学位申請論文

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主論之1.

On the Mechanism of the Activity Rhythm of the Sea-Pen, Cavernularia obesa Valenciennes

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Introduction

The physiological mechanism of the circadian rhythm is not yet clarified, though efforts have been made by many investigators, especially since 1960. In the present circumstances, it is generally suspected that there might be something independent of rhythmic phenomena but controlling them. Such a mechanism underlying the rhythmic phenomena such as the locomotory activity in rats (Rawson, 1960), mating reactivity in Paramecium (Imafuku, 1972), sporulation in Oedogonium (Bühnemann, 1955) and so on, was called the "clock" (Bünning, 1973, Sweeney, 1969). In all these experiments, rhythmic phenomena were suppressed by chemical or other treatments but recovered without any phase-shift when the effect of treatments was diminished or wholly removed; this suggested some time-keeping mechanism maintaining its running under the effect of treatments.

On the other hand, a different idea was presented as to the expansion-contraction rhythm of the sea-pen, Cavernularia obesa Valenciennes, in which the rhythmic phenomenon itself was involed in the time-keeping mechanism demanding no underlying clock (Mori, 1947), as this sea-pen was expanded by the increase of hydrogen ion concentration of the body fluid due to the accumulation of metabolites during the contracted state, but contracted by the decrease of hydrogen ion concentration due to the excretion of accumulated metabolites in the expanded state.

The present author has pursued the mechanism of the expansion-contraction rhythm of this sea-pen from the view point of the

above-mentioned working hypothesis. A part of the work was published in 1973, the results were summarized in the following four points: (1) A phase-shift was induced by the injection of acidified sea water. (2) The temperature coefficients of oxygen-consumption and the period of rhythm were 2.53 and 1.00 respectively in the range from 20 to 30°C. (3) The period of rhythm was temporarily changed by a sudden change in a certain temperature range. (4) Rarely, an active expanded state was omitted but without inducing any subsequent phase-shift. The first point (1) supports the working hypothesis mentioned above but the last (4) is seemingly left unexplained by this hypothesis.

The present paper deals with the results of further experiments with this sea-pen on the mechanism of the expansion-contraction rhythm, made after 1972 at the Seto Marine Biological Laboratory. Materials used were collected from Hatakejima island and near the laboratory. Methods were the same as reported in 1973.

Results

Hydrogen ion concentration of the body fluid at different temperatures:

If the rhythmic activity is based on the metabolism affected generally by temperature, the period of rhythm will be shortened at higher temperatures but lengthened at lower temperatures. However, the temperature coefficient of the period was calculated to be 1.00, although the metabolism represented by oxygen-consumption was greatly affected by temperature. Then it is necessary to examine whether or not the pH at the beginning of expansion is variable with temperature.

Several colonies newly collected were kept in the aquarium at 25-27°C and 14-16°C respectively under the condition of a light-dark cycle with the light phase from 20:00 to 8:00. The colonies

expressing a regular expansion-contraction rhythm in the aquarium and just expanding in the early dark phase were taken out and their body fluid was squeezed out by pressing respective colonies with hands after their surface was wiped with a towel. As far as they showed the expansion-contraction rhythm regularly related with the light-dark cycle, the same colonies were repeatedly used to obtain samples of the body fluid. The pH of body fluid samples was measured by the pH meter, Hitachi-Horiba M-5, or by colorimetry of hydrogen ion concentration using phenol red as an indicator.

The results are shown in Table 1. The pH of the body fluid was 7.2-7.4 at higher temperatures of 25-27°C, while it was 7.3-7.6 at lower temperatures of 14-16°C. Mean and standard deviations were calculated to be 7.30 ± 0.07 at higher temperatures and 7.44 ± 0.10 at lower temperatures. Thus, the pH of the body fluid was slightly lower at higher temperatures at the beginning of expansion. If it is supposed that the contracted period is the same in the two temperature ranges, hydrogen ions are produced at a constant rate during the period and then the hydrogen ion concentration at the beginning of expansion is correlated linearly with the hydrogen ion production, the temperature coefficient of the hydrogen ion production can be estimated to be 1.34 from the pH measurements at 26 and 15°C. In other words, if it is supposed that the metabolic rate is directly reflected on the hydrogen ion concentration of the body fluid, the pH difference between the two temperature ranges is calculated to be 0.44 and then the pH of the body fluid at 15°C will be calculated to be 7.74 on the basis of the actually measured pH value, 7.30, at 26°C. As the actually measured pH values differ from this, it is clear that the metabolic rate is not directly reflected in the hydrogen ion concentration of the body fluid. However, this does not always stand against

the working hypothesis (see page 13).

Hydrogen ion concentration of the body fluid of the colony that omitted an active expanded phase:

Occasionally an active expanded phase may be latent in the course of regular expansion-contraction rhythm of the sea-pen. Such a phenomenon was reported in the previous paper (1973) in the colonies kept under the condition of the light-dark cycle as well as the constant condition, and also observed in the present study as seen in Fig.4 (colony 2A, November 12, 20 and 23). This phenomenon is one of the reasons why questions are put to the working hypothesis that the expansion-contraction rhythm of the sea-pen is induced by the excretion and accumulation of hydrogen ions. According to this hypothesis, a regular rhythm is explained in a way that metabolites gradually accumulated during the contracted state to a certain amount in a relatively fixed time induce the contracted animal to expand. Therefore, the omission of an active expanded phase or a prolonged contracted state brought about by it seemingly can hardly be explained by the working hypothesis. One of the possible explanations of this phenomenon might be given by measurement of the pH of the body fluid in the colony just omitted an active expanded phase.

A colony skipped an active expanded phase in a dark phase of light-dark cycle was taken out at the beginning of expansion in the next dark phase to examine the pH of its body fluid in the same way as in the previous experiment. The pH of this colony was 7.4 at the temperature of 28.5°C, which was not different from that of the same colony presented later nearly the same pH value (7.3) at the beginning of an expanding phase in the following regular rhythm (Fig.1).

Thus, the pH in the expanding phase seemed constant regardless

of the omission of expansion. This result may be explained either by a sudden decrease by some unknown cause of the accumulation rate of hydorgen ions at the time of omission or by a supposition that the body fluid is provided with an ability to maintain the hydrogen ion concentration within a certain range by buffer action and therefore the expansion is triggered rather independently of the hydrogen ion concentration. The latter, suggesting a mechanism other than the change in hydrogen ion concentration, may be supported by the following experiment.

Effects of injection of alkalized, acidified, or natural sea water to contracted animals:

The working hypothesis is based significantly on a fact that the injection of acidified sea water induced the contracted colony to expand (Mori, 1945). Then, an experiment from the quite opposite view point, namely to see whether or not the injection of alkalized sea water induces the expansion of the contracted colony, must be very significant, too, to examine the validity of the working hypothesis. Thus, the experiments designed as follows were repeated three times.

(The first experiment in December '72 to April '73)

Ten colonies from 32 to 58 g in wet weight, which had been kept in glass cylinders under the condition of 12-h light and 12-h darkness, were injected with sea water alkalized by addition of sodium hydroxide or acidified by aeration with carbon dioxide or by addition of hydrochloric acid, or with natural sea water. The same colony was tested with various sea water many times. Treated or natural sea water of the volume 0.1 to 0.3 ml per gram of test animal was injected from the top of the contracted colony 2 to 4 hours after the beginning of the light phase of light-

dark cycle. The light intensity in the light phase was 330 lux at the surface of sand floor in cylinders, and the dark phase was constantly under the dim light of about 1 lux. The temperature of running sea water fluctuated in the range from 15°C to 23°C through the experiment.

Results of experiments are shown in Table 2 (A), in which the positive effect of injected sea water is shown by (+) and the negative effect by (-). Colonies reacted to injection differently; some without any reaction, while others showed a wide variation from a slight extension of the colony by 1-3 cm but without any reaction of polyps to a fully expansion of the colony with perfectly everted polyps through some intermediate states that are shown by extension of the colony by several centimeters with swelling of polyps corresponding to the degree of colony extension. As it is practically impossible to made any significant classification of such states, the effect of injection was judged quite arbitrary as positive when tested colonies exceeded half as long as in the normal expanded state, otherwise negative. The experiment with alkalized sea water, with pH from 9.20 to 10.70, was done in the period from December 11 to January 23 at the water temperature 15-22°C. Of 20 trials, two positive cases were met with. Therefore, the rate of positive effect was only 10 %, but anyhow the positive effect of alkalized sea water on expansion was seen clearly, because in expanding colonies there was observed the upward peristalsis as in natural expansion (Imafuku, 1975a) and the extension by alkaline sea water attained nearly to the fully expanded state with numerous everted polyps.

Injection experiment with the sea water acidified by carbon dioxide aeration to pH 4.42 to 4.75 was carried out in five weeks from February 7 to March 16 at 18.0-20.5°C, the volume of injected

sea water ranged from 0.1 to 0.3 ml per gram of animal. Of 19 trials, 7 gave the positive result and then the rate of positive effect was 37 %, clearly higher than in the case of alkalized sea water. The injection of sea water acidified by hydrochloric acid induced contracted animals to expand once in five trials.

The effect of natural sea water, with pH value 8.19, was tested in a week from March 21 to 27 on the same colonies at 20.8-22.8°C. Four of 15 trials were found positive, then the rate of positive effect was 27 %. In some colonies, the expansion was achieved as perfectly as in the natural state, as some colonies in the case of the injection of alkalized sea water.

From these results, it is apparent that each of alkalized, acidified and natural sea water can induce the contracted colony to expand and that there are differences in the sensitivity to injection; some colonies (colonies 5A, G1 and E2 in the experiments) never reacted to the injection, while others did to some extent, sometimes perfectly.

(The second experiment in the days from August 12 to 24, 1975)

Five colonies (01. 02, 03, 04 and 06, weighing 14 g to 22 g), kept together in a 100 liter aquarium and exposed to the natural daylight through the laboratory windows facing the north, were each injected on August 12 and 17 with sea water adjusted by carbon dioxide and liquid ammonia to maintain pH at 8.2, on August 19 and 21 with carbonated sea water, and on August 24 with sea water alkalized with sodium hydroxide.

The results are shown in Table 2 (B). The rate of positive effect of the injection of pH-adjusted sea water was 20 %; namely two colonies expanded and their polyps everted, though the colonies never attained the fully expanded length and polyps were not extended as perfectly as in the natural state, with tentacles

somewhat contracted. At the injection of carbonated sea water, 2 colonies were induced to expansion on August 19 but only one reacted perfectly, some others only extended the colony outside the sand bed by 1-5 cm on August 21. No colonies reacted to the injection of alkaline sea water on August 24.

(The third experiment in a month from September 15 to October 15 '73)

Two colonies K2 (73 g in wet weight) and K3 (68 g) kept in glass cylinders respectively and exposed to the artificial illumination of 12-h light and 12-h dim light as in the first experiment, were injected with a 0.54 M solution of sodium chloride insotonic to sea water, alkalized with sodium hydroxide or acidified with carbon dioxide or hydrochloric acid.

The results are shown in Table 2 (C). The colony K2 never reacted significantly to any treatments, but only slight lengthening of the colony. Reactions of the colony K3 to respective treatments are illustrated in detail in Fig.2. On September 15, 0.02 ml of alkalized saline solution per gram of the tested colony was injected and this induced a prominent expansion as large as in the natural expansion and lasting longer than in usual rhythm. On September 19, isotonic saline solution was injected, but this caused only a little bit expansion. However, such a small expansion was repeated without any treatment on the next day, September 20. This is a very interesting phenomenon that reminds us of what was quoted by Brown (1970) from the Bünning's work on the leaf movement of Phaseolus. Acid saline solution injected on September 23 did not induce any expansion. The second injection of saline solution on September 29 induced a clear expansion, the volume of saline solution injected being greater than in the first treatment on September 19. The second injection on October 3 of saline solution acidified with hydrochloric acid and the first

treatment with carbonated saline solution on October 10 resulted in negative. Finally, the injection of 0.1 ml of saline solution containing hydrochloric acid per gram of tested colony induced a great expansion.

Results of all these experiments are summarized in Table 3. From this table, it may be concluded safely that acidified or alkalized sea water or saline solution induced the contracted colony to expand to some extent and that the greatest positive effect was brought about by sea water aerated with carbon dioxide. A clear correlation between the volume of injected sea water and the rate of positive effect was not observed. Reasons for this are unknown at present, although it seems likely that the injection of a small amount of treated sea water is sufficient to induce an expansion and that the differences in reaction to the same treatment as seen between on August 12 and 17 on colony 01 or 04 (Table 2B) are attributable to the process of injection itself; the needle point may reach inside the canal of contracted colonies in some cases, but remanin in the coenosarc in the others, though this can not be confirmed actually.

The fact that not only the carbonated sea water but also natural sea water, isotonic saline solution, alkalized sea water and so on could induce the contracted colony to expand is evidently contradictory to the working hypothesis that the hydrogen ion concentration plays an important role in the expansion-contraction rhythm of the sea-pen.

Effect of injection of alkaline sea water to expanded colony:

If the increase of the hydrogen ion concentration of the body fluid induces the contracted colony to expand and the decrease induces the expanded colony to contract, the injection of alkalized

sea water to the expanded colony should cause a contraction. This was examined on a colony kept under the light-dark cycle and maintaining the regular active expansion in the dark phase. Ten ml of sea water alkalized by sodium hydroxide was injected to the expanded colony in the dark phase on September 25 and 30. At injection, the top of the colony was held with fingers and the needle was inserted from the top of the colony. This caused many open polyps to close, with a beautiful greenish-blue fluorescence, and the colony itself to contract slightly for a while. In one to three hours, however, the colony recovered the usual expanded state, with many open polyps, that lasted till the end of respective dark phases (Fig.3). Therefore, slight contraction just after the injection was clearly due to a mechanical stimulation at injection rather than the direct effect of alkalized sea water injected, because it was confirmed that simple touch to the expanded colony caused a similar reaction (Fig.5). Thus, it is impossible that the injection of alkalized sea water induces the expanded colony to contract perfectly. This conclusion also throws doubt on the working hypothesis. If the working hypothesis were true, the injection of alkalized sea water would have to induce the expanded colony to contract according to a rise of pH of the body fluid.

Effect of the injection of alkalized sea water on the rhythm of colonies kept under the constant condition:

It has been found already that a regular expansion-contraction rhythm is disturbed by the injection of acidified sea water, shifting the phase of rhythm, changing the period of rhythm, or bringing about an irregular rhythm. Then, it was examined if the injection of alkalized sea water brought about any changes in the rhythmic behavior of colonies kept under the constant dim light.

The colony 2A, showing a clear expansion-contraction rhythm, was injected with alkalized sea water in the contracted phase on November 14 and this caused an expansion immediately (Fig.4). After the injection, the active expanded phase moved to earlier hours of the day and the period of rhythm was slightly shortened to 21.7 hr. The colony 5A was injected with alkalized sea water in the expanded phase on November 8 and this caused a prominent prolongation of that state that ceased on the next day nearly at the closing time of the expanded phase, accustomed in the regular rhythm. The third colony, no. 50, which has been showing the rhythm persistently for a long time, was injected with alkalized sea water two times on November 8 and November 14 respectively; the former injection induced the contracted colony to expand and brought about some prolongation of expanded state, while the latter was ineffective and the colony remained contracted.

From the behaviors shown in three colonies by the treatment with alkalized sea water, it was noted that the injection of alkalized sea water also might affect a regualr rhythm by changing the period or phase of rhythm in some colonies, but quite ineffective in some others. Here again, colonies seem to react differently to the injection.

Effect of mechanical stimulation on the rhythmic behavior:

As described already, only a touch by fingers at injection caused a slight contraction in a colony. Then, to see the effect of pure mechanical stimulation on the rhythmic behavior, a colony kept under the constant condition of dim light and at the constant temperature of 28-29°C was experimented with in the days from July 23 to August 12 (Fig.5).

The colony which had been showing a regular expansion-contraction

rhythm was stimulated at night on July 26 by finger touch to the colony surface from the top to base of the rachis and then by pressing the colony strongly. This caused fluorescence, closing of polyps in a short time and shortening of the colony itself, that went on just similarly as in the usual contraction. But, the colony never contracted into the sand as usual. The colony contracted by stimulation began to recover the expanded state slowly and returned to the original fully expanded state in about 5 hours. The recovered expanded state was maintained for a long time till a perfectly contracted state appeared on the following day. The regular expansion-contraction rhythm with the active expanded phase at midnight was somewhat disturbed by this stimulation, but no phase-shifting was definable as the active expanded phase seemed unchanged as a whole.

The second stimulation was given to the same colony on August 2. This time, the stimulation was not so strong as in the first experiment; only light touch was given from the top to base of the rachis, but strong pressing was not applied to the colony. This stimulation caused fluorescence and closing of open polyps as in the first experiment, but shortening of the colony was not so strong as in the first treatment and the contracted colony recovered in 3 hours the usual expanded state that was followed 8 hours later by a usual contraction. This time, the active expanded phase of the colony was shifted to the daytime.

The third stimulation was given to the expanded colony on August 7 in a way to pinch the subapical portion of the colony with thumb and forefinger three times in a few seconds. Fluorescence was transmitted from the stimulated part to all rachidal area and all polyps were retracted into the coenosarc in a short time. Even this slight press made the colony contract largely, but

the colony recovered in about 3 hours the usual expanded state which lasted to next noon. The active expanded phase was maintained in the daytime.

Throughtout the three experiments, it was concluded that the active expanded phase might be shifted by mechanical stimulations from midnight to the daytime as seen clearly in the case of the second stimulation, and therefore, the expansion-contraction rhythm of the sea-pen was regarded to be relatively unstable to mechanical stimulations.

Discussion

The present study was made to clarify the physiological mechanism of the expansion-contraction rhythm of the sea-pen, Cavernularia obesa, and focussed especially on examination of the working hypothesis that the increase of the hydrogen ion concentration of the body fluid, due to accumulation of metabolites in the contracted state, induces the expanded state of the colony, that allows the excretion of accumulated hydrogen ions, read by pH rise in the body fluid, and is followed by the contracted state.

As, already, the temperature coefficient of the metabolic rate had been estimated to be 2.53 and the period of rhythm had been confirmed to be temperature-independent (Imafuku, 1973), the pH of the body fluid was measured at different temperatures. The pH of the body fluid in the expanding stage was 7.30 at 26°C and 7.44 at 15°C (Table 1). As the temperature coefficient of the hydrogen ion production was calculated to be 1.34, the metabolic rate was slightly reflected on the pH of the body fluid. Such a tmperature effect on the pH, related with the colony expansion, may be explained with regard to the working hypothesis by postulating that the sensitivity of muscle and nerve to the hydrogen ion concentration of the body fluid is also affected compensatingly

by temperature, that is, the muscle and nerve may react to lower pH at higher temperatures but to higher pH at lower temperatures. If this is true, the results of pH measurement at different temperatures do not always stand against the working hypothesis.

The pH of the body fluid in the expanding stage was 7.3-7.4 not only in the regular rhythm but also after skipping an expansion (Fig.1). Thus, it is suspected that metabolism might be somewhat depressed at the time of skipping an expansion or the pH of the body fluid might not be changed probably by the buffer action of the body fluid beyond a certain level even if the metabolism proceeds steadily when an expansion is omitted.

At any rate, the results of pH measurements may be explained with regard to the working hypothesis. However, the results of injection experiments evidently throw doubt on the working hypothesis. As the injection of alkalized sea water or even of natural sea water caused contracted colonies to expand (Tables 2, 3, Fig.2), the expansion can not be attributed limitedly to the increase of hydrogen ion concentration, rather it may be due to the infusion of foreign matters into the colony. This is supported also by results of the other experiments, in which the injection of alkalized sea water to the expanded colony induced no contraction (Fig.3) but resulted in a prolongation of the expanded state (Fig.4, colony 5A), by which the injected substance might be wholly excreted. The working hypothesis is, therefore, seemingly unacceptable.

A modification of the working hypothesis may be possible as seen, for example, in next idea that the accumulation of metabolites, but not always the increase of hydrogen ion concentration causes an expansion and the excretion of accumulated metabolites a contraction However, this idea should be put aside as far as the phenomenon of skipping an active expanded phase in a regular expansion-contraction

rhythm is noted, because the phenomenon can not be explained sufficiently by the idea. Generally, the metabolic rate is more or less affcted by temperature and probably by other environmental factors, and such an effect of environmental factors may cause naturally some slight fluctuation in the period of rhythm, though the period cannot be changed suddenly from 24 hours to just 48 hours, as seen repeatedly in the cases of omitting an expansion (Figs.1 and 4). Examples of other organisms, similar to the omission of an expansion in the sea-pen, were discussed in the previous paper (1973), refering the works on Oedogonium (Bühnemann, 1955), Paramecium (Imafuku, 1972), Gonyaulax (Hastings and Sweeney, 1958) and some mammals (Rawson, 1960). Another example is the locomotory activity of cockroaches, in which a cluster of activity was observed at intervals of about 24 hours in the constant condition of medium temperature, but every other cluster was suppressed under the condition of low temperature, and this resulted in the appearance of a 48 hour rhythm (Bünning, 1959). These examples are common in the following aspect that there cannot be found any fixed correlation between the period and amplitude of rhythm, and this might be a strong basis to postulate a time-keeping mechanism independent of rhythmic phenomena.

Furthermore, it is expected that circadian rhythms in various organisms are based on the same mechanism, because they have some common aspects. The expansion-contraction rhythm of the sea-pen, Cavernularia obesa, has the following aspects common to other organisms. It is entrained to a light-dark cycle (Mori, 1944) as well as to a temperature cycle (Imafuku, unpublished data). It can be entrained to different periods of environmental cycle in a certain range, but the period will become circadian when the period of light-dark cycle out of the range is applied (Mori and

Ondo, 1957). Of course, such a range is not always identical among differnt organisms (Imafuku, 1975b). The period of rhythm is temperature-independent (Imafuku, 1973) in a certain temperature range (Mori, 1960), though it is changable by sudden temperature changes (Imafuku, 1973).

On the other hand, the expansion-contraction rhythm of the sea-pen has some specific features. One is that the rhythm was easily affected by injection of alkalized or acidified sea water. This contrasts prominently with the leaf movement rhythm of Phaseolus, that was not affected by pH change in the range of 3.2 to 8.0 (Bünning, 1956). Generally circadian rhythms are stable to chemical treatments, except for a few substances that were listed up in the previous paper (Imafuku, 1973). Another specific feature is that the rhythm is affected by mechanical stimulations (Fig.5). The effect of mechanical stimulations such as agitation of sea water is reported already in the tidal rhythm of some shore crustacean (Palmer, 1974).

The final conclusion throughout the results of the experiments carried out so far and considerations given above is that the expansion-contraction rhythm of the sea-pen is controlled by a certain mechanism independent of the rhythm quite similarly as in other organisms, but the mechanism in the sea-pen is susceptible to influences from both the inside, such as changes in the body fluid components, and the outside such as mechanical stimulations as well as illumination or temperature.

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Abstract

Efforts have been paid in the present experiments to clarify the mechanism of the expansion-contraction rhythm of the sea-pen. Results are summarized as: (1) The hydrogen ion concentration of the body fluid at the beginning of expansion was slightly lower at higher temperatures in the regular rhythm as well as in the case of omission of an expansion. (2) Injection of alkalized, acidified or natural sea water or saline solution induced the contracted colony to expand to a certain extent. (3) Injection of alkalized sea water did not make the expanded colony contract. (4) The expansion-contraction rhythm was affected by both the injection of alkalized sea water and the mechanical stimulation. As far as the results obtained are concerned, it is concluded that the rhythmic behavior of the sea-pen is under the control of a time-keeping mechanism independent of the rhythmic behavior itself, and that the mechanism is susceptible in this sea-pen to influences from the inside and outside.

Fig.1. The pH of the body fluid at the beginning of expansion, after skipping an expansion (A) and in the regular rhythm (B). The body fluid of the colony kept in the light-dark cycle with the light phase from 20:00 to 8:00 and at the temperature 28.5°C was sampled at 9:25 on September 25 (A, 14 ml), when the colony was extended above the sand floor by 4 cm, and again at 9:15 on September 30 (B, 28 ml), when the colony was extended above the sand floor by 7 cm.

Fig.2. Record of the expansion-contraction rhythm of the sea-pen injected with saline solution (on September 19 and 29), alkalized by sodium hydroxide (on September 15), acidified by hydrochloric acid (on September 23, October 3 and 15) or by carbon dioxide (on October 10).

Fig.3. Effect of injection of alkalized sea water on expansion. A 10 ml of sea water alkalized with sodium hydroxide was injected to the expanded colony kept in the light-dark cycle.

Fig.4. Effect of injection of alkalized sea water on the expansion-contraction rhythm of the colony kept under the constant dim light. Colony 2A was injected with sea water alkalized with sodium hydroxide in a contracted phase on November 14, colony 5A in an expanded phase on November 8, colony 50 in a contracted phase on November 8 and 14. The active expanded phase is shown by a thick horizontal bar.

Fig.5. Effect of mechanical stimulations on the expansion-contraction rhythm. The colony kept under the constant dim light was stimulated by finger touch on July 26, August 2 and 7 as shown by arrows.

Table 1. The pH of the body fluid of colonies kept at temperature 25-27°C and 14-16°C, measured in the expanding stage in the early part of the dark phase of light-dark cycle with the light phase from 20:00 to 8:00. Date, time, temperature and length of colony as well as volume and pH of the body fluid at measurements are given. The mean and standard deviation of pH of the body fluid are shown at the bottom of higher and lower temperatures.

Table 2. Effects of injection of alkalized, acidified and natural sea water (A and B) or saline solution (C).
Injection was made 2 to 4 hours after the onset of the light phase or at dawn, to colonies kept in cylinders under the artificial light-dark cycle (A and C) or in the aquarium under the natural daylight (B). Sea water or saline solution was alkalized by sodium hydroxide, acidified by carbon dioxide or hydrochloric acid, or alkalized by liquid ammonia after once acidified by carbon dioxide. Positive (+) shows that the contracted colony was extended by injection more than half as long as in the usual expanded state.

Table 3. Results of injection experiments, summarized from Table 2. Number of positive injections / number of total injections is given, the rate of positive effect in percentage is given in parentheses.

Fig. 1.

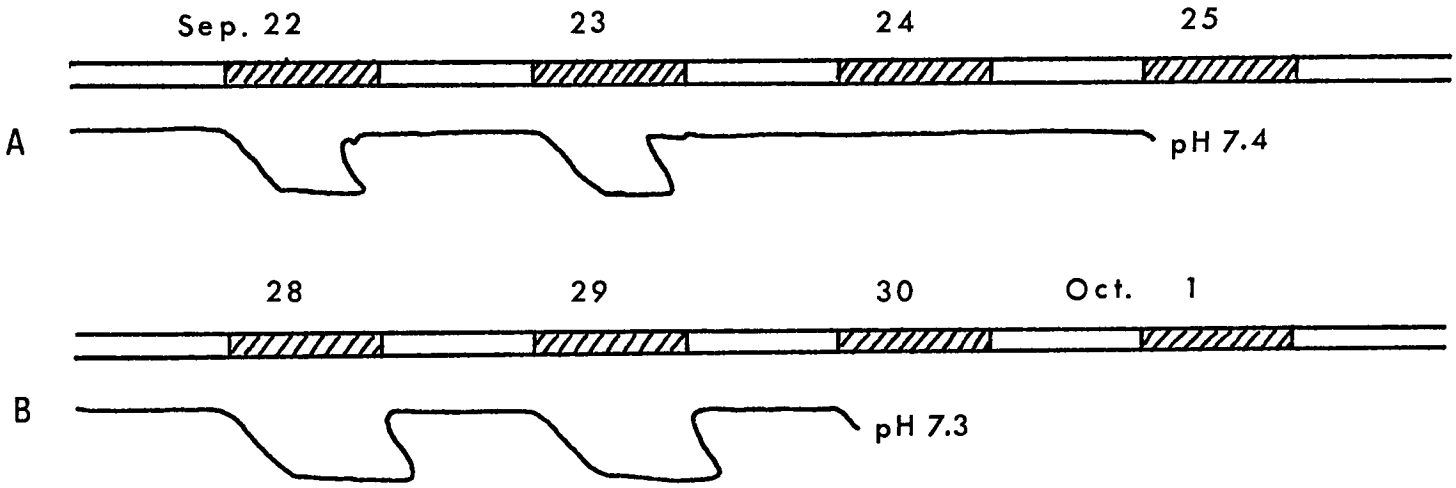
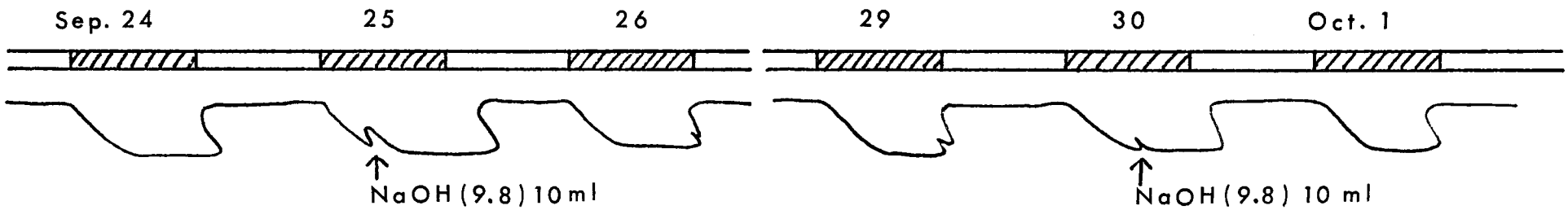
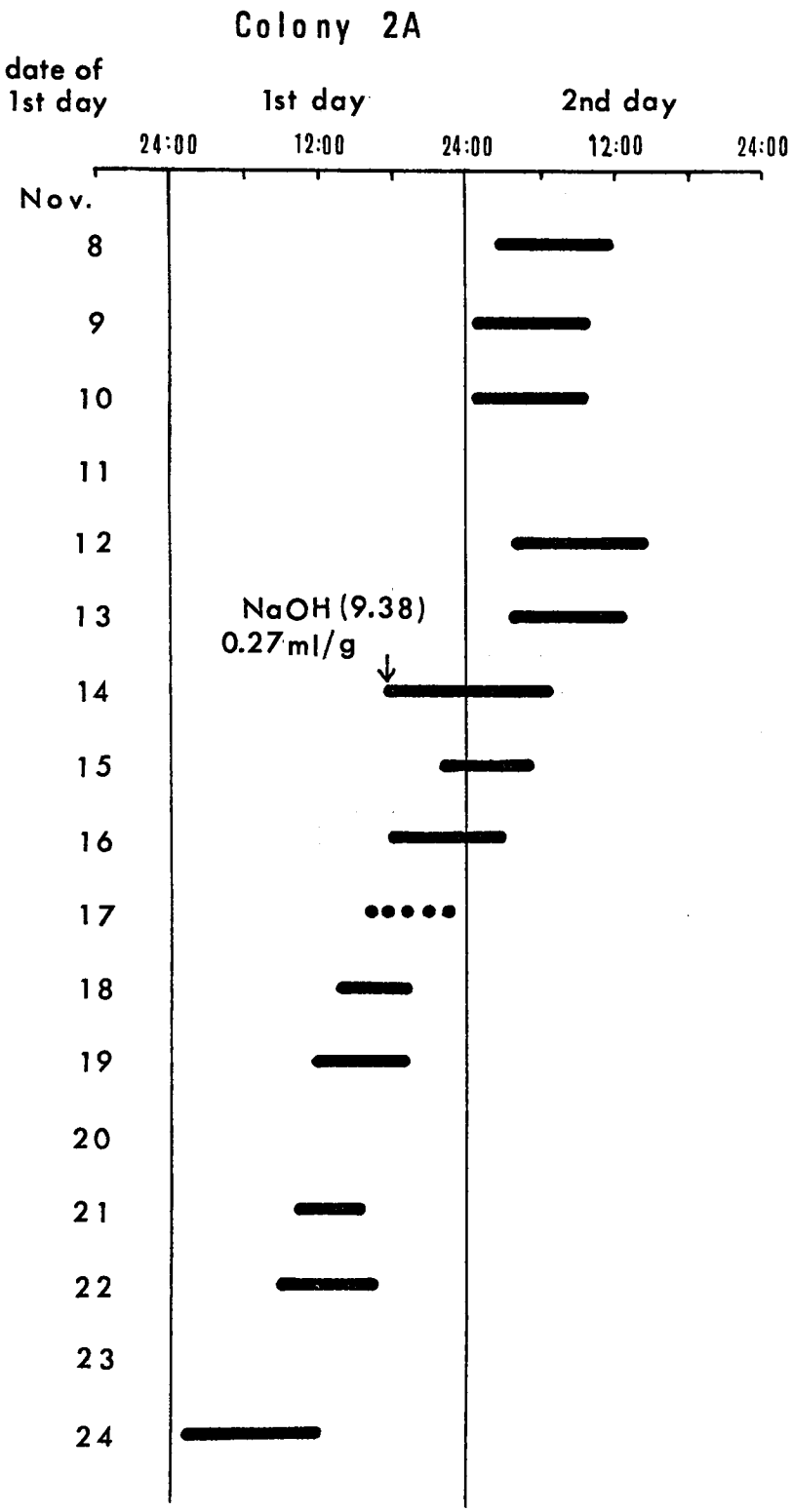


Fig 3




Colony 2A
date of 1st day
1st day
2nd day
24:00
12:00
24:00
12:00
24:00
Nov.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
NaOH (9.38)
0.27 ml/g

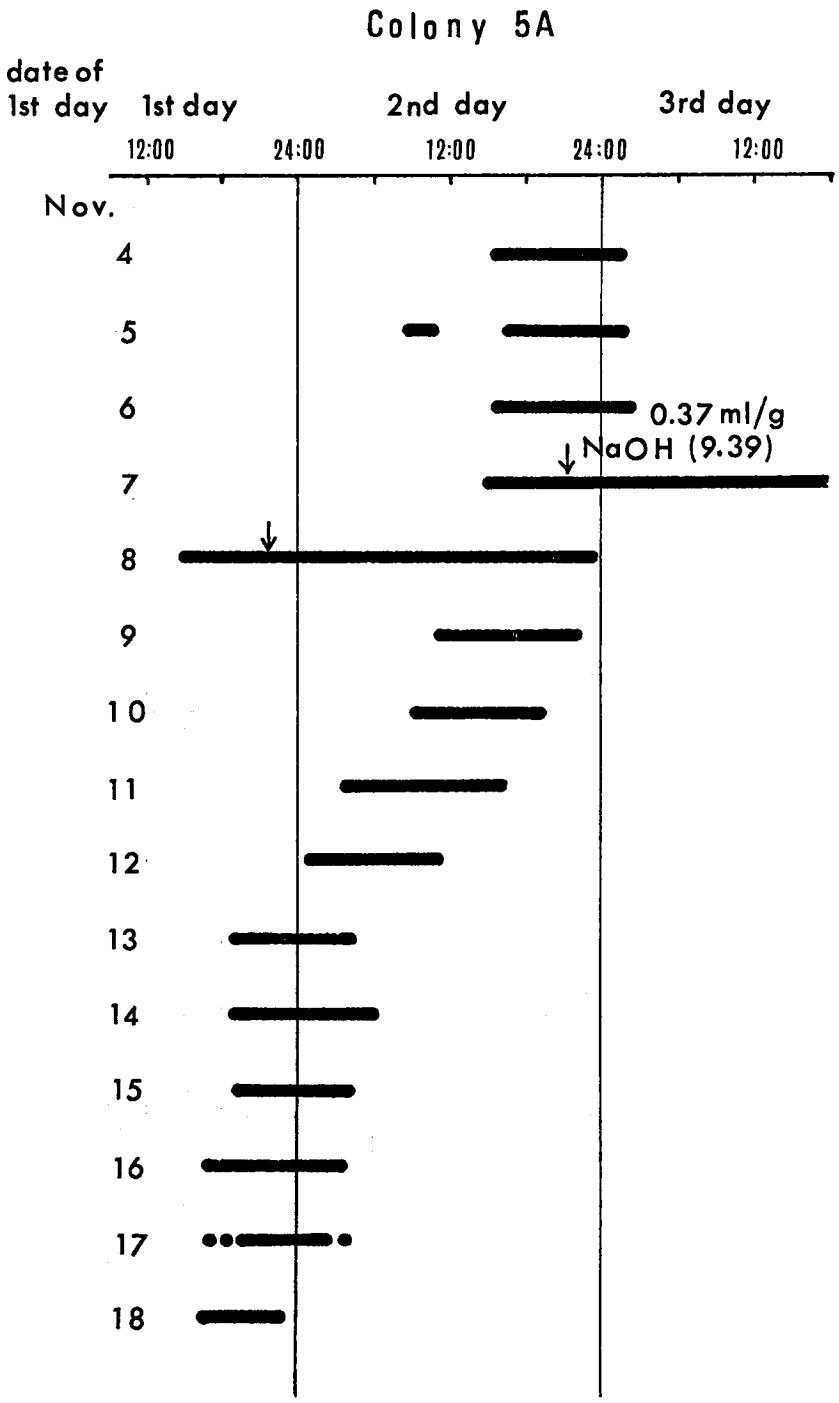

Colony 5A
date of 1st day
1st day
2nd day
3rd day
12:00
24:00
12:00
24:00
12:00
Nov.
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0.37 ml/g
NaOH (9.39)

Fig 4(2)

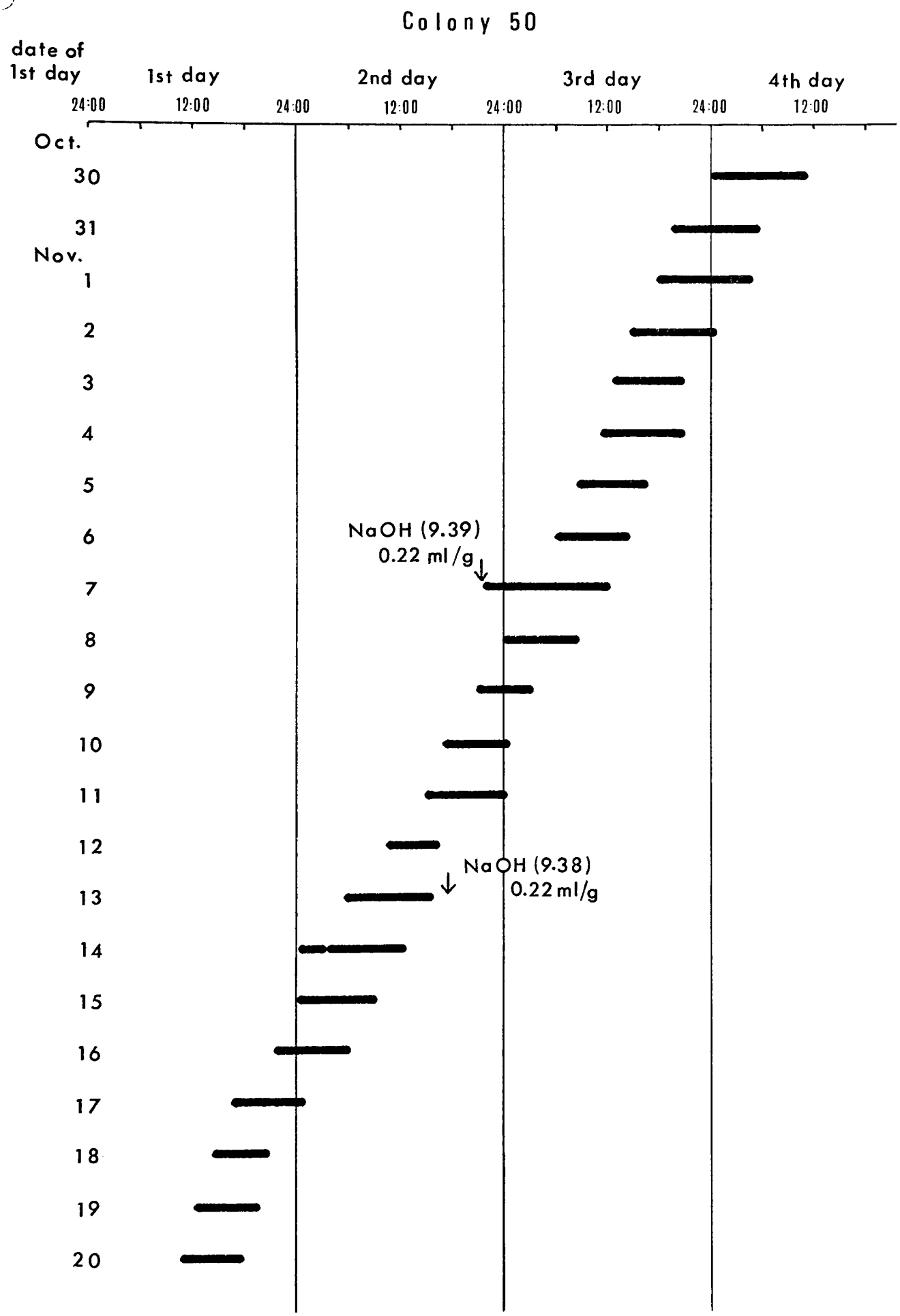
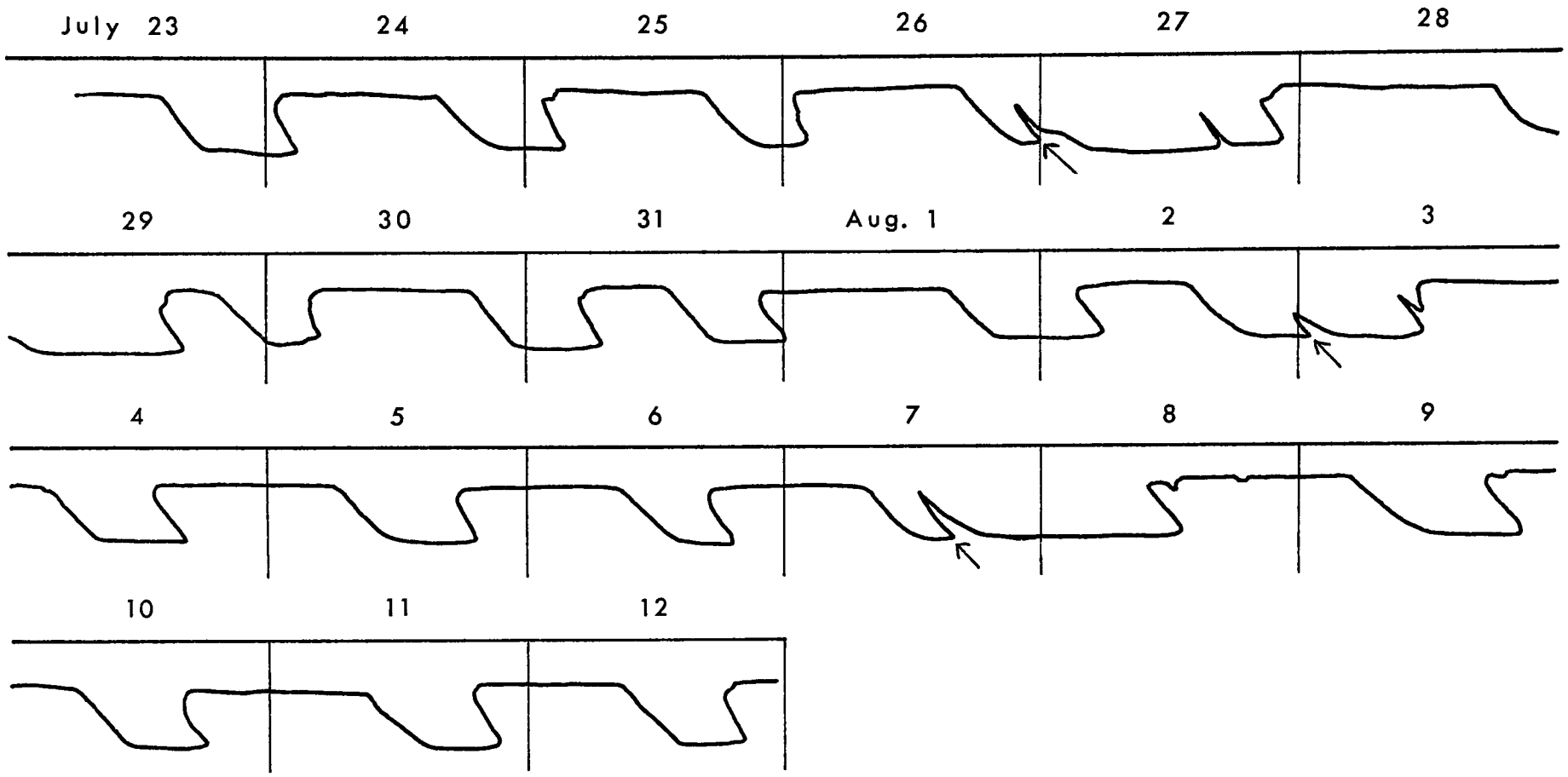

Colony 50
date of 1st day
1st day
2nd day
3rd day
4th day
24:00
12:00
24:00
12:00
24:00
12:00
24:00
12:00
Oct.
30
31
Nov.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
NaOH (9.39)
0.22 ml/g
NaOH (9.38)
0.22 ml/g

Fig. 5



ble 1.

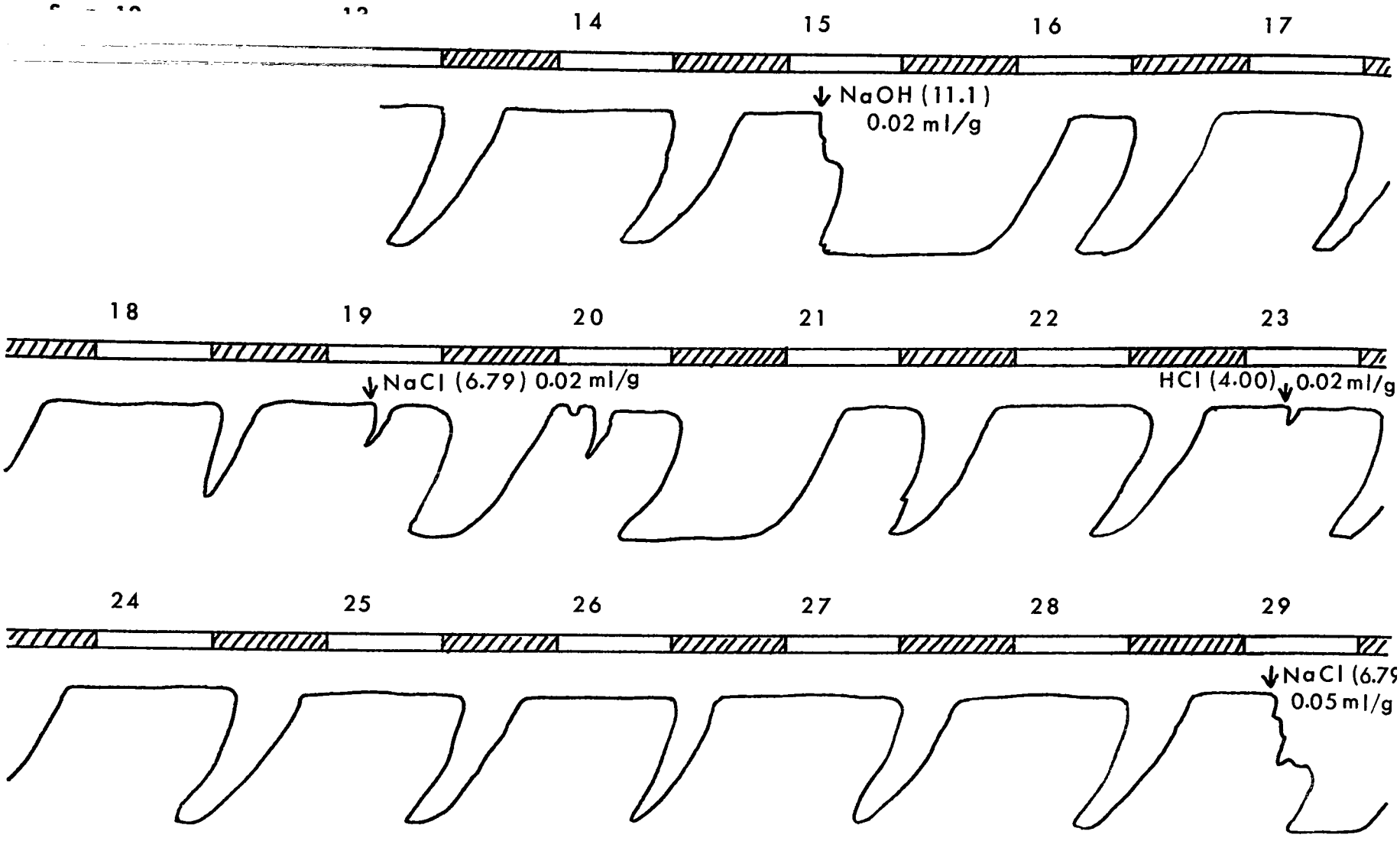
	date	time	temperature (°C)	colony length (cm)	body fluid	
					vol.(ml)	pH
Higher Temperatures	Sep. 28	9:20	26.8	10	19	7.4
	Sep. 28	9:35	26.8	15	44	7.3
	Oct. 4	9:oo	25.6	7	10	7.2
	Oct. 4	9:00	25.6	12	30	7.3
			25-27			7.30±0.07
Lower Temperatures	Sep. 28	9:00	15.1	10	51	7.4
	Oct. 4	9:00	15.1	20	74	7.4
	Nov. 2	10:30	14.8	10	30	7.5
	Nov. 2	12:30	14.8	10	50	7.3
	Nov. 2	12:30	14.8	5	15	7.6
			14-16			7.44±0.10

Table 3

pH / ml/g	HCl	CO_2	NaCl	CO_2+NH_3	S.W.	NaOH	
	3.0-4.0	3.1-5.1	6.8	8.2	8.2	9.2-11.1	
0.02	1/7		0/2			1/2	2/11 (18)
0.05	0/2	0/2	1/2				1/6 (17)
0.1	1/2	3/7			1/5	2/20	7/34 (21)
0.2		5/14		2/10	3/10	0/5	10/39 (26)
0.3		2/8					2/8 (25)
	2/11 (18)	10/31 (32)	1/4 (25)	2/10 (20)	4/15 (27)	3/27 (11)	22/98 (22)

	date	temp. (°C)		pH	vol. (ml/g)	5A (37)	2A (53)	6A (38)	50 (58)	E1 (47)	E2 (35)
A First Experiment	'72 Dec.11	15.1	NaOH	9.36	0.1	-	-	-	-	-	
	12	15-16	NaOH	10.70	0.1	-	-		+	-	
	'73 Jan.16	20.0	NaOH	10.32	0.1	-	-				
	19	20-22	NaOH	10.28	0.1	-	+	-	-		-
	23	21-22	NaOH	9.20	0.1	-	-	-	-		
	Feb. 7	19.0	CO_2	4.42	0.1	-	+	+			
	12	18.0	CO_2	4.66	0.3	-	-		-		
	14	20.5	CO_2	4.60	0.3	-	-	+	-	+	
						G1 (44)	G2 (57)	G4 (48)	G5 (32)	E1 (47)	E2 (35)
	'73 Mar. 2	18.0	CO_2	4.75	0.1	-	-	+			-
	16	19.8	CO_2	4.75	0.2	-	+	+			-
	21	22.8	S.W.	8.19	0.2	-		+	-		-
	24	21.5	S.W.	8.19	0.1	-		-	+	-	-
	27	20.8	S.W.	8.19	0.2	-	-	+	+	-	-
	Apr.18	18.0	HCl	3.04	0.2	-		-	+	-	-
B Second Experiment						01 (17)	02 (22)	03 (18)	04 (20)	06 (14)	
	'75 Aug.12	27.5	CO_2+NH_3	8.2	0.2	+	-	-	+	-	
	17	27.4	CO_2+NH_3	8.2	0.2	-	-	-	-	-	
	19	27.3	CO_2	5.0	0.2	+	+	-	-	-	
	21	27.3	CO_2	5.1	0.2	+	-	-	-	-	
	24	26.2	NaOH	9.6	0.2	-	-	-	-	-	
C Third Experiment						K2 (73)	K3 (68)				
	'73 Sep.15	21.9	NaOH	11.1	0.02	-	+				
	19	21.4	NaCl	6.79	0.02	-	-				
	23	22.1	HCl	4.00	0.02	-	-				
	29	20.0	NaCl	6.79	0.05	-	+				
	Oct. 3	19.0	HCl	3.30	0.05	-	-				
	10	20.0	CO_2	3.10	0.05	-	-				
	15	18.1	HCl	3.80	0.1	-	+				

Fig. 2


14
15
16
17
NaOH (11.1)
0.02 ml/g
18
19
20
21
22
23
NaCl (6.79) 0.02 ml/g
HCl (4.00) 0.02 ml/g
24
25
26
27
28
29
NaCl (6.79
0.05 ml/g
30
Oct. 1
2
3
4
5
HCl (3.30) 0.05 ml/g
6
7
8
9
10
11
CO2 (3.10) 0.05 ml/g
12
13
14
15
16
17
HCl (3.80) 0.1 ml/g