STUDIES ON ACID MUCOPOLYSACCHARIDES IN EXPERIMENTAL HYPER- AND HYPOPARATHYROIDISM

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SYNOPSIS

Changes in acid mucopolysaccharide metabolism were studied using ^35S^-sodium sulfate in experimental hyper- and hypoparathyroidism in rats. Urinary excretion of ^35S, reaching the maximum 12 hrs. after the administration of isotope, was higher in hyperparathyroidism and lower in hypoparathyroidism than in controls. In bone tissue, ^35S activity showed a marked increase in the groups with parathyroid extract administration, especially in the incisor and epiphyses during the process of growth. The concentration of ^35S also increased in the kidney, liver, muscle and skin in hyperparathyroidism, while the slight changes or a tendency to decrease was seen in hypoparathyroidism. ^35S in serum albumin and g-globulin fractions increased in hyperparathyroidism and showed a tendency to slight decrease in hypoparathyroidism. ^35S in a-globulin increased and ^35S in b-globulin showed a decrease in all experimental animals as compared with controls.

Parathyroid hormone has been known to exert an important action not only on bone but also on the ground substance of other connective tissue.

The relationship between parathyroid hormone and acid mucopolysaccharide (abbreviated as AMPS), as important constituent of the ground substance was suggested by the theory of Collip (1934) and Selye (1942) as regards the direct action of parathyroid hormone on bone, and by the theory of Gersh and Catchpole (1950), that depolymerization of the ground substance of connective tissue occurs in certain physiological states and in many pathological conditions and that this depolymerization results in the production of soluble glycoproteins from the connective tissue.

Engel (1952) explained the elevation of serum mucoid following parathyroid extract (abbreviated as PTE) administration with the appearance of degradation product of bone matrix in the blood stream. Shetlar et al. (1956) also suggested a specific action of PTE on elevation of serum mucoid.

Based on the elevation of ^35S in the long bone and the other tissues following PTE administration, Bronner (1957) concluded that PTE acts on the AMPS of bone matrix in addition to bone mineral.

Although changes in AMPS were already studied upon induction of experimental hyperparathyroidism through PTE administration, no corresponding study have been made upon restriction of dietary calcium and phosphorus or parathyroidectomy until the present study.

MATERIALS AND METHODS

Forty male wister rats were divided into three groups; the group with PTE administration, the
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Table 1. Contents of Diet*

<table>
<thead>
<tr>
<th>Ca : P Ratio</th>
<th>Control</th>
<th>High Ca</th>
<th>High P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 1.5</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>2.46 : 1</td>
<td>37.0</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td>1 : 4.42</td>
<td>10.0</td>
<td></td>
<td>4.5</td>
</tr>
</tbody>
</table>

Ca, P is contained in 0.38%, 0.57% Ca is contained in 1.40% P is contained in 1.57%

<table>
<thead>
<tr>
<th>Low Ca</th>
<th>Low P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>65.0</td>
</tr>
<tr>
<td>Casein sodium</td>
<td>18.0</td>
</tr>
<tr>
<td>Ebios (vitamin B complex)</td>
<td>3.0</td>
</tr>
<tr>
<td>Cod-liver oil</td>
<td>0.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>9.7</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>3.2</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>5.5</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>0.2</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium Phosphate (Na,HPO₄)</td>
<td>3.5</td>
</tr>
<tr>
<td>Potassium phosphate (K,HPO₄)</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Ca : P Ratio 0.375 : 1 1 : 0.66
Ca is contained in 0.15% P is contained in 0.20%

Diet-controlled rats were divided into four groups and had been reared for six months.

* Proposed by the National Institute of Nutrition, Japan.

parathyroidectomized group, and the group fed by a diet controlled for calcium and phosphorus content. The group with administration of PTE were further divided into three groups of 5 rats each, the group treated with a single dose of 300 USP units, the group treated with a single dose of 100 units, and the group with daily administration of 5 units for 20 consecutive days. "Parathyroid Injection (Eli Lilly and Co.)" was subcutaneously injected in the back.

In the second group, 5 rats were parathyroidectomized through the electric cautery under ether anesthesia.

In the third group, 20 rats were divided equally into four groups and were fed for six months the controlled diet shown in Table 1. All the diets were prepared according to the prescription of the National Institute for Nutrition.

Kawamura (1961) demonstrated parathyroid hyperfunction in animals maintained on low calcium and high phosphorus diet and hypofunction in those maintained on high calcium and low phosphorus diet with biochemical and histological methods applied on the parathyroids, sera and long bones. This experiment was designed according to the method.

As the radioisotope, 500μCi of Na₂^{35}SO₄ of
Dainabot was intraperitoneally injected. After isotope injection, animals were kept in a metabolic cage. Urine and feces were fractionally collected to examine the state of $^{35}$S excretion.

Animals were sacrificed 72 hrs. after injection of $^{35}$S through examination. Serum and various tissues were obtained for the test.

Serum protein fractions were analyzed with filter paper electrophoresis (150 volt for 7 hrs.) using barbital buffer ($\mu=0.05$, pH 8.6). The protein fractions on the filter paper stained by bromphenol blue were separated to each fraction. The radioactivity of each fraction was measured with Aloka (TDC-2 type) gas-flow counter (1300 volt, 2 babbles per sec. of Herium gas).

The tissues washed twice by water, were ashed separately by fire after it has been wiped off with filter paper. The radioactivity in the ashed measured with Geiger-Muller counter (1100 volt, counter per min.). Total excretion of $^{35}$S in urine and $^{35}$S content per 1g of each wet tissue was calculated.

### RESULTS

*State of urinary excretion of $^{35}$S in urine*

Injected $^{35}$S was rapidly excreted into urine. As for the changes during the first 24 hrs., $7.4\pm0.09\%$ (the average and S.E.) was excreted in 3 hrs., $9.9\pm0.21\%$ in 6 hrs., and the peak of $13.7\pm0.08\%$ in 12 hrs., $5.3\pm0.02\%$ in 18 hrs., and $3.7\pm0.02\%$ in 24 hrs. in the control group, while in the group given 300u of PTE $8.1\pm0.09\%$ was excreted in 3 hrs., $13.7\pm0.16\%$ in 6 hrs., $26.9\pm0.34\%$ in 12 hrs. showing the peak 1.9 times as high as the control, $7.9\pm0.10\%$ in 18 hrs., and $6.0\pm0.01\%$ in 24 hrs. Each value was higher in the PTE groups than in the control group.

A similar increase was noted in the group given 100u of PTE and the group given 5u of PTE for 20 days. Although the increase and decrease followed the same pattern as the controls, the height of the peak at 12 hrs. was 1.1 and 1.4 times the level of the control group respectively.

On the contrary, excretion of $^{35}$S was lower in parathyroidectomized group than in the controls (Fig. 1).

In the groups given the controlled diet, the high phosphorus and the low calcium with parathyroid hyperfunction showed the peak excretion of $15.1\pm0.03\%$ and $15.2\pm0.12\%$ at 12 hrs. respectively, representing higher values than those in the controls. However, the high calcium and the low phosphorus groups with parathyroid hypofunction gave lower values at each point (Fig. 2).

In the follow-up experiment for 5 days, the group given PTE always gave higher values of urinary excretion than in the controls. The group given 300u of PTE gave the highest value, followed by the group given 5u of PTE for 20 days and the group given 100u of PTE. On the other hand, the parathyroidectomized group gave lower values of $^{35}$S excretion than in the controls.
In the four groups given the controlled diet, the high phosphorus group showed a significant increase in excretion, while the low calcium group showed a slight increase 24 hrs. later followed by a pattern similar to that of the controls. The high calcium and the low phosphorus groups gave patterns quite similar to those in the controls, although total excretion was decreased.

**Tissue distribution of $^{35}$S**

While the administered $^{35}$S widely distributed throughout each tissue of the organism, specific changes were seen in organs which are intimately related to parathyroid hormone. The results are expressed as the rate of changes in $^{35}$S concentration when the control level was expressed as 1.0 (Figs. 3 and 4).

In bone tissue, $^{35}$S distribution showed an
increase in the groups given PTE, especially distinctly in the group given 300u of PTE. The changes were especially pronounced in epiphysis and incisor. Values as high as 1.55 to 2.2 were obtained in the groups given PTE. The low calcium and the high phosphorus groups also showed similar tendency.

Nevertheless, the parathyroidectomized, the high calcium and the low phosphorus groups gave lower values than in the controls. The changes were more distinct in the incisor and epiphysis during the process of bone growth.

In the kidney, an increase was seen in the groups given PTE. The increase was especially pronounced, 1.8±0.10 in the group given 300u of PTE. The group given 5u of PTE for 20 days gave 1.4±0.09. The low calcium and the high phosphorus groups showing chronic parathyroid hyperfunction showed also marked changes. The high calcium and the low phosphorus groups showed slight changes, 0.8±0.09 in the low phosphorus and 0.7±0.08 in the parathyroidectomized group. Generally speaking, a decrease in 35S concentration in the kidney was seen in the group with parathyroid hypofunction.

In the liver, a slight increase was seen in the groups given PTE. Changes were more pronounced in the low calcium and the high phosphorus groups, giving values of 1.4±0.04 and 1.3±0.06 respectively. On the contrary, specific changes were not seen in the high calcium group showing 1.1±0.04 and the low phosphorus group showing 1.0±0.06. The parathyroidectomized group decreased slightly to 0.9±0.01.

In the muscle, an increase was seen in all groups given PTE, especially in the group given 300u of PTE showing the high value of 1.6±0.10. In the groups given the controlled diet, the low calcium and the high phosphorus groups showing parathyroid hy-
perfunction gave increased values. The high calcium and the low phosphorus groups did not give a significant difference from the control, while the parathyroidectomized group gave a decrease to 0.7±0.01. In the groups with hypofunction of the parathyroids, the changes were very little or slightly decreased.

The $^{35}$S concentration in the skin a distinct increase was noted in the groups given PTE, the low calcium and the high phosphorus groups, showing parathyroid hyperfunction. The group 300u of PTE showed 1.7±0.06, and the group given 100u of PTE and the group given 5u of PTE for 20 days showed also higher values. In the groups fed on the controlled diet, the low calcium and the high phosphorus groups gave higher values of 1.5±0.06 and 1.5±0.10, respectively.

However, the high calcium group failed to give a significant difference, giving the value of 1.1±0.10, while the low phosphorus group gave a low value of 0.7±0.03 and the parathyroidectomized group gave 1.0±0.06, which was similar to the control value.

**Distribution of $^{35}$S among serum protein fractions**

In the control group, the distribution of $^{35}$S in albumin fraction was 43.4±0.58%. Among the groups given PTE, the group given 300u of PTE gave the highest value of 45.9±0.36%, the group given 5u of PTE for 20 days showed a slight elevation, and the group given 100u of PTE showed no significant difference from the control (Fig. 5).

On the other hand, the high phosphorus group indicating parathyroid hyperfunction showed the highest distribution of 46.1±0.11%. The low calcium group also showed 45.0±0.42%, a value similar to the group

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**Fig. 5.** Distribution of $^{35}$S in serum fraction. Electrophoretic distribution of radioactivity of serum from rats administered $^{35}$S-labeled sulfate. PTE300 and 100 units was injected only once and 5 units daily for 20 days.

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- PTE300u
- PTE100u
- PTE5u×20
- PTX

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- Average of control group.
- PTX: Group of parathyroidectomy.
Albumim

% 50
40
30
20
10

Fig. 6. Distribution of $^{35}$S in serum fraction. The groups fed by a diet regulated for calcium and phosphorus.

Given 300u of PTE. Groups indicating hypofunction of parathyroid, the parathyroidectomized, the low phosphorus and the high calcium all showed lower values of distribution than in the controls, the parathyroidectomized group did the lowest value of 37.8±0.52% (Fig. 6).

$^{35}$S in $\alpha_1$-globulin fraction was 9.2±0.12% in the controls. A slight increase was seen in groups given PTE: 9.5±0.07% in the group given 300u of PTE, 9.9±0.13% in the group given 100u of PTE, and 10.3±0.20% in the group given 5u of PTE for 20 days. The groups fed with controlled diet all showed high rates except for group the high phosphorus which gave a value as low as 8.2±0.16%. The parathyroidectomized group also showed an increase to 12.4±0.57%.

$^{35}$S content in $\alpha_2$-globulin fraction was 6.6±0.05% in the control group, while increase was seen in the groups given PTE. The highest rate of 8.7±0.11% was exhibited by the group receiving 5u of PTE for 20 days. However, the parathyroidectomized group also showed a high rate of 8.5±0.15%. In the groups given controlled diet, a decrease was noted in the low calcium and the high phosphorus showing parathyroid hyperfunction, while an increase was seen in the high calcium and the low phosphorus showing parathyroid hypofunction. The distribution as high as 8.8±0.07% was seen in the high calcium group.

In $\beta$-globulin fraction, the distribution in the control group was 18.7±0.08%. Distribution was lower in all experimental groups than in the controls. The groups given PTE gave the lowest value of approximately 13.5%. The parathyroidectomized group gave 18.2±0.21% and the low phosphorus group gave 18.0±0.42%, indicating minimal variation in the groups of parathyroid hypofunction.
Distribution of $^{35}$S in $\gamma$-globulin was $21.9 \pm 0.23\%$ in the controls. An increase was seen in all the groups given PTE, the group given 100u of PTE showing the highest value of $24.0 \pm 0.09\%$. Slight increase to $23.1 \pm 0.29$ was also seen in the parathyroidectomized group. Increase was also seen in all the groups given controlled diet. The high phosphorus group showed $25.0 \pm 0.33\%$ and the low phosphorus group showed $24.0 \pm 0.21\%$. Distribution in $\gamma$-globulin showed a tendency of increase regardless of the hyper- or hypofunction of the parathyroids.

**DISCUSSION**

The report of Gersh (1950) on the changes in connective tissue leading to depolymerization suggested the significance of the metabolism of ground substance in various diseases.

Studies on the relationship between parathyroid and the ground substance were carried out mainly on hydroxyproline by Klein et al. (1962) and Dull and Henneman (1963), while only the studies by Shetlar et al. (1956) and Bronner (1957) are available concerning AMPS.

When $^{35}$S-sodium sulfate is given to an organism, most of the $^{35}$S are excreted as inorganic sulfuric acid rapidly into urine and a small portion is incorporated into AMPS and then excreted (Dziewiatkowski, 1952; Bronner, 1961; Koizumi and Mituya, 1964). Most of the $^{35}$S absorbed in the connective tissue are connected with the sulfuric acid of mucopolysaccharide (Bronner 1961).

Dziewiatkowski et al. (1957) and Shetlar et al. (1961) separated AMPS with $^{35}$S from tissue homogenate of kidney and bone of the rat by means of paper chromatography and identified it as chondroitin sulfuric acid A. Di Ferrante (1963) and Rich and Meyers also (1955) reported that most of the AMPS in urine is chondroitin sulfuric acids.

In this study, $^{35}$S excretion showed the highest peak at the 12 hrs. followed by a gradual decrease. Within the 48 hrs., 52.5% was already excreted in urine in the control group and more than 77% in the group given 300u of PTE, while higher values were also obtained in the groups given 5u of PTE for 20 days or 100u of PTE. Moreover, the low calcium and the high phosphorus groups also showed a high excretion rate.

It is, therefore, evident that urinary excretion of $^{35}$S increased in experimental hyperfunction of parathyroid, probably due to the effect on ground substance which released AMPS, causing an increased excretion of $^{35}$S in urine.

On the other hand, urinary $^{35}$S excretion rate was rather low in the hypoparathyroid groups maintained high calcium and low phosphorus diets. A decrease by 12% from the control level was seen in the parathyroidectomized group. From these results, changes in ground substance appear to be small in parathyroid hypofunction.

$^{35}$S concentration in bone tissue showed a marked increase in the hyperparathyroid groups especially in the incisor, epiphysis and diaphysis. On the contrary, the changes were mild in hypoparathyroid groups with a tendency of slight decrease.

Such changes in bone tissue were especially pronounced in the incisor and epiphysis on the process of growth rather than the diaphysis.

Metabolism of calcium and phosphorus (Bronner 1961) and AMPS (Engfeldt et al., 1954; Engfeldt and Hjerlquist, 1954; Kent et al., 1956; Dziewiatkowski et al., 1957) is known to be especially active in the epiphysis.

A pronounced increase in $^{35}$S following PTE administration might be due to the accumulation of AMPS as the repairing process of changes in bone induced by resorption.

In addition to the theory of Collip (1943) on the direct action of PTE on bone, Kawamura (1961) also observed appearance of osteoclasts and osteoclastic resorption of bone in response to PTE administration. On account of such influence of PTE, AMPS is released to blood stream in connection with
the early destruction of bone. Increase of urinary AMPS then takes place and a secondary increase in AMPS in bone was found as the repairing process of the destroyed bone.

Kawamura (1961) observed the appearance of osteoclasts in the low calcium and the high phosphorus groups. The increase of $^{35}$S in the low calcium and the high phosphorus groups in the present experiment is therefore probably due to the resorption and destruction of bone rather than the direct effect of diet itself.

An intimate connection was thus suggested between AMPS in bone and in urine upon parathyroid dysfunction. Such bone changes were even more pronounced in continuous parathyroid hyperfunction than in the temporary parathyroid hyperfunction. The high phosphorus diet group and the group given a small dose of PTE for a long period thus demonstrated more severe changes.

Shetlar et al. (1961) demonstrated an increase in AMPS in the kidney homogenate following PTE administration and this result was confirmed by radio-autography. Bradford et al. (1959) obtained similar result. Nishiyama (1962) further emphasized the important role of AMPS in the mechanism of pathological calcification.

Parathyroid hyperfunction induced by long term maintainance on low calcium is considered to be caused by the decrease in serum calcium due to calcium deficiency, while high phosphate diet probably causes hyperphosphatemia and secondary parathyroid hyperfunction in an effort to maintain the homeostatic mechanism of the organism.

From these results, the increase in $^{35}$S in parathyroid hyperfunction might be considered part of the reparatory process in the interstitial tissue accompanying renal damage although the possibility of non-specific uptake of $^{35}$S by tissues cannot be ruled out. Increase of AMPS mobilized in the process of renal calcification also remains a possibility.

Relationship between the parathyroid and liver was pointed out by Underhill (1914) and Seta and Obara (1950, 1952a, 1952b, 1953), who emphasized functional disturbance of the liver upon parathyroidectomy. Yoshinari (1964) prepared biliary fistula in dogs with abnormal parathyroid function to study the excretion of calcium and phosphate using isotopes and concluded the presence of an intimate relationship between the calcium and phosphorus metabolism of the organism and the liver.

In the liver, increase in $^{35}$S was demonstrated in the group with parathyroid hyperfunction. The change was more pronounced in the group with long term parathyroid hyperfunction due to controlled diet than in the groups given a single dose of PTE.

$\tilde{O}$ (1960) and Kawamura (1961) demonstrated degeneration and necrosis of the liver with histological and electron microscopic technic under parathyroid dysfunction. These changes were more intense in the group with parathyroid hyperfunction.

Balasubramanyan (1953), Lupu (1962) and Galambos (1963) reported the increase in AMPS in the liver during abnormal proliferation of the interstitial connective tissue of the liver. Such increase in $^{35}$S during parathyroid hyperfunction therefore appears to be due to the increase in AMPS in the reparatory process of the hepatic tissue.

Such chronic parathyroid hyperfunction makes the renal changes even more pronounced. Thus Kawamura (1961), $\tilde{O}$ (1960) and others demonstrated histologically necrosis of kidney parenchyma, degeneration of tubles, and calcium deposition. Tsuji and Fujita (1964) reported the increase of AMPS in the interstitial tissue of the kidney accompanying the organic changes in diseases of the kidney, interpreting these results as one of the reparatory phenomena.
during parathyroid hyperfunction especially concerning calcium and phosphorus metabolism.

$^{35}$S increased in the muscle during parathyroid hyperfunction, probably due to the intramuscular storage of AMPS which was increased in blood similar to other tissue.

Concerning the skin, increase of AMPS was pointed out when abnormal state of the interstitial tissue of the skin prevailed. Miki and Nagano (1964) and others pointed out the important role of the AMPS in the ground substance in various diseases of the connective tissue represented by collagen disease.

In the skin, a marked increase was noted in the group with parathyroid hyperfunction. Although the mechanism of such action is still obscure, the presence of abundant AMPS in the skin and the action of PTE on the bone, liver and kidney might indicate an intimate relationship between parathyroid function and AMPS metabolism.

Bollet et al. (1957), Schiller and Dorfman (1957) and Kerby (1958) had reported that AMPS is combine with serum protein and such complex apparently circulates through the blood stream, so that AMPS is present as chondroitin sulfuric acid in normal blood. In disease and infection, an elevation of AMPS was found (Hauss and Hauelsing, 1961).

In the present experiment, $^{35}$S concentration was increased in albumin and $\gamma$-globulin fractions in hyperparathyroid groups, the groups given PTE, the low calcium and the high phosphorus groups. A tendency of increase was noted in $\alpha$-globulin fraction in all experimental groups. The decrease of $^{35}$S in $\beta$-globulin fraction in all groups was at variance with the results of Shetlar et al. (1961), while other results agreed with theirs. But a tendency of decrease in $^{35}$S in albumin fraction was seen in the hypoparathyroid groups, especially in the parathyroidectomized group.

Among various serum protein fractions, albumin fraction fluctuate according to the changes in the synthetic mechanism in vivo and metabolic abnormalities, while $\alpha$- and $\beta$-globulins are more intimately related with metabolism of ground substance as complex proteins such as mucoprotein, glycoprotein and lipoprotein.

Changes in serum protein fractions which frequently take place in diseases of the connective tissue might be related to fluctuations of $^{35}$S in each fraction. Changes in $^{35}$S in these fractions in experimental abnormal parathyroidism might suggest the effect of parathyroids on ground substance.

Shetlar et al. (1956) inactivated the serum calcium elevating action of PTE with formalin and still observed the effect on serum mucoid. Besides the actions on calcium and phosphorus metabolism, PTE might exert a specific action on AMPS. Changes on $^{35}$S fractions in protein fractions due to PTE in the present experiment might be due to the changes in AMPS in response to such specific action of PTE.

From these results, it might be concluded that AMPS widely distributed in various tissues throughout the organism had an intimate relationship with parathyroids. Abnormal parathyroid function always influences AMPS metabolism in a close functional correlation between parathyroids and the ground substance.

SUMMARY

Relationship between parathyroid function and AMPS was studied in experimental hyper- and hypoparathyroidism in mature rats using $^{35}$S-sodium sulfate.

Urinary excretion of $^{35}$S showed a marked increase in animals with parathyroid hyperfunction. Increase of 43% was seen in the group given 300u of PTE. Urinary excretion was generally decreased in animals with parathyroid hypofunction. The parathyroidectomized group showed an especially low value of 15% less than the control level.

In bone tissue a pronounced increase in $^{35}$S was seen in the groups given PTE, especially distinctly in incisor and epiphysis during the process of growth. In groups with para-
thyroid hypofunction, the high calcium, the low phosphorus diet and the parathyroidectomy groups showed a decrease. Changes of $^{35}$S concentration was most pronounced in bone tissue, where the metabolism of AMPS mainly took place.

In the kidney and liver, $^{35}$S concentration was increased in parathyroid hyperfunction, probably representing the increase of AMPS in the reparatory process of the tissue injured by hyperfunctioning parathyroids.

ACKNOWLEDGEMENT

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REFERENCES


