FLUCTUATION OF NA- AND K-CONCENTRATION IN BODY FLUIDS IN THE COURSE OF “EKIRI” PARTICULARLY IN THE SERUM AND IN THE SALIVA

[III REPORT] THE INFLUENCE OF AUTONOMOUS NERVOUS SYSTEM TOXINS, PARTICULARLY PILOCARPIN, ON THE NA- AND K-CONCENTRATION IN SALIVA OF “EKIRI” PATIENTS

BY

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INTRODUCTION

As reported in the previous papers, the author's co-workers, Miyamoto, Anan, Matsumura, Sugai and Osaki found an influence of autonomous nervous system on the Na-and K-concentrations in saliva from their day-fluctuation pattern. Furthermore, Miyamoto, Anan, Matsumura, Sugai, Osaki and Sudo, using various autonomous nervous system toxins and pituitary adrenocortical hormones, found that Na- and K-concentration in saliva were related to autonomous nervous system and these hormones, and that a fairly great day-fluctuation of Na- and K-concentrations were caused probably by the influence of autonomous nervous system itself. Following these studies, the present authors investigated the day-fluctuation of Na- and K-concentrations in saliva of “ekiri” and dysentery patients. The results were as follows:

Compared with dysentery patients, the divergence of the day-fluctuations of Na-concentration were greater in the acute stage (1-3. day of illness) and the early convalescence of “ekiri” patients. More conspicuous, however, were those of K-concentration. Namely, the K-concentration in these stages of “ekiri” and dysentery was as 2 times or more high as that of healthy persons. Consequently, the Na/K ratio, though “ekiri” demonstrated a little higher value than dysentery, in the acute stage of both diseases was markedly lower than that of healthy persons, as a result of a much greater increase of K- than Na-concentration. From these two characteristics; i.e. the striking increase of absolute value of K-concentration and the decrease of Na/K ratio, the predominance of sympathetic nervous system might be concluded in the early stage of “ekiri” and dysentery.

In order to confirm this conclusion, the autonomous nervous system toxin, particularly pilocarpin was administered to the patients of these diseases and variations of Na and K-concentration were examined. The influence of atropin could not be analyzed because atropin brought about the decrease of saliva

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excretion and in “ekiri” and dysentery patients the collection of saliva in successive hours was completely impossible. From this reason, the influence of pilocarpin alone was analyzed as reported here. The mechanism of initial and advanced stages of “ekiri” will be discussed in this report.

**Experimentals**

1. Twelve typical “ekiri” patients and for contrast 9 dysentery patients admitted in Tokyo municipal Honjo hospital from November 1956 to July 1957.

2. Methods of Investigation

Pilocarpin was administered to the patients in the acute stage (1-3. day of illness) and the convalescent stage (the 3. week).

The method of saliva collection: The oral cavity was cleaned up as described in the II report. And then, without any stimulating maneuver the saliva, which flew out naturally, the so-called “naturally flowing” mixed saliva was collected. Thereafter, pilocarpin (1% HCl-pilocarpin, Mohan Company) was subcutaneously injected (1.3 mg/10 kg of body weight) and at 5, 15, 30 minutes and 1, 2 and 3 hours after the injection the saliva was collected, centrifuged (3000 r.p.m. for 15 minutes), and the supernatant was diluted 50 times with distilled water. Because of difficulty of saliva collection in the acute stage of “ekiri” and dysentery only the cases in which saliva collection was possible, were analyzed.

Atropin (0.05% H₂SO₄-atropin, Tanabe Company) was subcutaneously injected (0.06 mg/10 kg of body weight) and the saliva was collected at the above intervals. In the acute stage, however, it was impossible to obtain saliva, as mentioned in the introduction. Therefore, the influence of pilocarpin alone is reported in this paper. Na and K were measured in the same way as in the II report.

**Results**

The fluctuation of Na- and K-concentration in saliva was shown in %, taking the value before the injection as 100%.

1). Fluctuation of Na-concentration in saliva of “ekiri” and dysentery patients after pilocarpin injection.

First the fluctuation of Na-concentration in saliva of healthy adults, investigated by the author's co-workers, Miyamoto, Anan, Matsumura, Sugai and Osaki, must be referred to for control. Figure I of the other experimental series shows that the Na-concentration begins to increase immediately after the injection reaches a marked peak in 15 minutes (average 350%), turns to decrease deeply in 30 minutes approaching 100% (the value before the injection), and then gradually lowers to 100% in 2-2.5 hours which keeps to fall furthermore.

**Acute stage:** (Figure 1a, 1b)

It was a very characteristic of the acute stage of “ekiri” (Figure 1a) that the
Na-concentration declined in 5 minutes after the injection except one case. In 15 minutes, except one case with a very high value, all cases demonstrated a mild tendency to elevate, but they were still below 100% or were only a little over 100%. And in 30 minutes it tended again to fall, showing a navicular curve from 1 to 2 hours, and in 3 hours for the first time began to elevate fairly well and approached 100%.

The Na-concentration in dysentery (Figure 1b) had a slight declining tendency in 5 minutes like in "ekiri", increased, however, rapidly and markedly
Figure 1a. Pilocarpin (1.3 mg/10 kg body weight)
The acute stage of "ekiri" Na

Figure 1b. Pilocarpin (1.3 mg/10 kg body weight)
The acute stage of dysentery Na
in 15 minutes and declined again in 30 minutes to the values over 100% as average. It continued to decline, thereafter, below 100% in 1 hour and then remained almost unchanged till 3 hours. This initial increase is regarded as completely identical with that of contrast group (healthy adults).

**Convalescent stage:** (Figure 2a, 2b)

"Ekiri" cases (Figure 2a) demonstrated in one half an increase and in the other half a decrease in 5 minutes (average an increase) and a fairly marked increase in 15 minutes, namely a so-called rapid initial increase characteristic of pilocarpin effect. The degree of increase, however, was distinctly lower than that of healthy adults. The value decreased again rapidly, but remained even in 30 minutes still higher than 100% and below 100% in 60 minutes lowered, then increased at a very slow rate to almost 100% in 3 hours. The curve, as a whole, showed thus the normal pattern, except that the initial increase was not so marked as the normal one.

The pattern of dysentery (Figure 2b) was similar to that of "ekiri". But the values in 5, 15 and 30 minutes were distinctly higher than in "ekiri", demonstrating thus almost the normal pattern after pilocarpin injection. The value in 60 minutes, as was in "ekiri" remained almost unchanged or showed a very slow elevation.

2. Fluctuation of K-concentration in saliva of "ekiri" and dysentery patients after pilocarpin injection.

![Figure 2a](image)
*Figure 2a. Pilocarpin (1.3 mg/10 kg body weight)
The convalescent stage of "ekiri" Na
First the experiments of our coworkers with healthy adults were here referred to as the control as in 1). The K-concentration (Figure II of the other experimental series) falls after the injection, reaches in 15 minutes the lowest value, then returns gradually in 60–90 minutes to near 100% and then remained almost unchanged. This initial fluctuation of K was in the opposite direction to Na, though not so marked as the latter is characteristic of pilocarpin effect.

**Acute stage:** (Figure 3a, 3b)

Both “ekiri” (Figure 3a) and dysentery cases (Figure 3b) demonstrated a fall of K-concentration in 5, 15 and 30 minutes after the injection, followed by a slow upward curve and returned in 3 hours almost to 100%, only the values of “ekiri” cases in respective time periods being not so convergent as in dysentery.
Figure 2. Pilocarpin (1%, 0.3 cc) Healthy adults K
(By Miyamoto, Anan, Matsumura, Sugai and Osaki)

Figure 3a. Pilocarpin (13 mg/10 kg body weight)
The acute stage of "ekiri" K

Figure 3b. Pilocarpin (1.3 mg/10 kg body weight)
The acute stage of dysentery K

Convalescent stage: (Figure 4a, 4b)
The K-concentration fell in "ekiri" (Figure 4a) as well as in dysentery (Figure 4b) from 5 minutes on after the injection, reached the lowest value in 15 minutes and then rose slowly till 60 minutes, approaching in 3 hours to 100%. That is to say, the K-curve of both diseases demonstrated in the acute
as well as in the convalescent stage the normal pattern of initial decrease observed in healthy adults.

3. Fluctuation of Na/K ratio in saliva of “ekiri” and dysentery patients after pilocarpin injection.

The general significance of Na/K ratio in analysis of the fluctuation of Na- and K-concentration in body fluids was discussed in detail in the I and II reports. The shift of Na/K ratio was shown in %, taking the value before the injection as 100%.

The Na/K ratio of healthy adults after the pilocarpin injection, (Figure III of the other experimental series) reported by the author’s co-workers demonstrated it’s peak in 15 minutes as easily seen from the marked initial increase of Na and the reverse moving of K. Thereafter, the ratio fell rapidly in 30 and 60 minutes and approached slowly to 100% in 3 hours.

Acute stage: (Figure 5a, 5b)

In the acute stage of “ekiri” (Figure 5a), Na/K ratio fell in 5 minutes after the injection once below 100%, like the Na-concentration did, on the contrary to the ratio in healthy adults. The ratio exceeded in 15 minutes again 100%, but the extent of increase was smaller than that of the healthy pattern. The ratio fell again sharply in 30 minutes, then followed the so-called vacuicular curve and then rose in 3 hours slightly, a little above 100% as average. This pattern is a striking contrast to the normal one.
Figure 3 of the other experimental series, Pilocarpin 
(1%, 0.3 cc) Healthy adults  Na/K 
(By Miyamoto, Anan, Matsumura, Sugai and Osaki)

In the acute stage (Figure 5b), the dysentery cases demonstrated in 5 minutes in one half an increase and in another a decrease of Na/K ratio, then increased fairly markedly in 15 minutes and fell again rapidly in 30 minutes, still showing an average value higher than 100%. It returned in 60 minutes almost to the original value (100%) before the injection and remained unchanged till 3 hours.
This initial increase of Na/K ratio in the acute stage of dysentery coincided well with that of healthy pattern, though the degree of increase was not so marked as in the latter.

Convalescent stage: (Figure 6a, 6b)

In this stage, "ekiri" (Figure 6a) as well as dysentery cases (Figure 6b) demonstrated a marked increase of Na/K ratio in 5 and 15 minutes after the pilocarpin injection and fell sharply down to the original value (100%) in 30 minutes. In 60 minutes the ratio of both diseases restored to the original value (100%) and then remained unchanged without any fluctuation till 3 hours.

Consequently, the fluctuation of Na/K ratio in "ekiri" and dysentery paralleled in general well with the normal pattern. The characteristic initial increase, which was observed with the control group, was lower in "ekiri" than in dysentery cases, whose elevated ratio was still lower than that in healthy adults.

SUMMARY

The characteristic of the fluctuation of Na concentration in the acute stage of "ekiri" was the initial decrease in 5 minutes after the injection and the rising tendency in 15 minutes, which stood, however, far behind the normal pattern.

The Na-concentration in the acute stage of dysentery fell once below 100%, but increased strongly in 15 minutes, whereby the extent of the increase was approximately the same as that of the normal group.

In the convalescent stage too, "ekiri" and dysentery demonstrated a dis-
tinct and marked initial increase of Na-concentration, the degree of which was lower in the former than the latter and even the value in the latter was still lower than that of the normal group.

Regarding the K-concentration, both diseases demonstrated in the acute and convalescent stages almost the same degree of initial decrease as observed in the healthy adults. However, the values in "ekiri" at respective time intervals appeared to be slightly more divergent than those of dysentery, and the initial decrease in both diseases was a little greater in the convalescent than in the acute stage.

Consequently, the fluctuation of Na/K ratio showed an amplified Na pattern, as easily concluded from the increase of Na and the decrease of K soon after the pilocarpin injection.
Figure 6a, Pilocarpin (1.3 mg/10 kg body weight) 
The convalescent stage of "ekiri" Na/K

One may summarize more briefly the above-mentioned results as follows:
The initial increase of Na-concentration is indistinct and markedly lower in the acute stage of "ekiri" than in the control group as well as in the acute stage of dysentery. This indicates unresponsiveness of "ekiri" patients to pilocarpin as a parasympathicomymetica. This unresponsiveness is not directly attributable to the stimulation by adrenalin, but is probably related to the excitement of sympathetic nervous system, as a result of which the effect of parasympathic nervous system becomes almost unrecognizable, as discussed in more details in the following chapter.
Figure 6b. Pilocarpin (1.3 mg/10 kg body weight)
The convalescent stage of dysentery
Na/K

SUMMARY AND DISCUSSION

As described in the preceding chapter, only the "ekiri" patients in their acute stage were unresponsive to the pilocarpin stimulation, in other words, to the parasympathetic stimulation, resulting presumably from a state of predominating sympathetic excitement which is expected in "ekiri" children. However, the cases investigated (1–3 days of illness) were not always acute (within 48 hours after the onset of the disease). Accordingly, our interpretation that the children
are still in the state of sympathe excitement, may be contradictory.

As to this apparent contradiction, the author’s coworkers, Miyamoto, Ada-
chi, Uesugi and Ozaki demonstrated that the adrenalin effect lasted much longer
than generally expected. They investigated the duration of adrenalin effect, by
taking one of its actions, i.e. eosinopenic action. The mice, when injected
0.001 mg adrenalin, demonstrated a reduction of eosinophils by 50% in 4 hours,
but the reinjection of 0.001 mg 4 hours after the first one did not reduce eosino-
phils at all on the contrary to the expectation, that further reduction of eosino-
phils by 50%, namely 25% of the original value would result. They restored
the original value in a certain period after the injection (16–20 hours), regard-
less of the second injection (Figure IV of the other experimental series).
Moreover, if the mice were reinjected 16–20 hours after the first injection, in
the period of recovery to the original eosinophils count, or in 24–40 hours;
when 20–24 hours elapsed after the adrenalin effect had perfectly disappeared,
they still did not respond to the adrenalin. This fact indicates that unrespon-
ding period against adrenalin lasts approximately as long as 52 hours (Figure
V, VI, VII of the other experimental series).

Accordingly the adrenalin effect, so far as it’s eosinopenic activity is con-
cerned, persists for an astonishingly long time. The effect of the first adrenalin

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**Figure 4.** The shift of eosinophil number of the white mice after frequent
adrenalin injections in time interval of 4 hours.
(By Miyamoto, Adati, Uesugi and Ozaki et al)

**Figure 5.** The shift of eosinophil number of the white mice in the second
adrenalin injection, 24 hours after the first adrenalin injection.
(By Miyamoto, Adati, Uesugi and Ozaki et al)
Figure 6. The shift of eosinophil number of the white mice in the second adrenalin injection, 40 hours after the first adrenalin injection. (By Miyamoto, Adat, Uesugi and Ozaki et al)

Figure 7. The shift of eosinophil number of the white mice in the second adrenalin injection, 52 ours after the first adrenalin injection. (By Miyamoto, Adat, Uesugi and Ozaki et al)

Injection exceeds not only the eosinopenic period, but also the recovery period of eosinophile count, when the animals do not show any signs of disturbances (30 hours).

Concerning the duration of the adrenalin action, one is obliged, however, to cite a contradictory fact, namely hyperglycemia induced by adrenalin injection. By the author's co-worker, Kumii, the transient hyperglycemia caused by adrenalin was to be readily produced again by a second injection. This adrenalin action lasts at most for some hours (Figure VIII of the other experimental series).

Anyway, the above-mentioned long unresponsive phase to adrenalin would be a strong support for the interpretation, that in “eikiri” patients also, the adrenalin effect may persist even after the real acute stage (48 hours). However, a problem, that how a possible persistence of adrenalin effect influences the pilocarpin action, is to be further studied. Another series of experiments is now in progress in a close contact with other groups of investigators.

The interpretation would be further supported by the following experiments performed by the author's co-workers. When ACTH and cortisone were administered to mice for examination of the fluctuation of eosinophils, there was no unresponsive phase. Consequently, the effect of ACTH and cortisone do not last so long as adrenalin. This evidence is an another support for the in-
I. The fluctuation of blood sugar level after the first injection

II. The fluctuation of blood sugar level after the second injection

III. The fluctuation of blood sugar level after the third injection

IV. The fluctuation of blood sugar level after the fourth injection

Figure 8 The shift of blood sugar level (Hagedorn method) of the white mice in the adrenalin injections in time interval of 4 hours (By Kunii)

terpretation, that the above-mentioned unresponsiveness to pilocarpin in the acute stage of "ekiri" might be caused by adrenalin persistency. These facts are not yet sufficient to explain all the phenomena in the height of "ekiri" only from the viewpoint of adrenalin effect. But one may definitely conclude that the acute stage of "ekiri" represents sympathetic stimulated status.

CONCLUSION

1. In the second report, the presumable existence of predominating control by sympathetic nervous system was concluded from the daily fluctuations of Na- and K-concentration in saliva in the "ekiri" and dysentery patients. In this report, the influence of pilocarpin, a stimulant of parasympathetic nervous system, on the Na- and K-concentration in saliva was investigated with the "ekiri" and dysentery patients in the acute (1–3 days of illness) and the convalescent stage (the 3. week of illness).

2. The initial increase of Na-concentration in saliva after the pilocarpin injection, which is common to healthy persons and dysentery patients, was not demonstrated characteristically in the acute stage of "ekiri".

3. Consequently, the "ekiri" patients seem to be unresponsive to the stimula-
tion of the parasympathetic nervous system, that is to say, to the stimulant against this system in the acute stage, which is probably explainable from the excitement of sympathetic nervous system.

4. On the other hand, our co-workers, following the shift of eosinophils in mice after the adrenalin injection, confirmed the existence of unresponsive phase to adrenalin. The lack of initial increase of Na-concentration in the acute stage of "ekiri" could be well correlated to this adrenalin insensitive phase. It has thus been confirmed that the "ekiri" patients are in the sympathicotonic state, in other words, under the predominant control of sympathetic nervous system.

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References

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