HYDROSTATIC PRESSURE EFFECTS ON MUSCLE
IN THE TADPOLE TAIL

BY

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ABSTRACT

It is very important to know the critical pressure which causes a living body to bring about histological damage or destructive metabolism when the hyperbaric effect is studied. Therefore, a histological examination was made on hydrostatic pressure effect on the muscle in the tadpole tail of the bullfrog (Rana catesbiana) by using an animal chamber which is able to compress a liquid from 1 to 500 atmospheric absolute (ATA). More than 200 tadpoles were compressed at the different depth of pressure between 7 and 500 ATA.

The results of the experiment may be summarized as follows: (1) The interstices between the muscular fibers are found at above 50 ATA for 10 minutes, and at 500 ATA for 30 seconds. (2) Stiffness is seen at 500 ATA for 60 seconds or over. (3) The "rounding fibers" are often found by exposure to 100 ATA for 10 minutes, and more often found at over 300 ATA. (4) The macroscopic stiffness is due to the histological "rounding fibers". (5) The damage to the muscle is caused more by decompression than by the compression effect.

INTRODUCTION

One of the main researches for underwater medicine is to find how to analyze the effect on a living body in hyperbaric status. Since Holdane's report1,2 was published in 1908, great efforts have been made to find what kind of decompression method is the best after diving, which means to find a good method of preventing the decompression sickness.

After putting the saturation diving effect to practical use, we have been able to stay at the sea-bottom for a long term. Studies on human life under a hyperbaric status have consequently been prosecuted, and on physiological adaptation, analysis of stress and so on.3-12 These researches are, however, mainly due to the effect-analyses of inert gases, for example, nitrogen or helium which are contained in the solution by breathing, and there are still many problems to be solved. When we conduct experiments on the effect of hyperbaric exposure, we should give special consideration to the fact that

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the influence of inert gas on an aquatic animal as a subject will be less than on a land animal, and that the higher pressure conditions required in the laboratories can be got more easily by using water pressure than gas pressure. Therefore, a tadpole was chosen as an experimental animal and histological examination was made on hydrostatic pressure effect on the muscle in the tadpole tail.

On the other hand, researches on hydrostatic pressure effect have been prosecuted in the fields of microbiology and biochemistry\textsuperscript{18}. However, there are very few histological data in fields. Although Fenn\textsuperscript{14} introduced the reports concerned with the hydrostatic pressure effect on the living body, detailed reports on researches in histological analyses have not been published, not to mention researches on a tadpole muscle.

It is an urgent necessity in the field of labor sanitation to know how to work safely under a hyperbaric status and how to prevent the decompression sickness\textsuperscript{15–17}, and it is also necessary to know about the basic hyperbaric pressure effect on a living body. No detailed discussions have been had on these problems in this field of underwater medicine. Such problems as bubble formation\textsuperscript{18–21} or aseptic bone necrosis\textsuperscript{22–26} have been studied mainly in relation to the hyperbaric pressure effect on a living body. However, histological destruction of a living body is expected to occur if the subject is exposed to hyperbaric status, and the present experiments on hydrostatic pressure effect were conducted.

**Material and Method**

To investigate the hydrostatic pressure effect, an animal chamber, in which a necessary pressure can be produced, is required. We manufactured an animal chamber in which the pressure can be controlled from 1 to 500 ATA. This chamber has two types of observation windows, through which the inside of the chamber can be seen, and has a medical electric system to record the data from the subject and the temperature of the liquid by using a multipurpose polygraph (Figs. 1 and 2).

A tadpole of the bullfrog (*Rana catesbiana*) was chosen as the subject, because a tadpole is one of the aquatic animals. As a tadpole grows into a frog, it is considered very convenient to use a tadpole for comparing the branchial respiration animal with the pulmonary respiration animal. Moreover, a tadpole of a bullfrog is easy to be obtained at any time of the year and it is more suitable for this experiment than any other kind of tadpoles.

The muscle in the tadpole tail begins to become atrophied at the stage of the metamorphosis of tadpoles into frogs, therefore the subjects must be chosen and exposed before the tail muscle becomes atrophied\textsuperscript{27}. 
Fig. 1. 500 ATA animal chamber

Fig. 2. Schema of animal chamber
1) Inside of animal chamber
2) Liquid tank
3) Multipurpose polygraph
4) Pressure gauge
5) Upper lid
6) Annex for fixation
7) Observation window
8) Window for source of light
9) Penetration cords to polygraph
10) Liquid supply pipe
11) Drainage pipe
12) 0 ring
13) Manual pump for compression
The muscle atrophy in the tadpole tail is generally considered to begin at the stage when the forelimbs appear. Therefore, the subjects must be chosen from among tadpoles at the stage II to XII in conformity with the classification of Taylor and Kollros\textsuperscript{28}. They say that the forelimbs appear at the stage XVIII. However, a tail muscle atrophy has been observed distinctly in some of the tadpoles at the stage XV during the experiments. Therefore, the tadpoles used as subjects are to be those whose forelimbs do not appear, whose hind legs are to be from 0.5 to 20 mm long, and whose bodies are to be from 65 to 95 mm in length. These tadpoles are exposed to high pressure if no abnormality is seen macroscopically. The experiments lasted for more than two years. The total number of the subjects of the exposure group was 204, that of the control group 51, the subjects being chosen from among more than 600 animals. The water temperature was set 20.0°C in the summer and 15.0°C in the winter. The same phylogenetic stage was required between the control group and the exposed group in each experiment. The experimental group was exposed to high pressure of between 7 and 500 ATA and exposure time was from 30 seconds to 120 minutes. After a few seconds of decompression, most of the subjects were fixed or frozen within 5 minutes and others were kept at normal laboratory temperature to investigate the effect of decompression during the period from 2 hours to 10 days.

Subjects were treated with such fluids as formalin, Bouin\textsuperscript{29} or Susa\textsuperscript{29} (Bouin=picric acid: formalin: glacial acetic acid=15 : 5 : 1, Susa=corrosive sublimate : 5%, trichloro acetic acid : formalin=5 : 4 : 3), and paraffin. These were observed microscopically after being stained by such methods as hematoxylin-eosin (HE) or Mallory’s stain.

On the other hand, some of the subjects were frozen instantaneously to −20.0°C. after decompression to investigate histochemically by acetone dry ice method and cut up into 6 μ by a criostat. These were stained histochemically by 8 kinds of methods\textsuperscript{80}: trichrome (TC), hematoxylin-eosin (HE), periodic acid Schiff's leuco fuchsin (PAS), reduced dephosphopyridine nucleotide (DPNH), succinic dehydrogenase (SDH), phosphorylase (PhR), adenosine triphosphatase (ATP), and cholinesterase (ChE). These are very useful methods to know what kinds of hydrostatic pressure effects on the muscle in the tadpole tail are recognized histochemically through the muscle structure, mitochondria, glycogen and so on.

These methods will be utilized histologically in future, even if these are new techniques and some of the biochemists are sceptic about studying the enzyme activity by these histochemical methods.
RESULTS AND DISCUSSION

The muscle in the tadpole tail is called "myotome", but it is a striated muscle and is functionally a voluntary muscle.

There is a "notochord" in the center of the tail and this is surrounded by a connective tissue. The muscle is present between the notochord and skin\(^{31-32}\).

The thickness of the muscular fiber is considerably different between the parts of the central and subcutaneous muscle (Figs. 3–6). Watanabe\(^{33}\) points out that the size of the subcutaneous muscle is small in diameter, it has a large quantity of mitochondria and the activity of succino-dehydrogenase is strong, but the muscle of the deeper strata is larger in diameter than the subcutaneous one, the quantity of mitochondria is small and the activity of succino-dehydrogenase is weak.

The skeletal muscle is generally classified into two kinds of fibers, morphologically and functionally. Type I fiber (red muscle) is rich in mitochondria and lipid, has strong activity of desmolase, and functionally controls the tonus and postural adjustment. Type II fiber (white muscle) is rich in polysaccharide, and controls the phasic and kinetic physiological functions. Moreover, there is the type III fiber in some animals which is considered to mediate between the type I and type II fibers, and the enzyme activity of the type III fiber is also intermediate\(^{30}\).

Watanabe\(^{34}\) has already discovered two types of muscles in the tadpole of *B. regularis*, which are type I and II, and has described that the red muscle (type I) is fine in diameter at the cross section and is of rich density, and the growth of the muscular vesicle is bad, but both mitochondria and lipid are rich, and the nuclei are generally present in the center of the cell. In the white muscle (type II), the muscular vesicle is grown well and surrounds the muscular fiber.

1) However, the type III fiber was found through the experiments on the muscle in the tadpole tail of the bullfrog (*Rana catesbiana*) by histochemical methods. Type I fiber is the smallest one, stain badly, and the activity is weak. Type II is the biggest and stains well, and moreover there is the type III which is an intermediate one, not only in size but in the activity according to the histochemical method of adenosine triphosphatase (ATP) (Fig. 7). These three kinds of fibers are reversely stained when such methods as reduced diprophosphopyridine nucleotide (DPNH) or phosphoryrase (PhR) are used (Fig. 8). This is also observed by periodic acid, Schiff's leuco fuchsin (PAS) stain (Fig. 9). The mitochondria in the muscle is clearly observed by the trichrome (TC) stain (Fig. 10). The general observations on the muscle
of the control group are shown by from Fig. 3 to 10.

2) Generally decompression sickness has been considered due to the bubbles which are formed from the surplus gas in the solution by unsuitable decompression after diving, but a tadpole is not a pulmonate animal, and considering that the gas solution is very little. A tadpole is not liable to take decompression sickness. An individual difference has been recognized in the sensibility to nitrogen among men and it is well known that some persons often have decompression sickness, while others do not even if the conditions of exposure to high pressure are the same. There is also an individual difference in the decompression effect among the tadpoles. However, no abnormality was found in all the subjects exposed to high pressure of less than 20 ATA, and no abnormality was found histochemically for 10 days after decompression, either. It is difficult to find the decompression effect in the tadpole under the conditions of within 20 ATA exposure for 10 minutes.

3) However, there were interstices between the muscular fibers which are considered to be formed in the subjects by decompression when exposed to 50 ATA for 10 minutes and treated 180 minutes after decompression. These interstices are evidently different from such ones in the control group made by histological fixation. These interstices have also been found in the subjects exposed to 500 ATA for only 30 seconds and treated from 120 to 180 minutes after decompression. All subjects are alive after decompression from 500 ATA, lying down for first 5 minutes, beginning to swim 12 minutes after decompression, and the macroscopical stiffness is not seen. However, the muscular damage is strong and the interstices are clearly seen (Fig. 11).

4) It is very important to know that stiffness is seen generally in the subjects in which the muscular fibers degenerate by pressure. However, any stiffness was not found in the subjects exposed to 50 ATA for 10 minutes or 500 ATA for 30 seconds (Table 1). The tail muscle is very tender. But the large interstices and the muscular damage are recognized histologically. The muscular fiber, nucleus and mitochondria became thin and activity was lowered when it was observed histochemically (Figs. 12 and 13).

5) However, the interstices have rarely been found in the subjects exposed to 500 ATA for 10 minutes if they are fixed or frozen within 3 minutes after decompression, and this will prove that the interstices are due to decompression. Therefore, subjects were tried to be fixed or frozen within 3 minutes after decompression when the hydrostatic pressure effect was observed (Table 1).

6) Subjects have been exposed to 500 ATA for from 60 seconds to 120 minutes. Stiffness was seen by 60-second exposure but never by 30-second exposure. The degree of stiffness was proportional to the genetical stage and sometimes hypodermal bleeding was seen in the abdominal region or tail fin.
The degree of stiffness increased by 3-minute exposure and apnea was seen for a few minutes. The longer the exposure time was, the greater was the degree. However, the tadpole showed a stronger resistance to exposure than the surface fish.

7) Muscular fibers were found histologically in the tail muscle at the cross section, which were compressed, degenerated and became round by hydrostatic pressure. The frequency of the occurrence was proportional to the macroscopical degree of stiffness and it was recognized that this muscular fiber degeneration could be found more widely if the subject was exposed to a higher pressure for a longer time. Therefore, this degeneration is presumed to be the hydrostatic pressure effect (Figs. 14 and 15).

8) On the other hand, there were seen crookedness or flexion and disordered arrangements of the muscular fibers in the stiffened subjects in the longitudinal section, which were found slightly in the control group. This variation will also be considered as pressure effect (Fig. 16).

These changes in the muscular fibers (Figs. 14 to 16) are sometimes recognized in such subjects in which the stiffness is not found although the frequency of the occurrence considerably decreases (Table 1). The muscular fiber degeneration changing from the normal shape to the round shape by pressure was also found in some of the subjects exposed to 100 ATA for 10 minutes even if the frequency is low, and the occurrence is due to the individual difference (Fig. 14). This muscular fiber which became round in shape will be called a “rounding fiber” in this paper hereafter.

It has already been mentioned that the frequency of the occurrence of the “rounding fiber” will increase if the conditions of exposure are severe, and the frequency will increase considerably at the pressure exposure of more than 300 ATA. It seems that the stiffness is due to the “rounding fiber”.

However, some risk will be involved in connecting the “rounding fiber” to the flexion of the longitudinal section, because the substance of the “rounding fiber” is transferred to the spoiled structure and appears as if it is uniformalized, but it is difficult to find the spoiled structure of the muscular fiber at the flexion. The size of the “rounding fiber” becomes larger when the conditions of exposure are severe. This fact is easily recognized by comparing Fig. 14 with Fig. 18. However, this interrelation will be investigated in the future work.

9) There were also found histochemical changes in the subjects in which “rounding fibers” were seen. Many “rounding fibers” were recognized at the condition of 500 ATA for 10 minutes, in which every tadpole became stiff (Fig. 18). Nevertheless, mitochondria in this subject meets less destruction than in the subject exposed to only 50 ATA for 10 minutes and frozen 180 minutes after decompression (Figs. 12 and 19). This fact will suggest that
the damage to mitochondria is due more to the decompression effect than to the compression one.

10) Moreover, the destruction of the muscle is so distinct in the subjects exposed to only 50 ATA for 10 minutes, and treated 180 minutes after decompression. However, the destruction of that is not so clearly seen in the subjects exposed to 500 ATA for 10 minutes and frozen immediately after decompression. And the subjects frozen immediately became stiff within a few minutes after the compression began in the animal chamber and many “rounding fibers” were recognized histologically. It is obvious that the destruction and damage of muscle will be caused not by the compression but by the decompression effect, and the “rounding fiber” will have problems when the conditions of pressure are severe.

No histological reports have been found regarding the hydrostatic pressure effects and it is important to realize that the interrelation between the macroscopical muscular stiffness and the histological muscular degeneration has been clear, although it has generally been recognized that the macroscopical stiffness in the surface fish is seen at a high pressure exposure.

CONCLUSIONS

The results of the present experiment may be summarized as follows: (The details of methods of exposure and the results are shown in Table 1).

1) Type III fiber was found in the muscle in the tadpole tail of the bullfrog (Rana catesbiana) by histochemical methods.

2) Abnormality was not found at under 20 ATA for 10 minutes.

3) The interstices between the muscular fibers were found at above 50 ATA for 10 minutes and at 500 ATA for 30 seconds.

4) The subjects exposed to 500 ATA for 10 minutes were alive, even if they lay down for a few minutes, and began to swim 12 minutes after decompression.

5) Stiffness was seen at 500 ATA for 60 seconds or over.

6) The “rounding fibers” were often found at 100 ATA for 10 minutes and more often at over 300 ATA.

7) There was the interrelation between the stiffness and the “rounding fiber”.

8) The damage to the muscle was caused more by the decompression than the compression effect. However, the “rounding fiber” had problems when the conditions of pressure were severe.

9) However, it was difficult to connect the decompression effect with the bubble formation theory. This shall be followed in the future work.
### HYDROSTATIC PRESSURE EFFECTS ON MUSCLE

#### Table 1. Methods of exposures and results

<table>
<thead>
<tr>
<th>Pressure (ATA)</th>
<th>Exposure time</th>
<th>Time after decompression</th>
<th>Number of subjects</th>
<th>Number of decompre. effects(1)</th>
<th>Number of compre. effects(2)</th>
<th>Number of stiffness</th>
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<td>3*</td>
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<tr>
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<tr>
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<td>2</td>
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<td>2*</td>
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<td><strong>115</strong></td>
<td><strong>57</strong></td>
<td><strong>46</strong></td>
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**Note:**

1) "Decompre. effects" means the interstices and the muscular damages without the "rounding fibers".  
2) "Compre. effects" means "rounding fibers".  
3) It is difficult to observe the "rounding fibers" on the subjects which are not treated immediately, because the muscular fibers have been badly destroyed. (*)

### ACKNOWLEDGEMENT

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of the Department of Neurology, Toranomon Hospital, for their kind advice and technical assistance in this study.

REFERENCES


EXPLANATION OF FIGURES

Plate 1

This plate from Fig. 3 to Fig. 6 shows the normal tail muscle histologically as the control.

Fig. 5 and Fig. 4. These are the cross sections of the tadpole tail. Notochord is present in the center of the tail and is surrounded by a connective tissue. The size of the subcutaneous muscle is smaller in diameter than the deeper strata. Fig. 3 is ×11, and Fig. 4 is ×500.

Fig. 5 and Fig. 6. These are the longitudinal sections of the tadpole tail muscle called the myotome. It is a striated muscle. Some parts of the fibers are slightly crooked, however, almost all the fibers run parallel to each other. Fig. 5 is ×15, and Fig. 6 is ×500.
Plate 2

This plate from Fig. 7 to Fig. 10 shows the normal tail muscle histochemically as the control, and all of the figures are of the cross sections.

Fig. 7. This is ATP (adenosine triphosphatase, Podykula and Herman). The smallest and badly stained fiber is type I, the biggest and well stained fiber is type II, and the intermediate fiber not only in size but in activity is type III. Muscular fiber is seen clearly and the interstices between the muscular fibers are very few. The classification of the muscular fibers is generally decided histochemically by the ATP stain. ×120

Fig. 8. This is PhR (phosphorylase). The smallest fiber (type I) is stained well and the largest fiber (type II) is badly stained. Type III fiber is intermediate. ×500

Fig. 9. This is PAS (periodic acid, Schiff’s leuco fuchsin). This is the same as Fig. 8. ×500

Fig. 10. This is TC (trichrome, Engel). Both the mitochondria and nucleus are stained well. ×500

Plate 3

This plate from Fig. 11 to Fig. 13 shows the decompression effects.

Fig. 11. This subject was exposed to 500 ATA for 30 seconds and fixed 180 minutes after 3-second decompression. Interstices are seen among the muscular fibers and some of the muscular fibers are broken by decompression, however the “rounding fiber” is never found. ×500

Fig. 12. This subject was exposed to 50 ATA for 10 minutes and frozen 180 minutes after 1.0-second decompression. This is a histochemical TC stain. Mitochondria and nucleus lack clearness when they are compared with not only the control (Fig. 10) but also the 500 ATA exposure (Fig. 19), and the interstices are considerably recognized. ×500

Fig. 13. This is DPNH (reduced diphosphopyridine nucleotide, tetrazolium reductase). This is the same subject as shown in Fig. 12. The stain is bad and this means that the activity is decreased. Interstices are also recognized. ×500

Plate 4

This plate from Fig. 14 to Fig. 16 shows the pressure effects.

Fig. 14. This is the cross section as shown in Fig. 4. This subject was exposed to 100 ATA for 10 minutes and fixed within 2 minutes after decompression. Stiffness was never seen, however a distinct “rounding fiber” was found. ×500

Fig. 15. This was exposed to 310 ATA for 10 minutes and fixed within 3 minutes after decompression. It is very easy to find the “rounding fiber”. All subjects lied down after decompression, however stiffness was not seen. ×500

Fig. 16. This is the longitudinal section. This is the same subject shown in Fig. 15. Muscular fibers are extremely crooked and the flexion is swollen, however the structure is clearly seen. It is very interesting to compare with the control (Fig. 6). ×500
Plate 5

This plate from Fig. 17 to Fig. 19 shows the pressure effects, however these figures are histochemical stains.

Fig. 17. This is the PAS stain. This subject was exposed to 300 ATA for 10 minutes and frozen within 3 minutes after decompression. This subject was treated with PAS 17 days after freezing, and the stain is bad, however the “rounding fibers” are seen. ×300

Fig. 18. This is the HE (hematoxylin-eosine) stain. This subject was exposed to 500 ATA for 10 minutes and frozen within 4 minutes after decompression. A large number of “rounding fibers” were recognized, however interstices were not found. It is obvious that the size of the “rounding fiber” at 500 ATA is larger than that at 100 ATA (Fig. 14). ×300

Fig. 19. This is the TC stain and this subject is the same one shown in Fig. 18. It is remarkable to know that mitochondria and nucleus have clearness when they are compared with the subject exposed to 50 ATA for 10 minutes and frozen 180 minutes after 1.0-second decompression (Fig. 12). This fact will suggest that the damage to the mitochondria and nucleus is due more to the decompression than the compression. ×500
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Fig. 3
×11

Fig. 4
×500

Fig. 5
×15

Fig. 6
×500