Cyclic AMP Metabolism in Cholesterol-rich Platelets*

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ASRU K. SINHA, SANFORD J. SHATTIL, AND ROBERT W. COLMAN

From the Coagulation Unit of the Hematology-Oncology Section, Department of Medicine, University of Pennsylvania School of Medicine, and the University of Pennsylvania Medical Service, Philadelphia Veterans Administration Hospital, Philadelphia, Pennsylvania 19104

The incorporation of cholesterol into human platelets by means of incubation with cholesterol-rich lecithin dispersions is associated with a decreased fluidity in the phospholipid hydrocarbon core of platelet membranes and an increased sensitivity of platelets to aggregating agents. We examined whether the platelet membrane enzyme, adenylate cyclase is influenced by changes in phospholipid fluidity. After incubation for 5 h at 37°C with cholesterol-rich lecithin dispersions, platelets demonstrated a 55% increase in membrane cholesterol content associated with a 5- to 10-fold decrease in the sensitivity of these platelets to the aggregation inhibitor and stimulator of adenylate cyclase, prostaglandin E1. However, these platelets were normally sensitive to direct inhibition of epinephrine-induced aggregation by dibutyryl adenosine 3':5'-monophosphate.

Platelet cholesterol content increased in platelets during incubation with cholesterol-rich lecithin dispersions, associated with a parallel increase in both platelet adenosine 3':5'-monophosphate content and the basal activity of adenylate cyclase. At 5 h, platelet cyclic AMP levels had increased 56% and basal adenylate cyclase activity had increased 2.5-fold. In contrast, platelets incubated with pure lecithin dispersions lost 21% cholesterol and their basal cyclase activity decreased 49%. The adenylate cyclase of control platelets was stimulated 3.5-fold by prostaglandin E1 (1.0 µM) and 4.5-fold by sodium fluoride (1.0 mM). In contrast, the adenylate cyclase of cholesterol-rich platelets was stimulated neither by prostaglandin E1 nor by sodium fluoride. Adenylate cyclase in cholesterol-depleted platelets was stimulated normally by prostaglandin E1. The kinetic constants of adenosine 3':5'-monophosphate for phosphodiesterases (EC 3.1.4.17) of cholesterol-rich platelets were normal.

An increase in the sensitivity of human platelets to aggregation by l-carniphrine or ADP can be induced in vitro by the incorporation of excess cholesterol into platelet membranes by means of incubation with cholesterol-rich lecithin dispersions (1). In platelets (2) as in other mammalian cells (3), an increase in the mole ratio of cholesterol to phospholipid in membranes is associated with a decreased fluidity in the phospholipid hydrocarbon core. This physical perturbation of the membrane bilayer might influence those events responsible for the reception or transmission of the aggregation stimulus. Moreover, decreased phospholipid fluidity might be expected to influence platelet membrane proteins whose functions are dependent upon the presence and physical state of specific phospholipids. The membrane enzyme adenylate cyclase (ATP pyrophosphate-lyase, cyclizing), EC 4.6.1.1) appears to be such a protein (4-6). The product of this enzyme, cAMP, is a potent inhibitor of platelet aggregation. Therefore, we examined cAMP metabolism in and the inhibitory effect of cAMP on cholesterol-rich platelets.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Adenosine 5'-[α-32P]triphosphate (tetra[triethylammonium salt]) (97 Ci/mmol) and adenosine 3',5'-[3H]monophosphate (triethylammonium salt) (20 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. [3H]Adenosine 3',5'-monophosphate (97 Ci/mmol) was obtained from Amersham/Searle, Arlington Heights, Ill. Dibutyryl cAMP was obtained from Sigma Chemical Co., St. Louis, Mo. Prostaglandin E1 was kindly provided by Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.) All other chemicals were reagent grade.

**Platelet Preparation**—All blood donors had abstained from medications for at least 2 weeks before blood donation and had normal serum lipoproteins according to standard criteria. Venous blood was collected through siliconized needles into plastic syringes and anticoagulated by mixing 9 volumes of blood with 1 volume of trisodium citrate (final concentration, 0.013 M). All blood processing was carried out in plastic ware at room temperature. Platelet-rich plasma was obtained by centrifugation of samples for 10 min at 170 g. The remaining blood was centrifuged for 15 min at 1,800 x g to obtain platelet-poor plasma which contained less than 10,000 platelets/µl. Platelets were counted using a Coulter counter model ZB equipped with a 50-µm aperture tube.

**Platelet Incubation System**—Cholesterol-rich, cholesterol-normal, and cholesterol-free phospholipid dispersions (with cholesterol to phospholipid mole ratios of 2.2, 1.0, and 0, respectively) were made from chromatographically pure cholesterol and L-a-dipalmitoyl lecithin (Sigma Chemical Co., St. Louis, Mo.). They were prepared by sonication in modified Tyrode's solution and assayed for purity and stability as reported previously (1, 7). Immediately prior to use, dispersions were centrifuged at 21,800 x g for 30 min to sediment undissolved lipid, primarily cholesterol. Cholesterol-rich dispersions

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(0.013 M cholesterol; 0.0039 M lecithin) incubated with an equal volume of platelet-rich plasma for 5 h at 37°C had been shown previously to increase platelet cholesterol content by 40% without changing platelet phospholipid content or the percentage of distribution of the major phospholipids (1). In contrast, cholesterol-free dispersions (0.0059 M cholesterol; 0.0059 M lecithin) or Tyrode's solution alone do not affect platelet cholesterol or phospholipid content.

In the present experiments, platelet-rich plasma was adjusted to a platelet count of 300,000 to 400,000/µl with platelet-poor plasma, and this mixture was incubated within 45 min of collection with an equal volume of either Tyrode's solution alone or with an equal volume of one of the lipid dispersions in Tyrode's. Mixtures were incubated for up to 5 h in a 37°C water bath with gentle inversion of the incubation tubes hourly. Samples were removed at various times for analysis of platelet aggregation, platelet lipids, and cAMP metabolism. Platelet cholesterol and phospholipid contents were determined on thrice-washed platelets as reported previously (1).

Platelet Adenylate Cyclase Assay—Adenylate cyclase activity was determined by Dowex 50 (H+) chromatography and ZnSO4-Ba(OH)₂ precipitation as described by Krishna et al. (8). Typically, platelets were centrifuged at 4°C at 2500 x g for 10 min, and washed twice with 12.5 mM Tris-HCl buffer, pH 7.5, 2.5 mM MgSO₄, 1 mM theophylline, and 50 µg of sonicated protein in a total volume of 0.1 ml. The reaction mixture (final pH 7.61) was incubated for 10 min at 37°C, then 1 µmol of cAMP was added, and the reaction was stopped by heating in a boiling water bath for 3 min. Recovery of cAMP was monitored in a Packard Tri-Carb scintillation counter (model 3380, with 45% and 100% counting efficiencies for ³²P and ³H, respectively) in Aquasol 2 (New England Nuclear, Boston, Mass.) solvent system. Under these assay conditions, formation of cAMP from ATP was linear for at least 15 min. The adenylate cyclase activity of cholesterol-rich and cholesterol-depleted platelets was verified by the protein kinase binding assay (9).

Cyclic Adenosine 3',5'-Monophosphodiesterase Assay—cAMP phosphodiesterase (EC 3.1.4.17) activity of the platelet sonicate was determined by the rate of hydrolysis of cAMP. A reaction mixture containing 30 µl of a 0.5 mg/ml sonicate in the range of 1 to 500 µM M, was incubated with [³²P]cAMP (50,000 cpm), 2.0 mM MgSO₄, 12.5 mM Tris-HCl buffer, pH 7.5, and 50 µg of sonicated protein in a total volume of 0.1 ml. The reaction mixture (final pH 7.61) was incubated for 10 min at 37°C. The reaction was terminated with the addition of 0.2 ml of 0.2 M ZnSO₄, followed by 0.2 ml of 0.2 M Ba(OH)₂. The precipitate was separated by centrifugation, and the radioactivity of the supernatant was measured. The amount of cAMP hydrolyzed was calculated from the rate of decrease of radioactivity in the supernatant.

Determination of Platelet cAMP Content—Platelet-rich plasma (5 ml) was incubated either with an equal volume of Tyrode's buffer or with Tyrode's buffer containing cholesterol-rich dispersions for various times at 37°C. Platelets were then isolated by centrifugation at 990 x g at room temperature for 30 min, washed twice with Tyrode's buffer, and their cAMP content was determined by the protein kinase binding method (9). Protein was determined according to Lowry et al. (10) using bovine serum albumin as the standard.

Platelet Aggregation Studies—Platelet mixtures (0.5 ml) were placed in a cylindrical cuvette 8 mm in diameter containing a sialic coated stirring bar. Aggregation was studied by adding a threshold concentration (minimal concentration necessary for complete aggregation) of l-epinephrine and measuring the decrease in light transmission as platelet aggregates form in an aggregometer (Chronolog Corp., Broomall, Pa.) with a stirring rate of 1200 rpm at 37°C. The apparatus was calibrated so that the difference in light transmission between platelet-rich and platelet-poor plasma was defined as 100%. Complete aggregation was defined as a greater than 75% increase in light transmittance and was monitored by a decrease in light transmittance in Tyrode (1). For studies of platelet aggregation inhibitors, platelet mixtures were incubated for 30 s at 37°C (with stirring) at various concentrations of PGE₂ or for 10 min (without stirring) with Bt-cAMP prior to addition of l-epinephrine. The minimum inhibitory concentration of PGE₂ or Bt-cAMP was defined as the lowest concentration of inhibitor which decreased the epinephrine aggregation response to less than a 20% change in light transmittance.

RESULTS

CAMP Metabolism in Cholesterol-rich Platelets—The cAMP content of platelets incubated with cholesterol-rich dispersions gradually increased, and at 5 h had increased by 56% (Table I). The cAMP levels of platelets incubated with Tyrode's buffer did not change. The increased level of cAMP in cholesterol-rich platelets suggested that the activity of enzymes responsible for the synthesis and/or degradation of cAMP might be altered by cholesterol incorporation. Several phosphodiesterases capable of degrading cAMP have been described in human platelets (11). The kinetic constants of cAMP phosphodiesterases in cholesterol-rich platelets were similar to those of normal platelets (Table II). Therefore, the elevated cAMP content of cholesterol-rich platelets could not be accounted for by defective catabolism.

In contrast, consistent abnormalities of adenylate cyclase were observed in cholesterol-rich platelets. Basal adenylate cyclase activity demonstrated a progressive increase during the 5 h incubation of platelets with cholesterol-rich dispersions, and this increase was proportional to the extent of cholesterol incorporation (Fig. 1). At 5 h, cholesterol-rich platelets had a basal adenylate cyclase activity 2.5-fold higher than that of platelets incubated for the same period of time with either Tyrode's buffer or cholesterol-normal dispersions (Table III) and this difference was significant (p < 0.01). In further control experiments basal adenylate cyclase was unchanged by the addition of cholesterol-rich dispersions to intact platelets or to platelet sonicates when the assay was performed immediately. In order to demonstrate further the effect of cholesterol on basal adenylate cyclase activity, normal platelets were partially depleted of cholesterol by incubation with 0.1% phospholipid dispersions, and the cAMP content of platelets was measured by the protein kinase binding method. The data presented are the means ± S.E. of two to four experiments.

Effect of cholesterol incorporation on cAMP content of intact platelets

Platelet-rich plasma was incubated either with an equal volume of Tyrode's buffer or with cholesterol-rich phospholipid dispersions at 37°C. At the indicated times, platelets were isolated by centrifugation and washed twice with Tyrode's buffer. cAMP content of the platelets was determined by protein kinase binding method. The data presented are the means ± S.E. of two to four experiments

<table>
<thead>
<tr>
<th>Time of incubation (h)</th>
<th>Platelets and Tyrode's buffer</th>
<th>Platelets and cholesterol-rich dispersions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/10⁶ platelets</td>
<td>pmol/10⁶ platelets</td>
</tr>
<tr>
<td>0</td>
<td>1.62 ± 0.15</td>
<td>1.53 ± 0.09</td>
</tr>
<tr>
<td>2</td>
<td>1.52 ± 0.09</td>
<td>1.74 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>1.50 ± 0.11</td>
<td>1.80 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.52 ± 0.05</td>
<td>2.30 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>1.45 ± 0.07</td>
<td>2.38 ± 0.05</td>
</tr>
</tbody>
</table>

Table II

Kinetic constants for platelet cAMP phosphodiesterase

<table>
<thead>
<tr>
<th>Km (µM)</th>
<th>Vmax (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Kₘ</td>
<td>Normal platelets 50</td>
</tr>
<tr>
<td></td>
<td>Cholesterol rich platelets 60</td>
</tr>
<tr>
<td>High Kₘ</td>
<td>Normal platelets 232</td>
</tr>
<tr>
<td></td>
<td>Cholesterol-rich platelets 243</td>
</tr>
</tbody>
</table>
Relationship between platelet cholesterol content and basal adenylate cyclase activity

Platelet-rich plasma (5 ml) was incubated with an equal volume of either cholesterol-normal, cholesterol-rich, or cholesterol-free phospholipid dispersions for 5 h at 37°C. After incubation the platelets were isolated by centrifugation and the homogenates were prepared as described under "Experimental Procedures." The adenylate cyclase assay system contained 2.0 mM ATP (adjusted to pH 7.2), 2 &mu;Ci of [alpha-32P]ATP, 12.5 mM Tris/HCl, pH 7.5, 2.0 mM MgSO4, 6 mM theophylline (pH adjusted to 7.5), and 50 &mu;g of homogenate protein in a total volume of 0.1 ml. After incubation at 37°C for 10 min, the reaction was terminated by heating in a boiling water bath for 3 min. [32P]cyclic AMP formed was isolated by chromatography on Dowex 50 (H+) and ZnSO4-Ba(OH)2 precipitation and the radioactivity was determined. The results presented here are the means ± S.E. of four to eight experiments. The enzymic activity of the platelets incubated in Tyrode's buffer for 5 h at 37°C (167 ± 1.0 pmol of cAMP formed/mg of protein/10 min) is expressed as the 100% activity.

Table III

<table>
<thead>
<tr>
<th></th>
<th>Adenylate cyclase activity</th>
<th>Cholesterol content</th>
<th>% Tyrode's control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal platelets (Tyrode's buffer)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Normal platelets (cholesterol-normal dispersions)</td>
<td>98.2 ± 4.0</td>
<td>97.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Cholesterol-rich platelets</td>
<td>253.0 ± 3.6</td>
<td>140.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Cholesterol-poor platelets</td>
<td>69.5 ± 0.98</td>
<td>78.6 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

Effect of PGE1 and NaF on Platelet Adenylate Cyclase - The hormonal responsiveness of adenylate cyclase to PGE1 was studied next. PGE1, 0.1 &mu;m, stimulated the adenylate cyclase activity of normal platelets by 2-fold and PGE1, 1.0 &mu;m, stimulated the enzyme 3.5-fold (Fig. 2). This degree of stimulation by PGE1 agrees with the results of Zappia et al. (12) and Krishna et al. (13) although Haslam (14) has reported a 30-fold stimulation. A 4.5-fold stimulation of the enzyme was observed with sodium fluoride (1.0 mM). In contrast, the adenylate cyclase activity of cholesterol-rich platelets was not stimulated by PGE1, sodium fluoride, or a combination of both (Fig. 2). Despite a depressed basal cyclase activity in cholesterol-poor platelets, PGE1 (0.1 &mu;m) stimulated the enzyme 3-fold.

Effect of PGE1 and Bt-cAMP on Aggregation of Cholesterol-rich Platelets - PGE1, inhibits &beta;-epinephrine-induced platelet aggregation by activating adenylate cyclase and increasing platelet cAMP levels (14). When normal platelets were tested immediately after mixing platelet-rich plasma with Tyrode's buffer or with cholesterol-rich dispersions or were tested after incubation with Tyrode's buffer for 5 h at 37°C, no change in platelet lipid composition occurred and the minimal inhibitory concentration of PGE1 was 5 to 25 nM (Fig. 3). The differences between the three groups was not significant. In contrast, when platelets were incubated with cholesterol-rich dispersions for 5 h at 37°C, a 56% increase in the cholesterol content of platelet membranes was observed (1). Concomitant with this, platelets became refractory to the inhibitory effect of PGE1 on epinephrine-induced aggregation.
and the mean minimal concentration of PGE, required to inhibit aggregation was 5- to 10-fold higher than normal (Fig. 3). This difference was significant ($p < 0.01$). These results could not be accounted for by interaction of PGE, with lipid dispersions since preincubation of PGE, with cholesterol-rich dispersions did not affect its inhibitory potency against normal platelets. Platelets incubated for 5 h with cholesterol-normal lecithin dispersions did not differ significantly from controls with respect to their sensitivity to inhibition by PGE.

The concentration of $\mathrm{BaCl}_2$ required to inhibit l-epinephrine-induced aggregation of cholesterol-rich platelets was similar to that required for platelets incubated with Tyrode's buffer for 5 h (Fig. 4). Therefore, cholesterol-rich platelets, although resistant to the inhibitory effect of PGE, on aggregation, were not intrinsically more resistant to cAMP.

**DISCUSSION**

These studies demonstrate that incorporation of cholesterol into platelet membranes resulted in selective changes in the enzymatic activity and function of the platelets. Cholesterol-rich platelets became resistant to the inhibitory effect of PGE, on aggregation, but they showed no change in sensitivity to $\mathrm{BaCl}_2$, suggesting an abnormality of cAMP metabolism rather than a change in the responsiveness of these platelets to this cyclic nucleotide. Basal adenylate cyclase activity increased in proportion to the degree of cholesterol incorporation, and this was associated with an increase in the total level of platelet cAMP. However, platelet phosphodiesterase activities were unaffected. Cholesterol not only increased basal adenylate cyclase activity but also rendered the enzyme unresponsive to stimulation with PGE, or sodium fluoride. This decrease in PGE, responsiveness of adenylate cyclase correlated well with the resistance of cholesterol-rich platelets to the inhibitory effects of PGE, on platelet aggregation. Although GTP levels can modulate the effect of PGE, on adenylate cyclase, the effects observed herein are probably independent of this nucleotide since GTP potentiation only occurs at levels of ATP (0.1 mm) much lower than that employed in this study (13).

Cholesterol is a major structural component of the plasma membrane of mammalian cells and it functions in part to maintain membrane phospholipids in an "intermediate fluid state" (15). Therefore, it is not unexpected that bulk changes in membrane cholesterol content would lead to significant alterations in membrane structure and function. Indeed, using lipid dispersions identical with those employed here, cholesterol enrichment of both human red cell and platelet membranes directly increases microviscosity and the degree of order within the membrane phospholipid core as measured by fluorescence polarization of hydrophobic probes (2, 16). In contrast, microviscosity and the degree of order within these membranes are decreased by cholesterol depletion. In the red cell, cholesterol-induced increases in microviscosity correlate with an inhibition of carrier-mediated co-transport of sodium plus potassium (17) and a decrease in membrane permeability to hydrophilic polyols and organic acids (18). On the other hand, cholesterol-depletion and a decrease in microviscosity correlates with an increased cold-induced compression of membrane surface area and an increase in ion permeability (7). In our previous studies with platelets, cholesterol enrichment resulted in increased membrane microviscosity (2) and an increased sensitivity of platelets to aggregating agents (1), whereas cholesterol depletion decreased microviscosity and the responsiveness to aggregating agents (1, 2).

Taken together, these previous observations suggest that the increased resistance of cholesterol-rich platelets to PGE, is somehow related to the physical effects of excess cholesterol on the platelet membrane. Moreover, the cholesterol effect appears mediated through an alteration of the membrane enzyme, adenylate cyclase. This hypothesis is supported by (a) the known stimulatory effect of PGE, on the adenylate cyclase of normal platelets (19), (b) the inability of PGE, to stimulate this enzyme in cholesterol-rich platelets, and (c) the apparent requirement of phospholipid for maximal hormonal responsiveness of adenylate cyclase (4, 5). Levey has demonstrated that adenylate cyclase solubilized from cat myocardium requires reconstitution with certain phospholipids for optimal stimulation by hormones (4). This regulatory role of phospholipids appears to be operative in intact membranes as well. The glucagon responsiveness of the adenylate cyclase of rat liver plasma membranes correlates with the physical state of phospholipids as modified by temperature (6). In platelets, cholesterol might inhibit the interaction of membrane phospholipid and adenylate cyclase by competing directly with cyclase for interaction with critical phospholipids. Alternatively, cholesterol might restrict the mobility of phospholipid fatty acids and thus impair the transmembrane events necessary for hormonal stimulation of the enzyme. Inhibition of enzyme-phospholipid interaction by cholesterol has been demonstrated for the membrane enzyme (Na$^+$ + K$^+$)-ATPase (20, 21).

The adenylate cyclase of cholesterol-rich platelets was not only unresponsive to hormonal stimulation but also failed to increase in response to high concentrations of sodium fluoride. The mechanism of sodium fluoride stimulation is unclear but is believed to involve directly the catalytic unit of the enzyme rather than surface receptors (22). As with hormonal stimulation, a cholesterol-phospholipid interaction may in some way be responsible for the lack of fluoride stimulation. Fluoride responsiveness of adenylate cyclase in membrane preparations from 3T3 mouse fibroblasts is markedly inhibited by lysol-ecithin (23) which in high concentration acts as a nonionic detergent and thus decreases the interaction of phospholipid with enzyme protein (24). The lack of fluoride responsiveness of the enzyme in cholesterol-rich platelets appears to be a direct effect of cholesterol and not of lysol-ecithin, as this lysophospholipid is present in normal amounts in these platelets (1).

A consistent finding in the present study was an increase in the basal level of adenylate cyclase which was proportional to the degree of cholesterol incorporation. This observation could be explained by (a) a direct effect of cholesterol on adenylate cyclase, (b) a change in conformation of the enzyme as a result of increased microviscosity within the hydrophobic lipid core of the membrane, or (c) a decrease in a "tonic" inhibitory role of phospholipids on adenylate cyclase. The data do not allow a distinction among these possibilities.

Despite the fact that cAMP is a potent inhibitor of platelet aggregation, the increased basal level of adenylate cyclase and cAMP in cholesterol-rich platelets is not inconsistent with our previous observations that these platelets are more sensitive than normal to aggregating agents (1). To understand this, it is necessary to consider the factors which are believed to control platelet aggregation. Aggregation appears to be a result of stimulatory and inhibitory humoral messengers. Aggregating agents, such as l-epinephrine and ADP, are believed to stimulate aggregation by initiating prostaglandin synthesis and the formation of cyclic endoperoxides and thromboxane A2 (23). Cyclic GMP may have a role in platelet
aggregation as well (26). It is unclear whether these substances cause aggregation directly, or whether they in turn mediate shifts in intracellular calcium (27). Moreover, the precise mechanism(s) whereby cholesterol incorporation accentuates these membrane-associated events is unknown.

Opposing these stimulatory influences is cAMP (14), which may inhibit aggregation by increasing calcium binding by membrane proteins (28) and by inhibiting prostaglandin synthesis (29). This duality of control mechanisms makes it essential to consider the state of the stimulatory limb of platelet function when evaluating the net effect of a given level of platelet cAMP. For example an elevation of platelet cAMP occurs in response to vasopressin and thrombin, both of which aggregate platelets (12, 30, 31). Thus, vasopressin and thrombin appear to activate both the stimulatory and inhibitory pathways controlling aggregation, but the net effect is aggregation. Similarly, the increased sensitivity of cholesterol-rich platelets to aggregating agents and their increased levels of inhibitory cAMP may be independent effects of cholesterol on membrane components which affect the process of platelet aggregation in opposite directions.

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REFERENCES
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