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# Biphasic kinetics of inositol 1,4,5-trisphosphate accumulation in gastrin-stimulated parietal cells

## Effects of pertussis toxin and extracellular calcium

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The effects of Pertussis toxin (PTx) and extracellular $Ca^{2+}$ were investigated on gastrin-induced $Ins(1,4,5)P_3$ mass level in isolated gastric parietal cells. Basal $Ins(1,4,5)P_3$ content was 5.48±0.49 pmol/500 000 cells. Gastrin (10 nM) induced a rapid increase in $Ins(1,4,5)P_3$ content which was maximal after 15 s and corresponded to 2–2.5-fold basal level; this $Ins(1,4,5)P_3$ content then decreased within 30 s. After a longer time of gastrin exposure (> 1 min), a sustained and unexpected increase in $Ins(1,4,5)P_3$ accumulation was observed which was maximal at 7.5 min (corresponding to 2.3–2.8-fold basal value) and slightly decreased thereafter. PTx treatment of cells (200 ng/ml) for 3 h or removal of extracellular $Ca^{2+}$ did not affect the rapid rise, but drastically reduced the sustained increase in $Ins(1,4,5)P_3$ content (60–100% inhibition); this inhibition was not evident after 10 min of hormone stimulation. Furthermore, diltiazem, a $Ca^{2+}$ channel blocker, led to a similar inhibition of the sustained increase. We concluded that: (i) gastrin induced a rapid increase in $Ins(1,4,5)P_3$ content via a mechanism insensitive to PTx and to extracellular $Ca^{2+}$, and (ii) gastrin induced a sustained increase in $Ins(1,4,5)P_3$ level via a mechanism sensitive to PTx and to extracellular $Ca^{2+}$. Even though the rapid rise in $Ins(1,4,5)P_3$ content may be involved in the intracellular $Ca^{2+}$ mobilization occurring after the first seconds of hormone stimulation, the physiological role of the sustained $Ins(1,4,5)P_3$ increased level remains to be elucidated.

Gastrin; Inositol 1,4,5-trisphosphate content; Gastric parietal cell; Pertussis toxin; Extracellular $Ca^{2+}$

## 1. INTRODUCTION

Receptor-mediated phosphoinositide breakdown is a wide-spread mechanism of action of hormones on secretory cells which use $Ca^{2+}$ as an intracellular messenger [1]. The gastrointestinal hormone gastrin, stimulates acid secretion from gastric parietal cells through activation of specific receptors [2,3], coupled to a phospholipase C (PL-C) [4–6] which causes $PtdInsP_2$ hydrolysis leading to the production of intracellular messengers: $Ins(1,4,5)P_3$ [6] and 1,2 *sn*-diacylglycerol. This reaction is concomitant with a rise in cytosolic $Ca^{2+}$ concentration, resulting from a dual mechanism: a rapid and transient mobilization of $Ca^{2+}$ from intracellular stores [7–10] probably induced by $Ins(1,4,5)P_3$, and a more sustained rise dependent on extracellular $Ca^{2+}$ [7,9,10].

Further reports evidenced a role for GTP-binding proteins (G-protein) in receptor mediating PL-C activated phosphoinositide metabolism [11]. Three types of G-proteins involved in this process have een described according to their sensitivity to bacterial toxins: (i) a G-protein insensitive to Pertussis toxin (PTx) and Cholera toxin (CTx) [12–15], (ii) a G-protein sensitive to PTx (named '$G_i$-like' protein) [16–19] and a G-protein sensitive to CTx but insensitive to PTx (named '$G_s$-like' protein) [20,21]. Recently, in pituitary cells or adrenocortical cells, several reports evidenced a role for a PTx-sensitive G-protein in the mediation of hormone-stimulated inward $Ca^{2+}$ current [22,23]. Furthermore, some authors concluded the existence of distinct G-proteins involved in the action of angiotensin II or vasopressin on rat glomerulosa cells: a PTx-insensitive G-protein mediating PL-C activity and a PTx-sensitive G-protein probably involved in the coupling process between receptor and $Ca^{2+}$ influx [23,24].

The mechanism whereby gastrin triggers phosphoinositide breakdown is yet poorly understood. Recently, we reported the involvement of G-proteins in gastrin-induced phosphoinositide breakdown since GTP potentiated inositol phosphate release from permeabilized parietal cells stimulated by gastrin [25]. Furthermore, alteration of gastrin effects following PTx treatment of intact cells led to conclude to the existence of a G-protein '$G_i$-like' involved in the action of gastrin on parietal cells [25]; nevertheless, PTx treatment caused 15–50% inhibition of gastrin-induced inositol phosphate accumulation or acid secretion; this observa-

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*Abbreviations:* $Ins(1,4,5)P_3$, inositol (1,4,5)trisphosphate; PTx, pertussis toxin; PL-C, phospholipase C; $PtdInsP_2$, phosphatidylinositol bisphosphate.

tion could be explained by the existence of more than one G-protein involved in the mediation of gastrin effects. Therefore, to specify the role of this '$G_i$-like' protein in gastrin-elicited phosphoinositide breakdown, we have studied the effects of PTx and extracellular $Ca^{2+}$ on Ins(1,4,5)$P_3$ content of rabbit gastric parietal cells following gastrin stimulation.

## 2. MATERIALS AND METHODS

Unsulphated gastrin, [Ahx[1]-HG-17 was a gift of Prof. E. Wünsch and Prof. L. Moroder (Max Planck Institüt für Biochemie, München, Germany). Pertussis toxin and Diltiazem were from Sigma (Saint-Louis, USA). Ins(1,4,5)$P_3$ radioreceptor assay kit was from Amersham (UK). Collagenase was from Serve (Heidelberg, Germany). Nycodenz was from Accurate Chemical (U.S.A.). Standard medium comprised Earle's balanced salt solution without bicarbonate containing 10 mM HEPES and 0.2% bovine serum albumin (pH 7.4). Earle's balanced salt solution was from Biomerieux (France).

### 2.1. *Preparation of isolated rabbit gastric parietal cells*

Cell isolation was carried out following the collagenase/EDTA procedure as already described [3]. Cell separation was performed by counterflow centrifugation with a Beckman elutriator rotor JE6-B. Elutriation fraction containing 70–75% parietal cells was layered onto Nycodenz gradient ($d$ = 1.04–1.08 g/ml) according to the method of Chew et al. [27]. The top fraction of the gradient which contained 90 ± 5% pure parietal cells (≥90% viability) was used for subsequent studies.

### 2.2. *Pretreatment of parietal cells with Pertussis toxin*

Cells ($5 \times 10^6$ cells per ml) were incubated for 3 h at 37°C with or without PTx (200 ng/ml) in standard medium under continuous gassing (95% $O_2$/5% $CO_2$). Cells were then centrifuged (200 $g$, 5 min) and resuspended in indicated medium for subsequent studies. At the end of the incubation period, cell viability was always about 85–95%.

### 2.3. *Ins(1,4,5)$P_3$ content measurement*

Cells ($4 \times 10^6$ per ml) were equilibrated for 5 min at 37°C in standard medium or $Ca^{2+}$-free medium containing 0.2 mM EGTA, without or with agents to be tested, and then incubated with 10 nM gastrin for various periods of time. The reaction was stopped by addition of perchloric acid (5% w/v) and the pH of the supernatant was adjusted to pH 7 by addition of 3 M KOH. 100 µl of supernatant was incubated for 15 min at 4°C with [$^3$H]Ins(1,4,5)$P_3$ (≈ 7 nCi) and with a specific Ins(1,4,5)$P_3$ binding protein. After centrifugation (12 000 × $g$, 3 min), the supernatant was removed and the pellet was assayed for radioactivity content. The amount of Ins(1,4,5)$P_3$ in the test sample was calculated from a standard curve using authentic Ins(1,4,5)$P_3$.

### 2.4. *Statistical analysis*

Results were reported as means ± SE. Data were analyzed for statistical significance using Student's $t$-test (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

## 3. RESULTS

### 3.1. *Effect of gastrin on Ins(1,4,5)$P_3$ content*

In gastric rabbit parietal cells, basal Ins(1,4,5)$P_3$ content was 5.48 ± 0.49 pmol per 500 000 cells. As shown in Fig. 1a, gastrin (10 nM) caused a rapid Ins(1,4,5)$P_3$ accumulation which was maximal after 15 s of hormone stimulation and corresponded to 2–2.5-fold basal level ($P<0.001$ vs. basal level). This stimulation rapidly

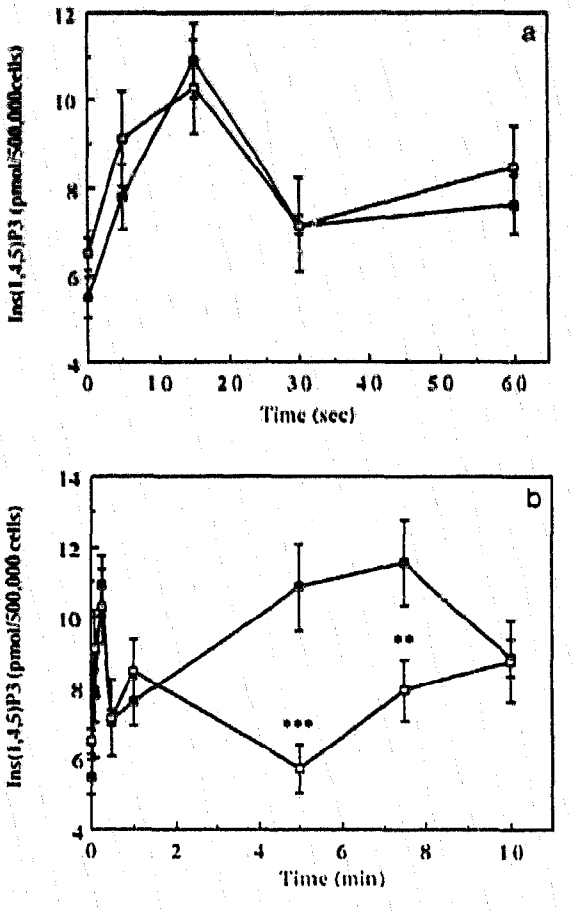


Fig. 1. Effect of Pertussis toxin (PTx) on time course of Ins(1,4,5)$P_3$ accumulation in gastrin-stimulated parietal cells. Cells preincubated for 3 h at 37°C with (□) or without (■) PTx (200 ng/ml) were then incubated with 10 nM gastrin for indicated times; Ins(1,4,5)$P_3$ accumulation was quantified as described in section 2. Results are expressed as pmol per 500 000 parietal cells and are means ± SE from 5 separate experiments. Statistically different from data from experiments without PTx at $P<0.01$** and $P<0.001$***.

decreased within 30 s. After a longer time of gastrin incubation, a sustained increase in Ins(1,4,5)$P_3$ content was observed; this increase was maximal after 7.5 min of stimulation which represented 2.3–2.8-fold basal level ($P<0.001$ vs. basal level), and slightly decreased thereafter (Fig. 1b).

### 3.2. *Effect of PTx treatment on gastrin-induced Ins(1,4,5)$P_3$ accumulation*

Pretreatment of parietal cells for 3 h with PTx (200 ng/ml) led to a weak but significant increase of basal Ins(1,4,5)$P_3$ accumulation: Ins(1,4,5)$P_3$ mass level was 6.48 ± 0.36 pmol per 500 000 treated cells. However, no significant difference on gastrin-induced Ins(1,4,5)$P_3$ accumulation could be observed between PTx treated and untreated cells within the first minute of stimulation (Fig. 1a): a rapid increase in Ins(1,4,5)$P_3$ content occurred, maximal within 15 s, and represented about two fold basal level (Fig. 1a). On the other hand, PTx

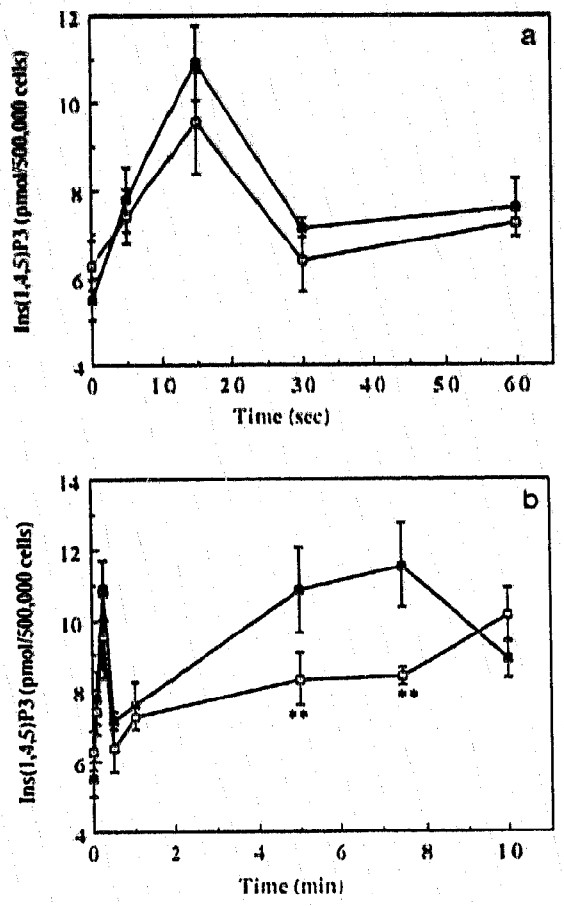


Fig. 2. Effect of removal of $Ca^{2+}$ medium on the time course of $Ins(1,4,5)P_3$ accumulation in gastrin-stimulated parietal cells. Cells were preincubated for 5 min in standard medium (■) or in $Ca^{2+}$-free medium plus 0.2 mM EGTA (□) and then incubated with 10 nM gastrin for indicated times; $Ins(1,4,5)P_3$ accumulation was quantified as described in section 2. Results are expressed as pmol per 500 000 parietal cells and are means ± SE from 5 separate experiments. Statistically different from data from experiments in $Ca^{2+}$-free medium at $P<0.01$**.

treatment drastically reduced the sustained increase in $Ins(1,4,5)P_3$ accumulation produced by gastrin (Fig. 1b): a total inhibition of hormone-induced $Ins(1,4,5)P_3$ accumulation was observed after 5 min of stimulation and 70% of inhibition after 7.5 min. It can be noticed that this inhibition was no more evident after 10 min.

### 3.3. *Effect of extracellular $Ca^{2+}$ on gastrin-induced $Ins(1,4,5)P_3$ accumulation*

Preincubation of parietal cells for 5 min in a $Ca^{2+}$-free medium had no significant effect on basal $Ins(1,4,5)P_3$ content: $Ins(1,4,5)P_3$ accumulation was $6.28 \pm 0.56$ pmol per 500 000 cells. When gastrin was added, no significant difference could be observed on $Ins(1,4,5)P_3$ accumulation in $Ca^{2+}$-free medium compared to 1.8 mM $Ca^{2+}$ medium within the first 60 s of stimulation (Fig. 2a): a rapid increase in $Ins(1,4,5)P_3$ content occurred, maximal within 15 s and represented 1.7-2.0-fold basal level. Nevertheless, removal of extracellular $Ca^{2+}$ led to a reduction of the sustained increase in $Ins(1,4,5)P_3$ intracellular level produced by gastrin (Fig. 2b): 60% inhibition of hormone-induced $Ins(1,4,5)P_3$ content was observed after 5 min of stimulation and 65% inhibition after 7.5 min. This inhibition was no more evident at 10 min of gastrin stimulation.

As shown in Table I, removal of extracellular calcium did not significantly modify the inhibitory effect of PTx on gastrin-induced $Ins(1,4,5)P_3$ accumulation. Furthermore, preincubation of cells for 5 min with Diltiazem, a $Ca^{2+}$ channel blocker, did not alter gastrin-induced $Ins(1,4,5)P_3$ accumulation within 15 s but caused 70% inhibition after 5 min.

## 4. DISCUSSION

The aim of this study was to investigate the influence of PTx treatment and of extracellular $Ca^{2+}$ on the $Ins(1,4,5)P_3$ content regulated by gastrin in isolated parietal cells from rabbit gastric mucosa. $Ins(1,4,5)P_3$ basal level, as measured by the radioreceptor assay, is relatively high, but closely resembles those recently found in NIH3T3, NG108 [27], human SH-SY5 neuroblastoma cells [28], or bovine tracheal smooth-muscle slices [29]. This $Ins(1,4,5)P_3$ level may not be attributed to other inositol phosphates such as $Ins(1,3,4,5)P_4$, $Ins(2,4,5)P_3$ or $Ins(1,3,4)P_3$, since these inositol phosphates give less than 1% cross reactivity with $Ins(1,4,5)P_3$ in the radioreceptor assay. Thus, in resting gastric parietal cells, $Ins(1,4,5)P_3$ intracellular concentration could be, if homogeneously distributed within the cells, $>2$ μM (if one assumed that intracellular volume corresponded to cellular volume). Nevertheless, since $Ins(1,4,5)P_3$ releases $Ca^{2+}$ from intracellular stores at submicromolar concentrations in permeabilized cells [30–32], substantial compartmentalization of this second messenger in resting parietal cells could be expected.

As already observed in experiments using [$^3$H]inositol prelabelled parietal cells [6], gastrin provoked an increase in $Ins(1,4,5)P_3$ content. Nevertheless, the measurement of $Ins(1,4,5)P_3$ mass level evidenced a biphasic increase of gastrin-induced $Ins(1,4,5)P_3$ content: (i) a rapid rise within 30 s of stimulation (maximal after 15 s) and (ii) a sustained increase between 1 and 10 min (maximal after 7.5 min). No reports, at this time, described such a profile of action for an agonist on $Ins(1,4,5)P_3$ production; but a similar biphasic regulation of $Ins(1,4,5)P_3$ intracellular content was observed in isolated gastric smooth muscle cells stimulated by gastrin or carbachol (Magous et al. unpublished observations). Furthermore, Lambert et al. reported an $Ins(1,4,5)P_3$ level above the basal value in a human neuroblastoma cell line after long periods of carbachol incubation ($>5$ min) [28].

Table 1

*Effect of Pertussis toxin (PTx), extracellular $Ca^{2+}$ and Diltiazem on $Ins(1,4,5)P_3$ accumulation in gastrin-stimulated parietal cells.* Cells were preincubated without (control) or with PTx (3 h), 10 μM Diltiazem (5 min) in standard medium or $Ca^{2+}$-free medium plus 0.2 mM EGTA and then incubated with 10 nM gastrin for 15 s and 5 min; $Ins(1,4,5)P_3$ accumulation was quantified as described in section 2. Results are expressed as pmol per 500 000 parietal cells and are means ± SE from 3-5 separate experiments.

| | $Ins(1,4,5)P_3$ accumulation (pmol per 500 000 cells) | | |
|---|---|---|---|
| | Incubation with 10 nM gastrin for: | | |
| | 0 s | 15 s | 300 s |
| 1.8 mM $[Ca^{2+}]_{ex}$ | | | |
| Control cells | 5.48 ± 0.48 | 10.9 ± 0.74 | 10.86 ± 1.22 |
| Cells + 10 μM Diltiazem | 6.00 ± 0.49 | 9.90 ± 0.85 | 7.06 ± 0.73** |
| PTx-treated cells (200 ng/ml) | 6.48 ± 0.36* | 10.3 ± 1.07 | 5.73 ± 0.68*** |
| 0 mM $[Ca^{2+}]_{ex}$ | | | |
| Control cells | 6.28 ± 0.56 | 9.53 ± 1.17 | 8.4 ± 0.23** |
| PTx-treated cells (200 ng/ml) | 4.48 ± 0.61 | 9.69 ± 0.56 | 6.89 ± 0.62** |

Statistically different from control (control cells in standard medium) at $P<0.05$*, $P<0.01$**, $P<0.001$***.

The rapid increase in $Ins(1,4,5)P_3$ level induced by gastrin seems to be unrelated to a PTx-sensitive G-protein, since PTx treatment of cells was devoid of effect on this phenomenon. Nevertheless, we showed recently that gastrin required GTP to induce inositol phosphate release from permeabilized parietal cells [25]. Taken together, these results suggest the presence of a PTx-insensitive G-protein, coupled to gastrin receptors and to PL-C mediating hormone-stimulated phosphoinositide breakdown. It seems likely that this rapid increase in $Ins(1,4,5)P_3$ content may be related to the already described [7–10] peak increase in intracellular $[Ca^{2+}]$ induced by gastrin: (i) the time course of $Ins(1,4,5)P_3$ accumulation fits in well with that of intracellular $[Ca^{2+}]$ increase, (ii) both events are independent on extracellular $Ca^{2+}$ [9] and (iii) $Ins(1,4,5)P_3$ was shown to induce a rapid rise in intracellular $[Ca^{2+}]$ in permeabilized gastric parietal cells [33,34].

Conversely, the sustained $Ins(1,4,5)P_3$ accumulation observed in the presence of gastrin may be related to a PTx-sensitive G-protein, since toxin treatment of cells led to a drastic reduction of this increase. These results confirm our previous observations indicating the existence of a PTx-sensitive G-protein involved in the effects of gastrin on parietal cells [25]. Therefore, the dual effect of PTx on the hormone-induced $Ins(1,4,5)P_3$ content (no inhibition of the rapid increase and inhibition of the sustained response) strongly suggests that gastrin receptors could regulate $Ins(1,4,5)P_3$ level via both pertussis toxin-sensitive and -insensitive mechanism. In this regard, several studies have evidenced a similar mechanism for $P_2$-purinergic receptors mediated $PtdInsP_2$ breakdown [35–37].

Gastrin-induced $Ca^{2+}$ influx may also account in part for the sustained increase in $Ins(1,4,5)P_3$ mass level since removal of extracellular $Ca^{2+}$ or treatment of cells with Diltiazem, a $Ca^{2+}$ channel blocker, caused a reduction of this increase. Thus, the sustained $Ins(1,4,5)P_3$ accumulation could be the result of $PtdInsP_2$ hydrolysis, secondary to a $Ca^{2+}$ entry produced by gastrin [9,10] since calcium enhanced PL-C activity [14,24,38]. In this way, an increase in inositol phosphate accumulation was observed in gastric parietal cells when incubated with the calcium ionophore A32187 (not shown).

Both inhibitions observed following PTx treatment of cells or removal of extracellular $Ca^{2+}$ appeared to be similar; furthermore, inhibitory effect of PTx seemed to be unmodified in a $Ca^{2+}$-free medium. Taken together, these results suggest a possible relationship between $Ca^{2+}$ influx observed after gastrin stimulation and a PTx sensitive G-protein: an attractive hypothesis would be the existence of a '$G_i$-like' protein involved in the coupling process between receptor and $Ca^{2+}$ influx. Such a PTx-sensitive G-protein related to a ligand-induced $Ca^{2+}$ entry has already been reported in rat glomerulosa cells [22,24], pituitary or adrenocortical cells [22]. Nevertheless, the relationship between extracellular $Ca^{2+}$ and his PTx-sensitive G-protein has to be confirmed.

It can be noticed that removal of extracellular $Ca^{2+}$ or PTx treatment of cells did not totally inhibit the increase in $Ins(1,4,5)P_3$ level produced after prolonged gastrin stimulation. Such an effect could be due to the fact that some of '$G_i$-like' proteins were not ADP-ribosylated because of the relatively brief toxin treatment (3 h) or that $Ca^{2+}$ was not completely removed from the medium. Another explanation could be that these factors did not totally account for this sustained increase. Thus, a modulation of the enzymatic activities responsible of $Ins(1,4,5)P_3$ metabolism can not be excluded.

The role of this sustained accumulation is yet unknown; however, this event may be involved in the acid secretory process induced by gastrin, since PTx caused a partial reduction of the hormone-induced acid secretion in this system [25]. However, further experiments are needed to explain the mechanism whereby this biochemical process could be involved in the biological action of gastrin on gastric parietal cells.

In conclusion, this present study clearly showed a biphasic increase in Ins(1,4,5)$P_3$ content following gastrin stimulation of isolated gastric parietal cells: a rapid increase insensitive to extracellular $Ca^{2+}$ or PTx treatment, and a sustained increase produced after prolonged gastrin stimulation (1–10 min), related to $Ca^{2+}$ influx and to a PTx-sensitive G-protein. Furthermore, the effects of PTx led to suggest that gastrin could regulate Ins(1,4,5)$P_3$ content via both pertussis toxin-sensitive and -insensitive mechanisms. Finally, although the rapid increase in Ins(1,4,5)$P_3$ mass level may be involved in the hormone-induced intracellular $Ca^{2+}$ mobilization, the physiological role of the sustained increase remains to be elucidated.

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