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Parasynchronous Division of Strain L Mouse Fibroblast Cells Induced by Cooling

by

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INTRODUCTION

In 1958 Newton and Wildy reported that an exposure of the cultures of HeLa cells to a temperature of 4°C for one hour is followed by a stepwise increase in cell number sometime after replacement of the cultures at 37°C, preceded by a rise in mitotic index and an increase in DNA uptake of the cells. They proposed the term "parasynchronous division" to designate the stepwise increase in cell number in the cultures.

Takahashi, one of our collaborators, showed that an exposure of tumor cells growing in mice to a hypothermia at 20°C for six hours results in an increase in thymidine uptake of the tumor tissue followed by a burst of cell division sometime after release from the hypothermia, and that the anticancer agents administered after the hypothermia are more effective in inhibiting the tumor growth as well as in prolonging the survival period of host animals.

On the mechanism of the intensification of anticancer effect by this method, Takahashi supposed that the tumor cells, brought to parasynchronous division after exposure to hypothermia, come in contact with the anticancer agents at the most sensitive phase of their mitotic cycle. In fact, it is observed by Takahashi, Miura and Kato that the effects of anticancer agents administered at an appropriate time after hypothermia are more intensified when the ratio of parasynchronous cell division induced by hypothermia is higher. In view of this, the degree of parasynchronous division might be directly related to the degree of intensification of the anticancer effects.

In the present experiment, using strain L mouse fibroblast cells in suspension culture, the relation between various types of cooling of the cultures and the degree of consequent parasynchronous cell division is studied.

MATERIALS AND METHOD

Strain L mouse fibroblast cells used in this study were obtained from Dr. Higashi’s laboratory in the Institute for Virus Research Kyoto University, in which the cell strain had been cultured in suspension by friction.

Culture vessels: Test tubes (23 cm long, 24 mm in inner diameter) with glass stopper were employed. The inner surface of the tubes was coated with silicon. During incu-
bation, the upper part of the tubes was covered with almofoil sterilized.

Rotation apparatus : The roller tube apparatus was kept in an incubator at a temperature of 37°C. The rotation drum, placed at an angle of 12°, was rotated at 51.5 rpm.

Growth medium : YLE medium contained 5.5 g of lactalbumin hydrolysate, 1.1 g of yeast extract, 1.3 g of NaHCO₃ and 15 mg of phenol red per litter in EARLE's saline solution. Larger amount of lactalbumin hydrolysate and yeast extract by 10 per cent over than usual prevent the cells from destruction during the procedures of friction and cooling. Bovine serum, used throughout the present experiment, was taken from the same pooled sample, stored in a freezer and was inactivated at 57°C for one hour just prior to use. One volume of the bovine serum and nine volume of YLE medium were mixed to make the growth medium of a amount of 1500 cc at a time and was passed through Seitz filter under pressure. Thereafter, the medium was dispensed into several flasks with rubber stopper and stored in cold to be used within two weeks. The flasks containing the growth medium was kept in an incubator for over 12 hours prior to use.

Method of cooling and rewarming : In order to subject the cells to hypothermia, the culture tubes were rotated in water at a designed low temperature. In this way, the temperature of the growth medium in the culture tubes reached a temperature 0.5°C higher than the designed temperature within 5 minutes, and the period of time while the temperature of the medium was in a range of 0.5°C of the designed temperature was designated as the period of cooling. After the cooling, the culture tubes were rewarmed in water at 37°C for 5 minutes within which the temperature of growth medium usually restored to higher than 35°C, and they were kept in an incubator at 37°C.

Counting of cell numbers : From cell suspensions, each 2 ml of samples was withdrawn by a pipette coated with silicon and its cell number was counted by a hemocytometer of Burker-Turk type without staining.

Subculture : Each 30 ml of cell suspensions, containing 20 to 40 x 10⁴ cells per ml in growth medium, was dispensed into several test tubes for culture and incubated at 37°C for two to three days until the cell number reached 80 to 120 x 10⁴ per ml. Then the cell suspension was diluted with growth medium to a density of 20 to 40 x 10⁴ cells per ml and incubated again. The cells used for the experiments of cooling were taken from the successive subcultures of older than one week and were in a state of logarithmic growth.

Cultures used for experiments of cooling : Cell suspension of 80 to 120 x 10⁴ cells per ml derived from the subcultures was diluted with culture medium to a density of 12 to 20 x 10⁴ cells per ml. Each 40 ml of diluted suspension was put into test tube for culture and incubated at 37°C for 20 to 30 hours. After the incubation, the cell density usually reached 25 to 40 x 10⁴ cells per ml and the procedures of cooling were begun. Throughout the experiments, the control cultures were kept at 37°C and the cells continued to be in a state of logarithmic growth.

RESULTS

When the cultures were cooled at a temperature under 20°C, the cell number did not change during the period of cooling. After rewarming the cultures at 37°C, the cell number changed in various ways according to the conditions of cooling.

1. Cooling at 4°C
At this temperature, cooling was performed for 1/4; 1/2; 1 or 2 hours. The growth curves obtained by counting cell numbers are shown in Fig. 1 and 2.

a) Cooling for 1/4 hour (Fig. 1)

The growth curve suggests that little number of cells divided within 2 hours after return to 37°C. During the period from the 2nd to 5th hour after return to 37°C, the cell number increased and the inclination of growth curve at this time seems to be a little steeper than that of the control culture. During the period from the 5th to 7th hour after rewarming, the cell number stayed almost unchanged. Then an increase in cell number was noted during the period from the 7th to 12th hour and at the 12th hour the cell number reached nearly equal to that of the control. Thereafter, the cell number kept unchanged until the 15th hour after rewarming. Under this condition, the cell number of the cultures increased in a fashion of two steps in 15 hour after return to 37°C.

b) Cooling for 1/2 hour (Fig. 2)

Until the 5th hour after return to 37°C, the cell number was almost unchanged. During the period from the 5th to 13th hour the increase in cell number was rather marked and at the 13th hour the cell number became larger than that of the control. During the period from the 13th to 19th hour, the increase in cell number was rather lower than that of the control and the number of the cells fell to under the control level. Under this condition, the cell number increased in a one step fashion.
c) Cooling for one hour (Fig. 2)

Following a slow increase in cell number within 8 hours after rewarming at 37°C, a rapid increase in cell number occurred during the period from the 8th to 12th hour and the cell number reached over the control level. After the 12th hour, the rate of increase in cell number reduced and the actual cell number fell to under the control level.

d) Cooling for 2 hours (Fig. 2)

The results were almost the same as that obtained by the cooling for one hour.

2. Cooling at 10°C

Cooling for 2, 4 or 6 hours was performed at 10°C and the results are shown in Fig. 3.

a) Cooling for 2 hours

A slow increase in cell number was observed during the period from the first to 8th hour after return to 37°C. During the period from the 8th to 12th hour after rewarming, the increase in cell number was more rapid than that of the control, and at the 12th hour the cell number reached over the control level. After then the cell number increased slowly and temporarily fell to under the control level, followed by a gradual increase.

b) Cooling for 4 hours

Until the 6th hour after return to 37°C little change in cell number was observed. During the period from the 6th to 12th hour a rapid increase in cell number was obtained and at the 12th hour the cell number reached over the control level. During the period from the 12th to 20th hour the cell number increased very slowly and fell to under the control level.

c) Cooling for 6 hours

The results were nearly the same as that obtained by the cooling for 4 hours.

3. Cooling at 16-17°C

Cooling for 2, 4, 6 or 24 hours was made at a temperature of 16-17°C and the growth curves obtained are shown in Fig. 4, 5 and 14-a.

a) Cooling for 2, 4 or 6 hours at 16°C (Fig. 4)

In each case a slow increase in cell number was obtained for a short time after return to 37°C. Until the 6th to 9th hour the increase was slower than that of the control. After then the cell number increased more rapidly than that of the control and at the 12th hour exceeded the control level. Between the 12th and 20th hour the increase in cell
number slowed down and the cell number fell to under the control level. These patterns of increase in cell number were more marked when the cooling was made for a longer period.

b) Cooling for 2 hours at 17°C (Fig. 14-a)

Under the condition of cooling at 17°C for 2 hours, the cell number increased in a fashion of two steps as in the case of cooling at 4°C for 1/4 hour.

c) Cooling for 6 or 24 hours at 17°C (Fig. 5)

Cooling for 6 or 24 hours at 17°C gave almost the same results as those of the cooling for 6 hours at 16°C, but after the cooling for 24 hours the rate of increase in cell number was less than that obtained by the cooling for 6 hours. It is supposed that some of the cells were destroyed during the procedures of rotation and cooling.

As described above, in each experiment of cooling at the temperature of 4, 10 or 16-17°C the cell number did not increase during the period of cooling and in some case only a little increase in cell number occurred immediately after return to 37°C. Thereafter,
the cell number continued to be almost the same for a little while, then increased rapidly and finally exceeded the control level. Then the increase in cell number slowed down and the cell number fell to under the control level. The pattern of growth curve shows that the stepwise increase in cell number was more marked when the cells were cooled for a longer period or at a lower temperature. Cooling for over one hour at 4°C, for over 4 hours at 10°C or for over 6 hours at 16-17°C, so far as our experimental results were concerned, did not make a significantly characteristic increase in cell number. Cooling at 16°C for 6 hours and cooling at 4°C for one hour showed almost the similar growth curve to each other as shown in Fig. 6.

4. Cooling at 20°C

Cooling for 1, 7 or 31 hours was performed at 20°C and the results are shown in Fig. 7 and 8.

a) Cooling for 1 or 7 hours (Fig. 7)

The cell number increased by 10 per cent immediately after rewarming at 37°C and thereafter the rate of increase in cell number was kept to be the same as that of the control.

b) Cooling for 31 hours (Fig. 8)

For a short period after return to 37°C an increase in cell number was hardly observed, but thereafter the growth curve became almost the same as that of the control.

Fig. 6 Comparison of cooling at 4°C for one hour and at 16°C for 6 hours

Fig. 7 Cooling at 20°C for 1 or 7 hours
5. Cooling at 25°C

Cooling for 10 or 21 hours was made at 25°C and the results are shown in Fig. 9 and 10. In each case an increase in cell number occurred even during the period of cooling and the growth curve after return to 37°C was almost the same as that of the control.

In brief, when the cultures were cooled at 20 or 25°C, stepwise increase in cell number was not obtained after return to 37°C.

6. Repeated cooling at 4°C for one hour at an interval varying from one to 13 hours

To investigate the effect of repeated cooling, the cells cooled at 4°C for one hour were incubated at 37°C for a period of 13 hours and thereafter cooled again at 4°C for one hour. The results are shown in Fig. 11–13.

a) Control (Fig. 11-a, 12-d and 13-f)

Cultures which were cooled once at 4°C for one hour was examined as control. Their growth curves showed that the increase in cell number was almost next to none until the 7th hour after return to 37°C: the period being proposed as "the first lag
The increase in cell number was marked for the period between the 7th and the 12th hour after rewarming at 37°C; the period being proposed as "the period of rapid increase in cell number". Thereafter, the increase in cell number was little for several hours; this period being proposed as "the second lag period". At the end of the

Fig. 10 Cooling at 25°C for 21 hours

Fig. 11 Repeated cooling at an interval of 1 or 3 hours

Fig. 12 Repeated cooling at an interval of 5 hours
period of rapid increase in cell number, the cell number increased by 60 per cent when compared with that before cooling.

b) Repeated cooling at an interval of less than 7 hours (Fig. 11-b; c, 12-e and 13-g)
When the cells were cooled again at the first, third, 5th or 6th hour after return to 37°C, that is, in the first lag period following the first cooling, the only effect of the second cooling was delay of the increase in cell number by about one hour. The repetition of cooling did not intensify the degree of parasynchronous cell division.

c) Repeated cooling at an interval of 8 or 9 hours (Fig. 13-h and i)
When the cells were cooled again at the 8th or 9th hour after return to 37°C, that is, in the middle of the period of rapid increase in cell number following the first cooling, the rate of increase in cell number was less than that of the control.

d) Repeated cooling at an interval of 10 or 12 hours (Fig. 13-j and k)
When the cells were recooled at the 10th hour after the first rewarming, that is, at a terminal period of the rapid increase in cell number following the first cooling, the cell number did not increase for one hour after the second rewarming. For the next two hours the cell number increased markedly and reached to the same level of the control. Thereafter, the increase in cell number was delayed only by one hour when compared with that of the control.

The period rapid increase in cell number following the first cooling ended at the 12th hour after the first rewarming at 37°C. When the cells were recooled at the end of the period of rapid increase in cell number following the first cooling, slight increase in cell number occurred after return to 37°C.

7. Repeated cooling at 17°C for 2 and 4 hours at an interval of 2, 4 or 6 hours
The results described above showed that the cooling at 17°C for 6 hours effectively induced parasynchronous cell division. In this experiment, the cooling period for 6 hours was interposed with an interval of incubation at 37°C for a varying period of time; after the first cooling at 17°C for 2 hours the
cells were cooled again at 17°C for 4 hours at an interval of 2, 4 or 6 hours. The results were shown in Fig. 11. The cells cooled once at 17°C for 2 hours served as the control (Fig. 14-a). In the control culture, the cell number increased until the 4th hour after return to 37°C, then stayed at the same level for 3 hours. Thereafter, the cell number increased again.

When the second cooling was made at an interval of 2 hours (Fig. 14-b), the growth curve after the second cooling became similar to that obtained by the cooling at 17°C for 6 hours. The growth curve showed a characteristic pattern of the stepwise increase in cell number, although not so marked.

When the interval was 1 hour, the growth curve after the second cooling did not show any pattern of stepwise increase in cell number (Fig. 14-c).

When the interval was 6 hours, the cell number increased markedly even during the period of second cooling and following the second rewarming increased in the same way as that of the control (Fig. 14-d).

8. Cooling after incubation at 30°C, 34°C or 38°C

To investigate the influence of incubating temperature before cooling on the degree of parasynchronous cell division induced by cooling, incubation of the cells at 30°C for 10 hours, at 34°C for 30 hours or 38°C for 24 hours was performed. The cells incubated at a lower or higher temperature than the

![Fig. 14 Repeated cooling at 17°C for 2 and 4 hours at an interval of 2, 4 or 6 hours](image-url)
Asynchronous division of mouse fibroblast in cooling

Fig. 16 Cooling at 17°C for 8 hours after incubation at 34°C

Fig. 17 Cooling at 17°C for 8 hours after incubation at 38°C

Fig. 18 Cooling at 4°C for one hour during incubation at 37.5°C

standard of 37°C were cooled for 14 hours at 16°C or for 8 hours at 17°C. The results are shown in Fig. 15-17.

When the incubation before cooling was done at a lower temperature such as 30°C or 34°C, a stepwise increase in cell number was observed after return to incubation at 34°C, 37°C or 38°C (Fig. 15 and 16). By contrast, when the incubation before cooling was done at 38°C a stepwise increase did not occurred after rewarming (Fig. 17).

9. Cooling at 4°C for one hour during incubation at 37.5°C

The cells incubated at 37.5°C were cooled at 4°C for one hour and then rewarmed at the same temperature of 37.5°C. As shown in Fig. 18, the period of rapid increase in cell number appeared earlier and the doubling time estimated to be 13 hours. An apparent pattern of stepwise increase in cell number was shown. The rate of the increased cell number within one step of growth curve was about 85 per cent. At least, two series of stepwise increase in cell number were observed.
before the counting of cell number was stopped at the 22nd hour after rewarming. The number of the cells at the 22nd hour after rewarming was three times that of before cooling.

DISCUSSION

Newton and Miura investigated the effect of chilling on mitosis of the tissue culture cells in logarithmic growth, but their results were not entirely the same as ours. Newton and Wildy cooled HeLa cells at 4°C for one hour and found that the cells did not divide for over a period of 20 hours following return to 37°C and then bursted into division within next two hours with an increase in cell number by about 60-80 per cent. On the other hand, it was reported by Miura and Utakoji that FL cells after being chilled at 4°C for one hour decreased in number and three hours after the chilling the cell number began to increase, the growth curve during this period being almost parallel to that of the control. In about 12 to 18 hours thereafter growth stopped and the cell number remained almost unchanged for another 12 to 18 hours. One generation time after chilling the number of the cells increased rapidly and reached almost the same level of the control, the cell number at this point of time being doubled when compared with that before chilling.

In the present experiment, a slight increase in cell number was observed immediately after return to 37°C which was followed by a decrease in cell number in some case. It is supposed that the initial slight increase in cell number was caused by mitosis of the cells which had entered in the phase of cell division at the time of beginning of cooling, and that the cells entered in the phase of division might tolerate cooling so far as mitotic process is concerned but might be apt to degenerate after finishing cell division.

In the present experiment, the doubling time of the cell culture, namely the time required to double the number of cells, was 14 to 21 hours. On the other hand, the period of time from the beginning of rewarming to the end of the rapid increase in cell number was 13 to 15 hours and the largest proportion of the cells increased during this period was enumerated to 85 per cent. Moreover, after the next burst of cell division the cell number exceeded twice as much as that just after return to 37°C. The generation time of strain L mouse fibroblast cells was estimated at a period of time varying from 13 to 15 hours and a stepwise increase in cell number occurred during a period corresponding to a generation time. Thus it is supposed that all the cells which were able to enter mitosis after cooling had finished mitotic process by the end of the period of rapid increase in cell number. This supposition agrees with that of Newton but not of Miura who observed two series of stepwise increase in cell number before doubling of the cell number. In the present experiment cooling of strain L mouse fibroblast cells under such a condition as 1/4 or 1/2 hour at 4°C induced two series of stepwise increase in cell number. When the cells were cooled for a longer period of time such as more than one hour at 4°C, one series of marked stepwise increase in cell number was obtained. Therefore it can be said that the condition of cooling carried out by Miura was not satisfactory to induce a higher degree of parasynchronous division of FL cells. In general, so far as our experimental methods with strain L mouse fibroblast cells are concerned, the most adequate method to obtain parasynchronous cell division was cooling at 4°C for one
The results of the present experiment were almost the same as those of Newton, excepting the durations of the first lag period and of the period of rapid increase in cell number. Some of these discrepancies might be due to the difference of the cell strain used, culture medium or the incubation temperature.

On the mechanism of parasynchronous cell division induced by chilling, Newton supposed that chilling might place all the cells, which are not actually in mitosis, in a state corresponding to that of the end of telophase. Some mechanism which places the cells in such "pseudotelophase state" could result in a parasynchronous form of growth after return to 37°C.

In the present experiment, the rate of cell increase which was lower than that of the control during the first lag period became higher in the period of rapid increase in cell number. It is supposed that some of the cells which had been cultured at 37°C were put in a "younger state" by cooling and were delayed to enter into mitosis and divided later in an early period of the rapid increase in cell number.

In a later period of rapid increase in cell number, the cells which had been cooled increased in number more rapidly than the control cells and resulted in a larger number of the cells than the control. In the next several hours, that is in the second lag period, the number of the cooled cells did not increase while the number of the control cells continued to increase. Thus the number of the cells in cooled culture finally became less than that of the control. It is supposed that some of the cells which had been cultured at 37°C were put in an "older state" by cooling and divided earlier than the control in the late period of the rapid increase in cell number. Possible existence of these cells was not pointed out either by Newton nor by Miura. Fig 19 indicates that the time of the first lag period (A-B) is 7 hours, the period of rapid increase in cell number (B-C) is 6 hours and the time from the beginning of the second lag period to the time when the cell number of the treated culture becomes equal to that of the control (C D) is 3 hours. Generation time of the cells is estimated at 16 hours. Thus most cells which were not actually in mitosis when cooled might be put in a state 7-13 hours younger than the time of their last division and few cells were put in a state corresponding to the end of telophase.

In the present experiment, intensification of the degree of parasynchronous cell division was not obtained by repetition of the cooling at 4°C for one hour. When the second cooling was made within six hours after the end of first cooling, the increase in cell number
was delayed by one hour and the pattern of the growth curve was the same as that of
the control. This fact indicates that the cells which had been put either in the younger
or the older state by the first cooling were not affected by the second cooling. At the
period of the 8–9th hour after return to 37°C, most cells which had been put in the
younger state by the first cooling would finish mitosis. And it is interesting that the
second cooling performed at this period decreased the ratio of the increase in cell number
which could be observed following the first cooling. This indicates that the cells which
had been put in the older state by the first cooling and would enter into mitosis in the
late period of rapid increase in cell number were not affected when the second cooling was
made before the 6th hour following the first rewarming. In contrast, when they were
cooled at the 8–9th hour, they were put in the younger state and the time of their divi-
sion was delayed. When the second cooling was made at the 10th hour, that is, at a
terminal period of the rapid increase in cell number following the first cooling, the growth
was not affected for several hours after return to 37°C. This fact supports the present
author's supposition that the mitotic process of the cells which are dividing or about to
divide is not affected by cooling.

According to the influence of cooling, the cells in various stage of mitotic cycle might
be classified as follows. The cells which are actually in mitosis or about to enter mit-
osis stay at the same stage during cooling and proceed with their mitotic process after
rewarming but degenerate sometimes after finishing division.

The cells in interphase, although they were thought to be put “in a state correspon-
ding to that at the end of telophase” by NEWTON 1, might be affected in various ways
as follows.

A ; The cells placed in the “younger state” by cooling : These cells divide in the early
period of rapid increase in cell number after return to 37°C and are not affected by the
second cooling until they have finished the next division.

B ; The cells placed in the “older state” by cooling : These cells divide in the late
period of rapid increase in cell number. When the second cooling is performed in
several hours after the first rewarming, the cells are not affected. When the second
cooling is performed before they have entered in next division, they are put in the
“younger state” by the cooling.

C : The other cells which are not affected by cooling : These cells might be in a
state between those of the two groups described above and might be put in the “younger
state” later by the cooling.

Recently hypothermia has been widely used in heart and brain surgery 41,53, and even
such a low degree of temperature as below 10°C can be applied by use of an extracor-
poreal circulation system. In spite of this, before such a deep hypothermia as examined
in the present experiment is applied clinically to intensify the effects of anticancer agents,
more studies should be done on the method of hypothermia which can induce a higher
degree of synchronous mitosis with more safety and easy.

**SUMMARY**

Using the suspension culture of strain L mouse fibroblast cells in logarithmic growth,
parasynchronous cell division induced by various conditions of cooling is studied.
PARASYNCHRONOUS DIVISION OF MOUSE FIBROBLAST IN COOLING

1) The cells increase in number during cooling at a temperature of 30°C or 25°C and the growth curve after return to 37°C is logarithmic.

2) The increase in cell number stops during cooling at a temperature of 20°C but the growth curve after return to 37°C is logarithmic.

3) During cooling at a temperature of 4°C, 10°C or 16–17°C, the cells do not increase in number and parasynchronous division of the cells occurs after return to 37°C. When the period of cooling is longer or the temperature of cooling is lower, the following cell division is more synchronous. Cooling for one or two hours at 1°C, for 4 or 6 hours at 10°C or for 6 or 24 hours at 16–17°C, however, gives almost the similar degree of parasynchronous cell division with one another.

4) Repeated cooling at 4°C for one hour is examined but it does not intensify the degree of parasynchronous cell division.

5) When the second cooling for 4 hours is made after the first cooling for 2 hours at 16°C, the inclination of growth curve does not become steeper than that obtained after cooling once at 16°C for 6 hours.

6) When the incubation before cooling is made at a temperature higher than usual (37.5°C or 38°C), the rate of parasynchronous cell division observed after cooling is rather lower.

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REFERENCES

冷却により誘起される
L 株細胞の同調分裂に関する研究

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真鍋 拓

低体温度利用して制御する効果を高める方法については、既に高橋等の報告があり、それによると制御動物を20℃以下に一定時間冷却した後も正常体温度に復温し、その後適当な時間に制御剤を投与することが必要であり、しかる低体温度によって復温後に起こる細胞の同調分裂の程度が高かったことから、ための効果が得られるであろうとのことである。そこで著者は低体温度によって誘起される同調分裂の程度を高める方法について検討するため、in vitro で L 株細胞を用いて実験を行ない、複数の条件によってもたらされる同調分裂の程度を調べた。

25℃或いは30℃で冷却を行なった場合には、冷却直後に細胞増加がみられ、しかる37℃に復温後にも細胞増加に同調は認められなかった。

20℃で冷却すると、冷却中の細胞増加は停止するが、復温後の細胞増加には依然として同調が認められなかった。

17℃、10℃或いは4℃で冷却すると、冷却中の細胞増加が停止した後、37℃に復温後、細胞増加に同調化がみられる。この同調化の程度は冷却温度を低くするか又は冷却時間を長くするか高くなる傾向があるが、4℃では1時間、10℃では4時間、17℃では6時間の

冷却で同調化の程度は最高となり、これ以上の長時間冷却を行なつても同調分裂の程度はそれ以上には高くならない。なお16℃6時間冷却と1℃1時間冷却の効果を比較してみたが、両者による同調化の程度はほぼ同じであった。

総合し冷却を行う事による効果を検討するために、
1℃1時間の冷却を1～13時間の間隔を置いて反復してみたが、同調分裂の程度は高め得なかった。又16℃6時間の冷却を分割し、16℃2時間冷却後復温して2、4、或いは6時間培養の後再度16℃4時間の冷却を行ないつつ行なってみたが良結果は得られなかった。

次に冷却前の培養温度を変えて、34℃、37℃、37.5℃或は38℃で培養後冷却を行なったところ、37.5℃、38℃の比較的高い温度で培養した後に冷却すると同調化の程度が低くなる傾向があった。

以上の実験成績と臨床的低温温度を利用する際の難易を考慮に入ると、冷却によって同調化を得るには17℃で6時間の冷却を1回行なうのが最もよい方法とみられる。脾細胞治療の目的で低温温度を応用するために

は、脾細胞の性質、或いは冷却の方法等について更に検討する必要がある。