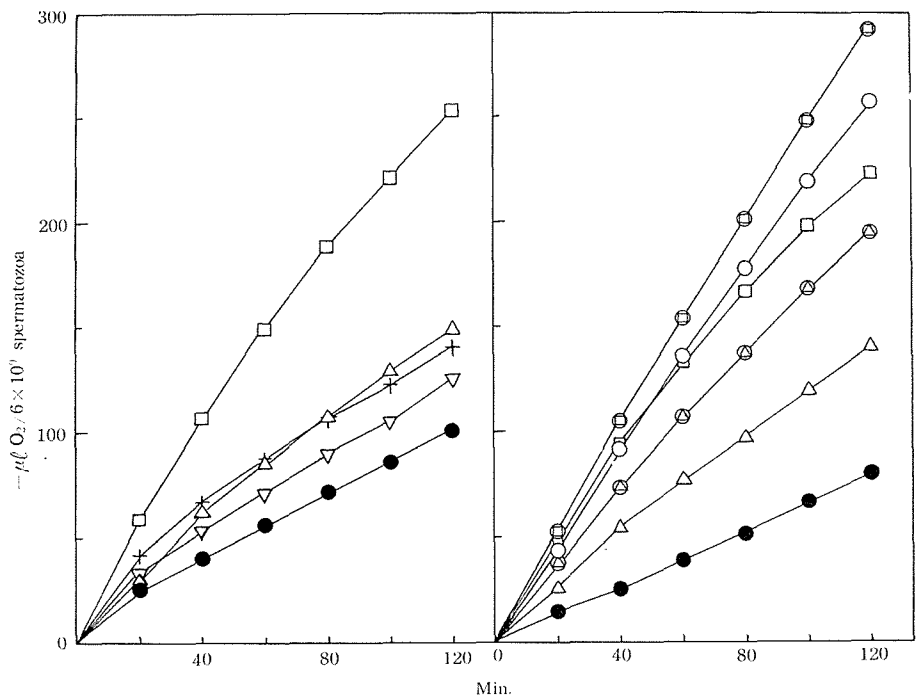

Fig. 1 Fig. 2

Fig. 1. Effects of $CuSO_4$ on the respiration of sea-urchin spermatozoa. Sperm density: 5×10^9 per ml. ●: Control (Artificial sea water); $CuSO_4$-concentration, △: 10^{-3}M □: 10^{-4}M, +:10^{-5}M, ▽:10^{-6}M.

Fig. 2. Effects of $CuSO_4$ and DMDTC on the respiration of sea-urchin spermatozoa. Sperm density : 5×10^9 per ml. ●: Control (Artificial sea water), ○: 10^{-2}M DMDTC, △: 10^{-3}M $CuSO_4$, □: 10^{-4}M $CuSO_4$, ◬: 10^{-3}M $CuSO_4$ +10^{-2}M DMDTC, ◫: 10^{-4}M $CuSO_4$ +10^{-2}M DMDTC.

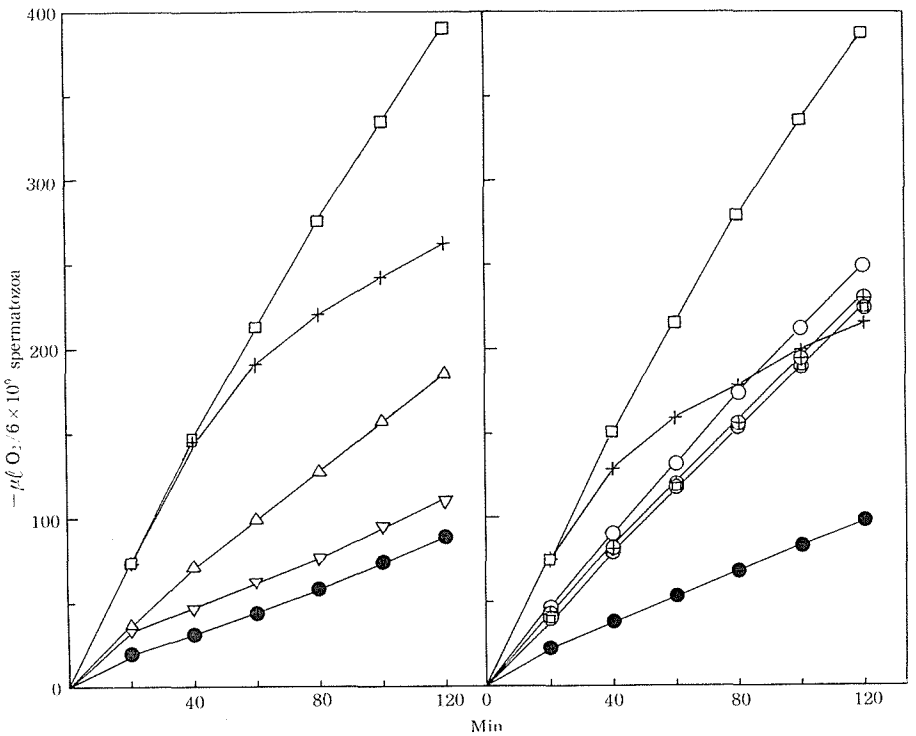

Fig 3. Fig. 4

Fig. 3. Effets of $ZnSO_4$ on the respiration of sea-urchin spermatozoa. Sperm density : 5×10^9 per ml. ● : Control (Artificial sea water); $ZnSO_4$-concentration, △: 10^{-3}M, □:10^{-4}M, +:10^{-5}M, ▽:10^{-6}M,

Fig. 4. Effects of $ZnSO_4$ and DMDTC on the respiration of sea-urchin spermatozoa. Sperm density : 5×10^9 per ml. ●: Control (Artificial sea water), ○:10^{-2}M DMDTC, □: 10^{-4}M $ZnSO_4$, +: 10^{-5}M $ZnSO_4$, ◎:10^{-4} $ZnSO_4$ +10^{-2}M DMDTC, ⊕: 10^{-5}M $ZnSO_4$ +10^{-2}M MDDTC,

To have some interpretations about the relation between the two different ways to augment the sperm respiration, one is the addition of heavy metal ions and the other is that of DMDTC, either Cu^{++} or Zn^{++} was given together with DMDTC. The concentration of DMDTC added was 10^{-2}M which had been established to be the most effective to activate the respiration. As shown in Fig. 2, the activating effect of DMDTC surpassed that of Cu^{++} and DMDTC further augmented the respiration enhanced by Cu^{++}. Such an effect of DMDTC as in the case with Cu^{++} did not occur when DMDTC was added with Zn^{++} (Fig. 4). The rate of respiration at 10^{-4}M $ZnSO_4$ was much higher than that at 10^{-2}M DMDTC during the course of measurement and the rate at 10^{-5}M $ZnSO_4$ was also somewhat higher for a while after initiation of

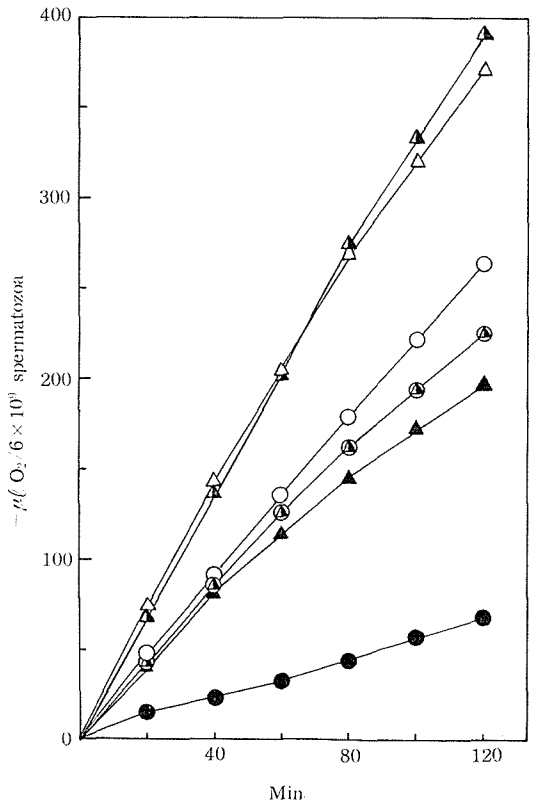

Fig. 5. Effects of $CuSO_4$, $ZnSO_4$ and DMDTC on the respiration of sea-urchin spermatozoa. Sperm density: 5×10^9 per ml. ●: Control (Artificial sea water), ○: 10^{-2}M DMDTC, ▲: 10^{-4}M $CuSO_4$, △: 10^{-4}M $ZnSO_4$, ◭: 10^{-4}M$CuSO_4$, +10^{-4}M $ZnSO_4$, ◬: 10^{-4}M $CuSO_4$ +10^{-4}M $ZnSO_4$ + 10^{-2}M DMDTC.

measurement and gradually became lower than DMDTC-respiration. When DMDTC was given with $ZnSO_4$ at 10^{-4} and 10^{-5}M respectively, differences in the effect of $ZnSO_4$, ascribed to the different concentrations, on the DMDTC-respiration were not observed but the rates of respiration became to almost the same level being slightly lower than the rate of DMDTC-respiration. Fig. 5 illustrates the result of the experiment in which 10^{-4}M $CuSO_4$, 10^{-4}M $ZnSO_4$ and 10^{-2}M DMDTC was added together. When Cu^{++} and Zn^{++} were simultaneously administered to spermatozoa, the rate of respiration was nearly equal to that obtained by the single addition of Zn^{++}. When DMDTC was further added to the sperm suspension containing Cu^{++} and Zn^{++}, the effect of DMDTC seems similar to the result of experiment shown in Fig. 4; the respiration rate enhanced by Cu^{++} and Zn^{++} was reduced to the rate some-

what lower than that of DMDTC-respiration.

Discussion

The enhancement of the respiration of sea-urchin spermatozoa in dense suspension, such as in the present experiments, is shown in this paper to occur both by the addition of heavy metal ions and by that of DMDTC. These facts suggest that the respiratory machinery of the spermatozoa involves at least two different components or sites, both concerned with heavy metals; one is activated by the addition of heavy metals and the other is suppressed to be in low activity by the presence of them. Cu^{++} an Zn^{++} may act on the former component and DMDTC may act on the latter one. Cu^{++} and Zu^{++} were found in sea water at 10^{-7}M (BARNES and ROTHSCHILD, 1950) and at 10^{-6}M (KINOSHITA, 1956) respectively and they seem to catalyze the respiration in the natural condition.

When Cu^{++} is employed as an activating agent for the respiration, DMDTC does not interfere with the action of Cu^{++}. When Zn^{++} is employed, the action is influenced by DMDTC. Such a difference in the effect of DMDTC between on the Cu^{++}-respiration and on the Zn^{++}-respiration does not seem to be attributed to the difference between in the degree of affinity of DMDTC to Cu^{++} and to Zn^{++}, for DMDTC combines with Cu^{++} to form complexes more easily than with Zn^{++} (GOKSØYR, 1955). An interpretation for the present results is that the rate-limiting factor in the Cu^{++}-respiration is the component activated by the addition of the metal and that in the Zn^{++}-respiration is the component activated by the removal of metals which is artificially realized by the addition of DMDTC.

There still remains another problem to be settled: which ion, Cu^{++} or Zn^{++}, plays more important roles in the sperm respiration? An answer may be drawn from Fig. 5. When these two ions were given together, the effect of Cu^{++} does not make its appearance and the effect of Zn^{++} only can be seen. This is also the case when DMDTC is given besides these ions. These findings indicates that Zn^{++} may participate more closely than Cu^{++} in the activation of the respiration of sea-urchin spermatozoa suspended in the medium containing both ions such as natural sea water.

Summary

There are two different ways to enhance the respiration of sea-urchin spermatozoa suspended at a high density. One of them is the addition of either Cu^{++} or Zn^{++} and the other is that of dithiocarbamate which is one of the metal chelating agents. Experiments were performed to elucidate the relation between these two apparently conflicting phenomena and the results suggest the existence of at least two components in the respiratory process of the spermatozoa. The important role of Zn^{++} in the sperm respiration is discussed.

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References

BARNES, H. & LORD ROTHSCHILD, 1950. J. Exp. Biol., **27**: 123–125.
GOKSφYR, J., 1955. Physiol. Plant., **8**: 719–835.
KINOSHITA, S., 1956. J. Fac. Sci. Univ. Tokyo (IV), **7**: 497–503.
MURAMATSU, S., 1963 a. Embryologia., **7**: 267–278.
——, 1963 b. Ibid., **7**: 331–343
ROTHSCHILD, LORD & P. H. TUFT, 1950. J. Exp. Biol., **27**: 59–72.
——, 1950. Ibid. **27**: 420–436.
—— & A. TYLER, 1954. Ibid., **31**: 252–259.
——, 1956. Ibid., **33**: 155–173.
TYLER, A., 1950. Biol. Bull., **99**: 324.
—— & E. ATKINSON, 1950. Science, **112**: 783–785.
—— & LORD ROTHSCHILD, 1951. Proc. Soc. Exp. Biol. Med., **76**: 52–58.
—— , 1953. Biol. Bull., **104**: 224–239.
UTIDA, S. and S. NANAO, 1956. J. Fac Sci. Univ. Tokyo (IV), **7**: 505–514.