Studies on Human Adaptability to Climatic Conditions
Report 6
Seasonal Variation in Serum FFA on Civilian Japanese
and Caucasians in Japan

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I. INTRODUCTION

This study was made to investigate whether or not there is any difference in seasonal
variations of physiological functions, especially of serum FFA level, between Japanese and
Caucasians exposed to the same climate in Japan, in reference to the seasonal variations of
basal metabolic rate (BMR).

Gotoh et al.1) studied the seasonal variations of serum FFA in Japanese males and females
by four measurements conducted in February, May, August and October. Serum FFA level
showed the variation of one-peak curve with a significant increase in February as compared
with the other months, the minimal value in May, and an increase in August and October.
Itoh et al.2) reported the result of their experimental study using Japanese and Ainu in
Hokkaido. The BMR and FFA of Japanese showed a significant positive correlation both in
summer and winter and those of Ainu showed a significant negative correlation. Among
Japanese, BMR was high in those with high FFA level, and among Ainu, BMR was low
in those with relatively high FFA level but on the contrary it was high in those with low
FFA level. In Yamaguchi’s report3) dealing with Japan Maritime Self-Defence Force per-
sonnel of group life and much outdoor activities and U.S. Marine Corps personnel having
stayed in Japan for less than one year, the monthly mean of serum FFA level was higher in
Americans throughout the year but its relation with BMR in both groups showed a positive
correlation in summer and a negative correlation in winter. In the present experimental
study, the subjects were, unlike those in Yamaguchi’s study, civilian Caucasian males who
had stayed in Japan for the period from one to over ten years living in Japanese houses
together with Japanese in the Japanese mode of living. It was also attempted to follow up
the result of Yamaguchi’s study in such civilians and to examine any ethnic difference in
adaptive changes of FFA to seasonal change of the climate in Japan.

II. METHOD OF EXPERIMENTAL STUDIES

1. Subjects

The subjects of the present study were the same persons selected for the studies in
Report 4 and Report 5, and they consisted of the following two groups.

Group 1: 10 healthy Japanese males consisting of the staff members of the Department
and medical students ranging in age from 21 to 55 at the commencement of the study.

Group 2: 10 healthy Caucasian males consisting of college teachers and priests ranging
in the period of stay in Japan from 0.7 to 16 years and in age from 24 to 64 at the com-
mencement of the study.
2. Procedures

After awakening early in the morning, respirated air was collected in a Douglas bag for BMR measurement by Chen in Report 4, and then, while the subjects were resting in bed, the blood sample was drawn from the cubital vein for determination of serum FFA level by Itaya-Ui's method modified by Kushiro et al. Since each determination curve shows a slight parallel movement, each determination was accompanied by the measurement of two tubes of chloroform, and two tubes each of chloroform solution of 250 μEq/l, 500 μEq/l, 750 μEq/l, 1,000 μEq/l and 2,000 μEq/l palmitic acid, totalling 12 tubes. A regression line formula was obtained from the colorimetric values of the blind crossover and the colorimetric value of each sample was applied to this formula to obtain FFA level.

3. Climatic conditions and life environments

Although a detailed description was made by Chen in Report 4, the outline is given here again.

During the period of the present experimental study, the mean external temperature was 16.7°C, mean atmospheric pressure 1,015.1 mb and mean humidity 73.3%. These climatic conditions were practically identical with the mean values during the past 20 years in Nagasaki.

The room temperature was measured by providing a self-recording thermometer in the house of each subject. As shown in Fig. 5, the monthly means of room temperature were generally lower in Caucasians than in Japanese and the annual mean room temperature was 24.75°C for Caucasians and 25.37°C for Japanese. The other experimental conditions were the same as those in the previous reports by Chen and Tsuchimoto.

III. RESULT AND DISCUSSION

1. Preliminary test for serum FFA

(a) In order to examine the accuracy of Itaya-Ui's method modified by Kushiro et al., for determination of FFA level, five kinds of samples (No. 1~No. 5) were repeatedly determined five times on the same day under the same conditions. As a result, as shown in Table 1-(1), stable values of relatively small variations were obtained and the coefficient of variation (CV) was as low as 2.75% in mean. The analysis of variance of the determined values by the number of times of determination and sample revealed no significant differences among the values of repeated determinations as shown in Table 1-(2).

(b) Experiment on preservability of serum FFA

According to Kushiro et al., the increased rate by preservation at frozen state was lower as compared with the rate by preservation at room temperature and at 4°C, and yet it increased to 19% on the third day and 43% on the eighth day but decreased to 10% on the 15th day and marked 20% on the 30th day. In this respect, the author conducted the following preliminary experiment. Five kinds of samples (A~E) were divided into 6 tubes and preserved in a frozen state from March 5 to June 11. The results of 6 determinations at certain intervals during the period were as shown in Table 1-(3). No significant difference was revealed during the period as shown in Table 1-(4). Hence, it is assumed that there was no change in the value of determination during such a period of preservation.

In the present study, blood samples collected within the range of a few days were preserved in a frozen state and examined collectively on monthly basis since simultaneous blood drawing from all subjects was technically difficult because of the experimental schedule. However, the time interval between the date of blood drawing and the date of determination seems to be of no problem according to the result of the above preliminary experiments.
Table 1-(1) Preliminary test for serum FFA determination values of repeated
determinations of the same sample and their statistic review

<table>
<thead>
<tr>
<th>Number of times</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
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<td>436</td>
<td>578</td>
<td>403</td>
<td>607</td>
<td>351</td>
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<td>±100.1</td>
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<tr>
<td>2</td>
<td>430</td>
<td>526</td>
<td>416</td>
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<td>345</td>
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<td>±98.6</td>
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<td>±107.2</td>
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<td>610</td>
<td>351</td>
<td>468.0</td>
<td>±94.3</td>
<td>20.15</td>
</tr>
</tbody>
</table>

Mean: 427.2 ±12.2 S.D.: standard deviation
C.V.: coefficient of variation

Table 1-(2) Preliminary test for serum FFA determination Analysis of
variance for time of determinations and number of samples

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variation S</th>
<th>Independence n</th>
<th>Variance S/n</th>
<th>Variance ratio F</th>
<th>Probability P</th>
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<tr>
<td>Times (M)</td>
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Table 1-(3) Preliminary test for serum FFA determination
Chronological change of serum FFA levels

<table>
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<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V. (%)</th>
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<td>355</td>
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<td>360</td>
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<td>±34.3</td>
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</tr>
<tr>
<td>3rd (March 27)</td>
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<td>383</td>
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<td>355</td>
<td>395.6</td>
<td>±51.9</td>
<td>13.12</td>
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</tr>
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<td>4th (April 10)</td>
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<td>±63.5</td>
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<tr>
<td>5th (May 12)</td>
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<td>±57.7</td>
<td>14.58</td>
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<td>6th (June 6)</td>
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<td>420</td>
<td>350</td>
<td>369.0</td>
<td>±31.7</td>
<td>8.57</td>
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</tr>
</tbody>
</table>

Mean: 423.8 ±23.1 S.D.: standard deviation
C.V.: coefficient of variation

Table 1-(4) Preliminary test for serum FFA determination Analysis of
variance for date of determinations and individuals

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variation S</th>
<th>Independence n</th>
<th>Variance S/n</th>
<th>Variance ratio F</th>
<th>Probability P</th>
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</table>

2. Serum FFA levels of subjects and their seasonal variations

Serum FFA levels of all subjects, mean values and 95% confidence intervals are shown
in Table 2-(1) and 2-(2) and Fig. 1.
Table 2-(1) Serum FFA levels in the Japanese Subjects (µEq/l)

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<tr>
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<td>390</td>
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</table>

Mean 29.4 530.5 520.8 517.8 449.5 439.3 440.0 438.5 435.9 457.0 423.6 434.8 4516.5 460.9

95% C.I. 120.1 193.8 167.1 103.5 82.7 82.8 72.3 73.3 65.4 75.3 109.1

The annual mean values for all subjects showed no significant difference between 477.1±66.4 µEq/l in Caucasian and 460.9±73.2 µEq/l in Japanese, the former being somewhat higher. The mean age of the 10 Japanese subjects was 29.4 years while that of the 10 Caucasian subjects was 39.5 years, the difference being about 10 years. Novak reported that fasting FFA level was high in infants and Okinaka reported that FFA level in adults showed a trend of increase with age. However, the difference in FFA level between the two groups in the present study may not be attributed to the difference in age between these groups.

The mean values in Yamaguchi’s study were significantly higher at 5% level in the U.S. Marine Corps personnel than in the Japan Self-Defense Force personnel. This difference was attributed mainly to the difference in room temperature in cold seasons between the two groups being so great as 10°C.

As shown in Fig. 1, the both Caucasian and Japanese groups showed a trend of gradual decrease during the period from August to January although there was some difference between the two groups. The Caucasian group as compared with the Japanese group showed a trend of gradual decrease during the period from August to January although there was some difference between the two groups.

Table 2-(2) Serum FFA levels in the Caucasian Subjects (µEq/l)

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Mean 39.57 613.9 642.9 473.8 531.4 446.7 436.3 452.5 356.0 410.4 561.4 558.8 563.1 477.1

95% C.I. ± 134.9 105.1 91.7 172.0 114.9 133.5 93.9 64.2 67.4 118.4 191.8 149.5
a sizeable but not significant decrease in February and March and a sizeable increase in April, May, June and July, indicating a trend of higher level in warm and hot seasons and lower level in cold seasons. In comparison of FFA levels among various months, the Japanese group showed no significant difference but the Caucasian group showed a significant difference in February as compared with April, May, June, July, August and September, and in March as compared with April and July ($P=0.05\sim0.01$). Accordingly, the annual variation rate was as great as 49.6% in the Caucasian group while it was 25.2% in the Japanese group.

In comparison among the four seasons, the seasonal mean FFA levels as seen in Fig. 2 were almost equal between spring and autumn, but higher in summer and lower in winter in both groups. In the Japanese group, the summer mean level 522 µEq/l was higher than the winter mean level 438 µEq/l but there was no significant difference between the two means. In the Caucasian group, the difference between the summer mean level 544 µEq/l and the winter mean level 415 µEq/l was significant ($P<0.01$).

Itoh et al.\textsuperscript{9} reported that serum FFA level tended to be lower in the subjects who seemed to be adapted to coldness and higher in those with little experience of coldness. Yamaguchi\textsuperscript{10} observed a trend to increase in summer and decrease in winter in the Japan Self-Defence Force personnel, but on the contrary an increase in winter in the U.S. Marine Corps personnel, and he attributed this to the difference of the room temperature in winter, being lower in the former group and higher in the latter.

In consideration of the FFA levels in the above two reports, the significant decrease in winter in the Caucasian group as observed in the present study may be attributed to the fact that the exposure to coldness of winter might have been greater in the Caucasian group than in the Japanese group. This will be discussed further in the latter part of this paper.

From the above findings it may well be said that the seasonal variation of FFA levels
in the Caucasian group was greater and statistically significant. This corresponds to the greater seasonal variation of BMR in the same Caucasian group in Report 40. It is likely that there is no essential difference in the FFA level between Japanese and Caucasians and that the FFA level in winter is affected by the temperature of the living environment.

3. Caucasian's period of stay in Japan and FFA level

Caucasians' period of stay in Japan ranged from 0.7 to 16 years. However, there was no significant correlation between their annual mean FFA level and their period of stay in Japan. As the Caucasians were grouped into those having stayed in Japan for over 3 years and those less than 1 year, and as their FFA levels represented in 3-month-moving-averages were compared, the decrease for the less than 1-year-group in the period of seasonal cooling was remarkable as shown in Fig. 3. In order to confirm this fact statistically, the regression coefficients for the monthly FFA values in both groups were obtained by plotting the individual values of FFA from July to December on the ordinate against each month on the abscissa, where the months of July to December were indicated by the numbers 1 to 6 respectively. The regression coefficient of FFA values against number of each month was -41.7 in the less than 1-year-group and significantly different from -14.4 in the more than 3-year-group. This suggests that the group staying for a shorter period in Japan had a quicker and greater reaction against the change of the climate from summer to winter.

4. Relation between FFA and RQ of BMR

Fig. 4 Correlation between FFA and RQ (July 1972~June 1973)
An attempt was made to check if serum FFA level was related to fat combustion rate. In correlation distribution (Fig. 4) of serum FFA levels of all subjects during the entire experimental period and RQ levels obtained in the study of BMR by Chen⁴, the Caucasian group as well as the Japanese group showed a trend of negative correlation, though not significant, that RQ decreased with the increase of FFA concentration, being the same as the result of Yamaguchi⁵ on the U.S. Marine Corps personnel and the Japan Self-Defence Force personnel. This indicates that fat combustion rate was high in proportion to serum FFA concentration.

5. Relation of serum FFA with values of other concurrent measurements

In addition to the serum FFA levels in the present study, BMR by Chen⁴, PBI by Tsuchimoto⁵ and other items measured for the same subjects are reproduced and illustrated together in Fig. 5.

Seasonal change is almost the same for serum PBI in both groups and more remarkable for BMR and serum FFA in the Caucasian group than the Japanese group. The pattern of the seasonal change is the same in BMR and PBI and that in FFA is reverse to the above two.

(a) Relation between serum FFA and BMR

A review was made on the relation of serum FFA to BMR determined by Chen⁴. In terms of all individual values during the year, a significant positive correlation \((r=0.22, P<0.05)\) was observed for the Japanese group and a trend of negative correlation, though not significant, was noted for the Caucasian group \((r=-0.19)\), as seen in Fig. 6. This fact suggests that the Japanese group is rather adapted to warmth comparing to the Caucasian group. In order to remove the variations in both items due to individual differences, the relation between FFA and BMR was seen in terms of monthly variation rate against the annual mean of the same subjects, as shown in Fig. 7. While there was hardly any correlation present in the Japanese group \((r=0.081)\), a significantly negative correlation was observed in the Caucasian group \((r=-0.25)\), suggesting that the Caucasian group was adapted rather to cold. This difference of trends of adaptation in

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Fig. 5 Changes in monthly means of BMR, serum PBI and FFA, Body weight and Temperatures
both groups may be attributed to the different temperatures of their living environments.

The correlation between serum FFA and BMR in summer and that in winter are shown in Fig. 8. In summer both Japanese and Caucasian groups showed a trend that BMR increased with the increase of serum FFA but hardly any correlation. In winter, whereas the Japanese group showed a trend of positive correlation ($r=0.33$), the Caucasian group showed a significant negative correlation ($r=-0.54$), wherein BMR decreased with the increase of FFA concentration. This is to say that the above mentioned difference of the trends for adaptation in both groups was revealed more remarkably in the cold seasons.

From the experiments on the rats, Hsieh$^{11}$ found the fact that increased heat production after the infusion of noradrenaline was accompanied with an increase of plasma FFA concentration in the warm-adapted rats, but decrease of that in the cold-adapted rats, suggesting the increased capacity of tissues to oxidize fatty acids. Itoh$^{10}$ found a positive correlation
between BMR and serum FFA in university students and reversely a negative correlation in Ainu in Hokkaido and he supposed that the Japanese subjects were adapted to the warmth and Ainu were adapted to the cold, from the view point of the above fact found by Hsieh. It is understood that the negative correlation between serum FFA and BMR found in the present study by the author means that the Caucasians were forced to adapt themselves to the cold more intensively than the Japanese, and this might have been because of the lower temperature of life environment of the Caucasian group in winter.

(b) Relation between serum FFA and PBI

In the experimental study by Tsuchimoto, the annual variation of PBI much resembled that of BMR, showing a significant positive correlation (r=0.31). In review of the relation between FFA in the present study and PBI in Report 5, both the Japanese and Caucasian groups showed a negative correlation (Fig. 9). The negative correlation in the Japanese group was significant (r = -0.285, P < 0.01). However, the correlations in both groups were significant, when FFA and PBI were expressed in the monthly variation rates to the respective annual means. Among the Caucasians, the over 3-year-group showed no significant correlation but the less than 1-year-group showed a significant negative correlation.
In the previous studies by Yoshida, Kuwano et al., and Yamaguchi, the annual variations of FFA and PBI showed a trend of negative correlation though not significant. Anyway it may not be a negligible fact that serum FFA level tends to be low in the seasons when serum PBI is elevated.

6. Relation between serum FFA and external temperature

Fig. 10 shows the relation between serum FFA level and external temperature. FFA level was high when temperature was high and the former was low when the latter was low. Thus, a significant positive correlation was demonstrated in both the Japanese and Caucasian groups. But, FFA levels of the Japanese were plateau below 20°C, being probably affected by the higher room temperature than that of the Caucasians.

7. Summary

Serum FFA levels were almost the same for the Japanese and Caucasians but their seasonal change was larger and the levels in winter were lower in the Caucasian group, contrary to the results of Yamaguchi.

However, these incompatible results by the author and Yamaguchi were probably attributable to the difference of the environmental conditions for the subjects in these two experiments. It is considered that the serum FFA values were lower in the subjects who lived in the lower temperature of the microclimate in winter than in the subjects living in the higher temperature whether they were Japanese or Caucasians. In review of the results by author and by the coworkers dealing with BMR and PBI, it was also confirmed that the Caucasians who had come to Japan lately tended to react more quickly and strongly to the seasonal cooling in Japan.

IV. CONCLUSIONS

Serum free fatty acid (FFA) levels and their seasonal variations were compared between 10 Japanese subjects (staff members of Hygiene Department and medical students of Nagasaki University) and 10 Caucasian subjects (college teachers and priests) living in Nagasaki City, Japan. Determinations were conducted monthly for one year from June 1972 using Itaya-Uii's method modified by Kushiro et al. After studying the FFA levels, seasonal variations and
relations with BMR and other items, the following conclusions were reached.

1. Serum FFA levels did not differ in mean throughout the year between the two groups. The seasonal variations were smaller in the Japanese group (25.2%) than in the Caucasian group (49.6%). The Caucasian group showed lower levels than the Japanese group in January and February indicating the variations of great decrease in winter. This result is incompatible with that of Yamaguchi on the U.S. Marine Corps personnel and Japan Self-Defence Force personnel. This difference between both results is discussed and attributed not to the ethnic difference but to the difference of the living room temperature for the experimental subjects.

2. Decrease of the serum FFA values were quicker seasonally and remarkable in the Caucasians who had come to Japan in recent one year than those who had stayed in Japan for many years, suggesting that the former were not accustomed to the vehement change of the climate in Japan.

3. Correlation between serum FFA values by the author and basal metabolic rates (BMR) obtained by coworker Chen was examined. Significant positive correlation for the Japanese, but not significant negative correlation for the Caucasians were found in the whole values through the year.

Seasonally, there was no correlation in summer, however in winter a significant positive correlation was found in the Japanese and a significant negative correlation in the Caucasians. These facts suggested that the Japanese group was adapted rather to the warmth and the Caucasian group rather to the cold, being attributed to the difference of the room temperature which was relatively high in the former and relatively low in the latter.

4. Between serum FFA level and PBI, a negative correlation was noted in both groups of Japanese and Caucasians.

5. In view of the results of the present study including Report 4 by Chen and Report 5 by Tsuchimoto, there seems to be no essential difference in seasonal variations of serum FFA, including BMR and serum PBI, between Japanese and Caucasians living under the same Japanese climatic conditions. The level and the pattern of the seasonal changes in these items are controlled rather by the temperature conditions of the microclimate of the environment than by ethnical factors.

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ヒトの気候順応能に関する研究 第6報
日本の気候下に於ける一般市民日本人と白人の
血清の遊離脂肪酸の季節変動

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先に陳・鎧本が報告したBMRと血清PBIに関する研究と併行して、同じ日本の気候に曝露される日本人10名と、白人10名の被検者について、血清遊離脂肪酸（FFA）濃度を1972年6月から毎月1回1年間にわたりItaya-Utの方法を用いて測定した。

（1）血清FFA値の年間平均値には日本人と白人の間に差がなかった。これは教室の山口の米軍人と日本自衛隊員と比べて前者が有意に高かったという報告とは異なる結果である。そして白人のFFA値は1月2月の暖冬期に日本人に比べて低く、かつこの季に大きく低下する動きを示し、その年間変動率（49.6％）は日本人（25.2％）よりも有意に大きかった。この点も山口の結果とは逆であった。また白人でも日本に移住して間もない者の寒期の低下は著しかった。

（2）FFA値とBMR値とは、日本人では有意の正相関を示したのに対し、白人では負の相関傾向を示した。両計測値で各個人の平均に対する各月の変動率でみると白人の負の相関係数は有意であった。また季節別にみると、夏期のFFA値とBMR値との関係は日本人、白人とも正相関の傾向にとどまるが、冬期には日本人は正相関傾向を示すのに反して白人では有意の負の相関を示した。

山口は冬の居住気温が日本人で10℃でも低かった事が主因となって、日本人の方がより強く寒冷に順応されたものと解しているが、（1）（2）の結果を総合すると、本研究では逆に白人被検者の方がより寒冷に順応したものと解される。したがってFFA値の水準や季節変動には人種的な差はなく、その寒冷期の居住環境が大きく関与すると推論された。血清PBI値とFFA値は両群とも負の相関傾向を示した。血清FFA値と基礎代謝の呼吸商（RQ）の間に年間を通じて有意ではないが負の相関傾向がみられた。

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