The pathway and kinetics of inositol 1,4,5-trisphosphate (IP₃) metabolism were measured in Xenopus laevis oocytes and cytoplasmic extracts of oocytes. Degradation of microinjected IP₃ in intact oocytes was similar to that in the extracts containing comparable concentrations of IP₃. The rate and route of metabolism of IP₃ depended on the [IP₃], and the intracellular free Ca²⁺ concentration ([Ca²⁺]). At low [IP₃] (100 nM) and high [Ca²⁺] (>1 mM), IP₃ was metabolized predominantly by inositol 1,4,5-trisphosphate 3-kinase (3-kinase) with a half-life of 60 s. As the [IP₃] was increased, inositol polyphosphate 5-phosphatase (5-phosphatase) degraded progressively more IP₃. At a [IP₃] of 8 μM or greater, the dephosphorylation of IP₃ was the dominant mode of IP₃ removal irrespective of the [Ca²⁺]. At low [IP₃] and low [Ca²⁺] (both ≤400 nM), the activities of the 5-phosphatase and 3-kinase were comparable. The calculated range of action of IP₃ in the oocyte was ~300 μM suggesting that IP₃ acts as a global messenger in oocytes. In contrast to IP₃, inositol 1,3,4,5-tetrakisphosphate (IP₄) was metabolized very slowly. The half-life of IP₄ (100 μM) was 30 min and independent of the [Ca²⁺] IP₄ may act to sustain Ca²⁺ signals initiated by IP₃. The half-life of both IP₃ and IP₄ in Xenopus oocytes was an order of magnitude or greater than that in small mammalian cells.

Ca²⁺ signals regulate a diverse array of cellular functions including secretion, cytoskeletal rearrangement, and gene transcription (1, 2). Modulations in [Ca²⁺] are used to transduce signals in nearly all cells including those of plants and animals (1–3). Generation and degradation of the second messenger IP₃, which opens the IP₃ receptor/Ca²⁺ channel on the endoplasmic reticulum, regulates the formation and termination of Ca²⁺ signals. Knowing the rates and pathways of IP₃ degradation is fundamental to understanding Ca²⁺ wave formation. The metabolism of IP₃ and its products by phosphatases and kinases is unfolding as an increasingly complex process (4–6). Two primary degradative pathways exist for IP₃, but they differ in their relative importance among cell types and between species. Several isomers of the 5-phosphatase dephosphorylate IP₃ yielding inositol 1,4-bisphosphate (4–8). IP₃ is also a substrate of the 3-kinase which phosphorylates IP₃ to form IP₄ (9–13). The binding of Ca²⁺/calmodulin to the 3-kinase enhances its activity (14–16). The 5-phosphatase also metabolizes IP₄ to inositol 1,3,4-trisphosphate (1-1,3,4-P₃). Current evidence suggests that the major function of the 5-phosphatase in the phosphoinositide cycle is to decrease the [IP₃] and the concentration of IP₃ ([IP₃]). In contrast, the role of the 3-kinase is to generate another second messenger, IP₄, as well as to decrease the [IP₃]. An increasing amount of evidence suggests that IP₄ is an important regulatory molecule in cells (6). Like IP₃, IP₄ may be involved in the regulation of the [Ca²⁺] (17–19). IP₄ binds to the IP₃ receptor/Ca²⁺ channel and releases Ca²⁺ from the endoplasmic reticulum although with a 10-fold lower potency than IP₃. More intriguing, IP₄ binds with high affinity to several intracellular proteins, synaptotagmin I and II, Gap1, Btk, and centaurin-α (20–24). The Ras GTPase-activity of Gap1 is stimulated by IP₄, and IP₄ may interact with synaptotagmin to inhibit synaptic transmission (20, 25). The pathway selected to metabolize IP₄ may not only influence its rate of removal but also alter subsequent signal transduction within the cell.

Much of our mechanistic knowledge of Ca²⁺ wave propagation is derived from studies of Ca²⁺ waves in oocytes and eggs of Xenopus laevis. Formation of the Ca²⁺ wave, which follows the fertilization of an egg and which follows the activation of plasma-membrane receptors in oocytes, requires IP₃ (26, 27). Work to date on IP₃ metabolism in Xenopus oocytes has yielded conflicting results. Microinjection of concentrated [³H]IP₃ into single Xenopus oocytes followed by separation of the metabolites by ion-exchange chromatography has suggested that the 5-phosphatase pathway prevailed (28). However, the addition of [³H]IP₃ to a homogenate of oocytes or microinjection into ovarian follicles followed also by high pressure liquid chromatography (HPLC) separation have provided strong evidence for the 3-kinase as the primary route of IP₃ degradation (29, 30). These discrepancies remain unresolved, despite the central role that the Xenopus oocyte has played in understanding Ca²⁺ wave propagation. All three of the studies discussed above found a surprisingly prolonged degradation time for IP₃, 20 min or longer. This contrasts with smaller cells that degrade IP₃ in a few seconds (31, 32). Establishing both the rate and pathway of IP₃ metabolism in oocytes is required to understand the role of the phosphoinositide pathway in the generation and termination of Ca²⁺ signals and in interactions with other signal transduction cascades. The purpose of this investigation was to determine how IP₃ and IP₄ were degraded in X. laevis oocytes. Moreover, the goal was to quantify the kinetic properties of these metabolic pathways in the cytoplasmic milieu.

Metabolism of Inositol 1,4,5-Trisphosphate and Inositol 1,3,4,5-Tetrakisphosphate by the Oocytes of Xenopus laevis

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‡The abbreviations used are: [Ca²⁺], free Ca²⁺ concentration; IP₃, inositol 1-phosphate; IP₄, all isomers of inositol monophosphate; I-1,4-P₃, inositol 1,4-bisphosphate; IP₅, all isomers of inositol bisphosphate; IP₆, inositol 1,4,5-trisphosphate; I-1,3,4-P₃, inositol 1,3,4-trisphosphate; IP₇, inositol 1,3,4,5-tetrakisphosphate; IP₅, concentration of IP₃ ([IP₃]), concentration of IP₃; IP₄, inositol 1,4,5-trisphosphate 3-kinase; IP₅, inositol polyphosphate 5-phosphatase; IP₆, inositol 1,4,5-trisphosphate 5-phosphatase; IP₇, inositol 1,4,5,6-tetraakisphosphate; HPLC, high pressure liquid chromatography; BAPTA, 1,2-bis(2-amino-phenoxyl)ethane-N,N',N'-tetraacetic acid.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Tritiated inositol and inositol phosphates were purchased from NEN Life Science Products. Nonradioactive IP$_3$ was purchased from Calbiochem and Alexis (Woburn, MA), and nonradioactive IP$_4$ and calpain inhibitor I (N-ac-Leu-Leu-norleucinal) were purchased from Calbiochem. Phorbol 12-myristate 13-acetate was supplied by Alexis (Woburn, MA). EGTA “puriss,” grade, was obtained from Fluka (Ronkonkoma, NY). Rhod-2, BAPTA, and calcium green-5N were supplied by Molecular Probes (Eugene, OR). All other reagents were purchased from Fisher.

**EGTA-buffered Ca$^{2+}$ Solutions**—An equimolar solution of EGTA and Ca$^{2+}$ was prepared by the method of Tsien and Pozzan (33). Cytoplasmic extracts with varying [Ca$^{2+}$] were made by addition of 10 mM EGTA and 10 mM EGTA with 10 mM Ca$^{2+}$. The [Ca$^{2+}$] in the EGTA-buffered extracts was estimated from the [Ca$^{2+}$] in buffer A (135 mM KCl, 5 mM NaCl, 1 mM MgCl$_2$, 10 mM HEPES, pH 7.4) which approximated the intracellular ionic environment and contained the same mixture of 10 mM EGTA and 10 mM EGTA with 10 mM Ca$^{2+}$ as the EGTA-buffered extract. The [Ca$^{2+}$] in the buffer solution was measured using the fluorescent Ca$^{2+}$ indicators, rhod-2 and calcium green-5N, as described by Allbritton and colleagues (34) and Haugland (35). The [Ca$^{2+}$] in buffer A containing 10 mM EGTA and 10 mM Ca$^{2+}$ was 10 mM.

**Preparation of Oocytes and Cytoplasmic Extracts**—Female *X. laevis* frogs were purchased from Nasco (Modesto, CA). Oocytes were surgically obtained and prepared as described previously (34, 36). Cytoplasmic extracts were also made as described previously with the following exceptions (34, 37). To minimize proteolysis the oocytes and extract were maintained at 4 °C throughout the preparation. Calpain inhibitor I (10 μg/ml) was added to the extract to block the Ca$^{2+}$-activated protease calpain. After isolation, the cytoplasmic extract was used immediately in experiments. Prolonged delays prior to use diminished the ability of the extract to metabolize inositol phosphates. This cytoplasmic preparation is ~90% pure cytoplasm (90 mg/ml protein) (34).

**Measurement of IP$_3$ Degradation in Intact Oocytes**—Oocytes were microinjected with 5–30 nl of [H]$IP_3$ (2 μM) contained in buffer A with or without BAPTA (100 mM). The volume of injectate was determined from the total number of counts contained in the oocyte. The oocytes were incubated at room temperature for the indicated times. Just prior (~10 s) to the end of the incubation period, the buffer solution surrounding the oocyte was removed to determine how much of the tritium label had leaked from the oocyte. Cells that leaked greater than 10% of the radioactive label were excluded from experiments. The intracellular reactions of the oocyte were terminated by rapid freezing with powdered dry ice. The frozen oocyte was then homogenized in chloroform: methanol (50 μl of 1:2) and buffer B (50 μl of 25 mM tetrabutylammonium hydrogen sulfate, 20 mM KH$_2$PO$_4$, pH 3.5) with 90 μg of high purity bovine serum albumin added as a carrier to prevent nonspecific loss of inositol phosphates. Hydrolyzed phytic acid was prepared as described by Irvine and colleagues (38). After addition of chloroform (50 μl), the mixture was centrifuged at 15,000 × g, and the aqueous phase was separated by reverse phase HPLC.

**Measurement of Inositol Phosphate Degradation in Cytoplasmic Extracts**—[Ca$^{2+}$] in the cytosol was buffered at the indicated concentration by addition of 10 mM EGTA and 10 mM EGTA with 10 mM Ca$^{2+}$. In some instances, phorbol 12-myristate 13-acetate (50 ng/ml) was included in the cytoplasmic mixture. [H]$IP_3$ or [H]$IP_4$ was then added to the oocyte cytosol. After the addition of each reagent to the cytosolic extract, it was gently mixed over a 5-s period by pipetting the mixture up and down. The cytoplasmic extract was incubated for the indicated time at room temperature (~20 °C). The reaction was stopped with a 16-fold excess volume of trifluoroacetic acid (10%) containing hydrolyzed phytic acid (0.8 mg/ml) and centrifuged at 15,000 × g. The supernatant was dried, resuspended in buffer B, and separated by reverse phase HPLC.

**Reverse Phase HPLC**—Inositol phosphates were separated on an analytical C18 column (Alltech, San Jose, CA) maintained at 45 °C with an elution protocol modified from that of Shayman and Bement (39) and Sulpice et al. (40). The flow rate was 1 ml/min. During the first 22 min, fractions were collected every 0.25 min and thereafter every 1.25 min. To monitor the separation efficiency of the column, a set of standards previously added to and then extracted from cytosol was chromatographed each day. [H]$IP_3$ coeluted with inositol 1-phosphate as did the other isomers of inositol phosphate. The isomers of inositol bisphosphate were not distinguishable.

**RESULTS**

To qualitatively determine the rate of IP$_3$ metabolism in *vivo*, stage VVI oocytes were microinjected with [H]$IP_3$ (2 μM) (n = 11). After incubation for 30 s, 1 min, or 5 min, the oocytes were rapidly frozen to terminate intracellular reactions. The inositol phosphates were extracted from the cytoplasm and separated by HPLC. Representative standard and experimental traces are shown in Fig. 1, A–C. Oocytes microinjected with small volumes of [H]$IP_3$ metabolized half of the [H]$IP_3$ in approximately 1 min (Fig. 1B). Longer times were required to degrade half of the [H]$IP_3$ when larger volumes were microinjected (Fig. 1C). In all of the experiments, the metabolic fraction that contained the most tritium was that of IP$_3$ or IP$_4$. *In vivo* and at an [IP$_3$] of 2 μM and lower, IP$_3$ was metabolized predominantly by the 3-kinase.

In other species the activity of the 3-kinase is increased by the binding of calmodulin and Ca$^{2+}$ (11, 14–16). To determine whether [Ca$^{2+}$] regulated the metabolism of IP$_3$ in Xenopus oocytes, [H]$IP_3$ (2 μM) was co-injected with BAPTA (100 mM) (n = 10) to diminish the IP$_3$-mediated increase in [Ca$^{2+}$]. After a 1, 5-, 15-, or 30-min incubation time, the inositol phosphates were extracted and separated. Remarkably, very little of the [H]$IP_3$ was metabolized by 5 min (Fig. 1D). The half-life of the microinjected IP$_3$ was ~13 min. In contrast to the oocytes without BAPTA, substantial amounts of tritium did not accumulate in the IP$_3$ fraction (Fig. 1, D and E). The activity of the 3-kinase was dramatically decreased in the oocytes containing BAPTA presumably due to a decrease in [Ca$^{2+}$]. In these *in vivo* experiments, both diffusion and degradation altered [IP$_3$] during the time course of the measurements. One minute after microinjection in the absence of degradation, the [IP$_3$] at the microinjection site would still be 10 times greater than the final equilibrium concentration (41). Since [IP$_3$] was also altered by diffusion, these experiments could not be used to measure quantitatively the rates and pathways of IP$_3$ metabolism.

To provide a quantitative description of the metabolism of IP$_4$, subsequent experiments were performed in a cytosolic extract. In past experiments, cytosolic preparations were greatly diluted during preparation, and the compartmentalization of proteins and membranes was abolished by homogenization (29, 30). To eliminate these disadvantages, we used a cytosolic extract that is ~90% of the concentration of undiluted cytoplasm (34). The cytosol contains nearly all of the intracellular organelles, and they retain many of their normal functions including the ability to transit through the cell cycle (34, 37, 42). These observations suggested that this extract could be used to define how [IP$_3$] and [Ca$^{2+}$] regulate the removal of IP$_3$ in an environment similar to the cytoplasmic milieu. [H]$IP_3$ (100 mM) was added to a cytoplasmic extract that contained 10 mM EGTA with 10 mM Ca$^{2+}$ ([Ca$^{2+}$] = 10 μM). At varying times an aliquot of this cytosolic mixture was removed, and the inositol phosphates were extracted and chromatographed. The half-life of the [H]$IP_3$ was 60 s (Fig. 2B). Under these conditions most of the [H]$IP_3$ was converted to IP$_3$. The half-life of IP$_3$ was considerably longer than that of IP$_4$. For incubation times of 5 min or less, the metabolism of IP$_3$ was negligible (see also Fig. 4). By 10 min much of the tritium (~30–40%) initially added to the cytosol was no longer soluble after acid extraction; the [H]inositol was recycled into a cellular component other than inositol or an inositol phosphate. These results are consistent with those obtained in the intact oocytes microinjected with [H]$IP_3$, suggesting that the cytosolic extract is a very good model for the intact oocyte.

For experimental time points of 5 min or less, the cytosolic reactions of IP$_3$ could be segregated into two distinct pathways without common metabolites (Fig. 2A). In the 3-kinase path-
way, IP₄ accumulated with very little conversion of IP₃ to the lower inositol phosphates. Consequently, [IP₄] was used as an estimate of the activity of the 3-kinase for time points of 5 min or less. Inositol bisphosphate (IP₂) was then formed almost exclusively by dephosphorylation of IP₃ rather than by dephosphorylation of I-1,3,4-P₃. Inositol and inositol monophosphate

FIG. 1. Metabolism of IP₃ by intact oocytes. An oocyte was microinjected with [³²H]IP₃ (7 nl (B) or 25 nl (C)) or [³²H]IP₃ and BAPTA (14 nl (D) or 20 nl (E)). After 1 (B), 5 (C and D), or 15 (E) min, the oocyte was frozen rapidly, and the inositol phosphates were extracted from the oocyte and chromatographed. Representative HPLC traces are shown in B–E. An HPLC trace of tritiated standards (~50 nCi each) which were added to and then extracted from cytoplasm is shown in A. The standards were inositol 1-phosphate (I-1-P₁) (fraction 11), inositol 1,4-bisphosphate (I-1,4-P₂) (fraction 19), I-1,3,4-P₃ (fraction 68), IP₃ (fraction 74), IP₄ (fraction 92), and IP₆ (fraction 105). B–E, the migration times of the accompanying set of standards are marked by arrows. The migration times of the standards in A–C differ from those in D and E since the samples were chromatographed on different HPLC systems. The standards eluted in the same order in all experiments. Since IP₆ was not included in the standard mix for the experiments in D and E, the migration time of IP₆ was not marked in these panels.
Metabolism of IP$_3$ and IP$_4$ by X. laevis Oocytes

The schematic of IP$_3$ metabolism was valid for incubation times of 5 min and less when [Ca$^{2+}$] in the extract was elevated (>5 mM) and for 10 min and less when [Ca$^{2+}$] was very low (<100 nM). B and C, [H]$^3$IP$_3$ (100 nM) was added to a cytoplasmic extract with [Ca$^{2+}$] buffered to 10 mM (B) or to less than 100 nM (C). After varying incubation times, the inositol phosphates were isolated and separated by HPLC. [IP$_4$] represents the concentration of IP$_4$. IP$_3$, or IP$_{2-4}$, IP$_{2-4}$ represents the sum of the concentrations of inositol, inositol phosphate, and inositol bisphosphate. The traces are the best fits to the data obtained with Origin (Microcal, Northampton, MA). B, the IP$_3$ and IP$_2$ data points were fit to an exponential function, whereas the IP$_{2-4}$ data points in B and all points in C were fit to a straight line. D, [H]$^3$IP$_3$ (100 nM) was added to a cytoplasmic extract with varying [Ca$^{2+}$] and incubated for 1 min. The concentrations of the inositol phosphates were quantitated and graphed as in B and C. The traces are hand drawn.
Metabolism of IP$_3$ and IP$_4$ by X. laevis Oocytes

IP$_3$ was degraded chiefly by the 5-phosphatase. To determine how a decrease in [Ca$^{2+}$] altered IP$_3$ metabolism at different [IP$_3$], the initial [IP$_3$] was varied from 100 nM to 30 μM in a cytosolic preparation containing 10 mM EGTA (Fig. 3B). The $[^{3}H]$IP$_3$ was incubated in the extract for 5 min followed by acid extraction and chromatographic separation. For all starting [IP$_3$], the rate of formation of IP$_3$ was greatly decreased compared with that in the presence of micromolar [Ca$^{2+}$]. In contrast, the rate of accrual of [IP$_{4,2}$] was nearly unchanged from that in the presence of high [Ca$^{2+}$]. As before the 5-phosphatase was independent of [Ca$^{2+}$]. Moreover, [IP$_{4,2}$] did not depend on [IP$_4$] indicating that these lower inositol phosphates resulted mainly from the action of the 5-phosphatase on IP$_3$. The rate formation of IP$_3$ became independent of [IP$_3$] when the initial [IP$_3$] was $\leq 10 \mu$M or greater. This rate was used to estimate the $V_{\text{max}}$ of the 3-kinase at low [Ca$^{2+}$] (1 nM/s). For most initial [IP$_3$], IP$_3$ was degraded mainly by dephosphorylation, and the rate of formation of [IP$_{4,2}$] was linearly related to the initial [IP$_3$] (Fig. 3B). For an initial [IP$_3$] of 1 μM or greater, [IP$_3$] decreased negligibly during the course of the reaction ($\approx 20\%$). Since [IP$_3$] was nearly constant during the reaction time and [IP$_{4,2}$] was linearly related to the starting [IP$_3$], the reaction of the 5-phosphatase with IP$_3$ was modeled as a first order reaction for [IP$_3$] greater than 1 μM. The $k$ of the 5-phosphatase for IP$_3$ ($6 \times 10^{-4} \text{s}^{-1}$) was then estimated from the rate of formation of [IP$_{4,2}$]. When [Ca$^{2+}$] was in the micromolar range, the metabolic pathway of IP$_3$ depended on [IP$_3$]. At low initial [IP$_3$], IP$_3$ was metabolized predominantly by the 3-kinase, whereas at high initial [IP$_3$], the 5-phosphatase dominated. The crossover point from the 3-kinase to the 5-phosphatase occurred at an [IP$_3$] of approximately 8 μM.

Since IP$_3$ may be an important signaling molecule, the rate and pathway of IP$_4$ degradation were determined. $[^{3}H]$IP$_4$ (100 nM or 10 μM) was added to a cytosolic preparation containing 10 mM EGTA with 10 mM Ca$^{2+}$. At the indicated times the inositol phosphates were extracted from an aliquot of the reaction mixture and separated. For both initial [IP$_3$], 50% of the $[^{3}H]$IP$_4$ was degraded by dephosphorylation in 30 min (Fig. 4). After a 5-min reaction time, only 5% of the IP$_4$ was metabolized. For reaction times of 5 min or less, destruction of IP$_4$ was negligible, and the reaction scheme depicted in Fig. 2A for IP$_3$ was valid. Varying the [Ca$^{2+}$] in the extract from <100 nM to 10 μM did not alter the dephosphorylation of IP$_3$ by the 5-phosphatase or phosphorylation of IP$_3$ by the 3-kinase.

The Michaelis constant ($K_{m}$) of the 5-phosphatase for IP$_3$ is greater than 10 times that of the 3-kinase in many different tissues (4, 5). Therefore, the metabolic pathway of IP$_4$ may depend on [IP$_3$]. To determine how the route of degradation in Xenopus oocytes changed with [IP$_3$], we varied the starting [IP$_3$] in the cytosolic preparation from 100 nM to 30 μM. $[^{3}H]$IP$_3$ was incubated in the extract for 5 min followed by extraction and separation of the inositol phosphates. In the first series of experiments, [Ca$^{2+}$] was maintained at 10 μM by addition of 10 mM EGTA with 10 mM Ca$^{2+}$. For a starting [IP$_3$] of less than 8 μM, the major metabolic pathway was conversion to IP$_4$ by the 3-kinase (Fig. 3A). At an initial [IP$_3$] of approximately 2 μM and greater, the rate of formation of IP$_4$ was independent of [IP$_3$] consistent with [IP$_3$] being much greater than the $K_{m}$ of the 3-kinase. The rate of generation of IP$_4$ at this high [IP$_3$] was used to estimate the maximal velocity ($V_{\text{max}}$) of the 3-kinase for IP$_3$ (4 nM/s). For a starting [IP$_3$] greater than 8 μM, [IP$_4$] continued to increase despite a plateau in the generation of IP$_4$ (Fig. 3A). The additional IP$_{4,2}$ must originate from dephosphorylation of IP$_3$ by the 5-phosphatase. At high [IP$_3$], most of the IP$_{4,2}$ was formed by dephosphorylation of IP$_3$ rather than IP$_4$.
μM did not alter the rate of degradation of IP₃. As in prior experiments, I-1,3,4-P₂ did not accumulate to a substantial degree although [IP₄]₀ increased with time. Conversion of IP₃ to IP₄ was not observed. Metabolism of IP₄ and formation of IP₄ was followed first order kinetics for initial IP₄ concentrations of 100 nM and 10 μM. The k obtained by fitting the [IP₃] or [IP₄] curves to an exponential was 4 × 10⁻⁴ s⁻¹.

**DISCUSSION**

Intact oocytes and a cytoplasmic extract of oocytes were used to determine the pathways and estimate the rates of IP₃ and IP₄ metabolism in *Xenopus* oocytes. The pathway of IP₃ degradation depended on both [IP₄] and [Ca²⁺]. The 3-kinase was regulated by [Ca²⁺]; the measured Vₘₐₓ and k of the 3-kinase decreased as [Ca²⁺] decreased. Lowering [Ca²⁺] from 1 μM to less than 100 nM diminished the k and the Vₘₐₓ by a factor of 25 and 4, respectively. The k of the 3-kinase for IP₃ in the presence of 10 mM EGTA ([Ca²⁺] < 100 nM) was estimated from the slope of the IP₃ trace in Fig. 6C and the initial slope of the IP₄ trace in Fig. 3B (k = 4 × 10⁻⁴ s⁻¹). The Kₘ calculated from the Vₘₐₓ and k increased from 400 nM to 2.5 μM as [Ca²⁺] declined from greater than 1 μM to less than 100 nM. [Ca²⁺] regulated the 3-kinase by altering both the Vₘₐₓ and Kₘ of the enzyme for IP₃. By releasing Ca²⁺, IP₃ enhances its own degradation. The Kₘ values of the 3-kinase and its regulation by Ca²⁺ in *Xenopus* oocytes are close to that reported in other species (4, 5, 10–12). These similar enzymatic features suggest that the *Xenopus* oocyte 3-kinase is regulated by calmodulin as is the case in other species.

The k value of the 5-phosphatase for IP₃ was approximately twice that of the 3-kinase when [Ca²⁺] was low (∼100 nM). However, when [Ca²⁺] was greater than 1 μM, the k value of the 3-kinase exceeded that of the 5-phosphatase by almost 20-fold. Although the Vₘₐₓ of the 5-phosphatase for IP₃ could not be determined, the Vₘₐₓ must be greater than 16 nM s⁻¹, the fastest rate measured for the 5-phosphatase (Fig. 3). The Kₘ of the 5-phosphatase for IP₃ must then be greater than 27 μM. The first order behavior of the 5-phosphatase at IP₃ concentrations up to 30 μM also suggests that the Kₘ is greater than 30 μM. The lower limit of the Kₘ value and the absence of regulation by [Ca²⁺] are consistent with the properties of the 5-phosphatase in other species (4–8, 48–50). For all [Ca²⁺], the Kₘ and Vₘₐₓ values of the 5-phosphatase for IP₃ were much greater than those of the 3-kinase for IP₃. The relative values of the Kₘ and Vₘₐₓ of the 5-phosphatase and 3-kinase divided IP₃ metabolism into three regions. The regions were defined by their [Ca²⁺] and [IP₃] and were characterized by their different metabolic routes for IP₃. At high [Ca²⁺] (>400 nM) but low [IP₃] (<8 μM), IP₃ was predominantly metabolized by the 3-kinase to IP₄. The 5-phosphatase and the 3-kinase degraded roughly similar amounts of IP₃ when both [Ca²⁺] and [IP₃] were low (<400 nM) and <1 μM, respectively. However, the 5-phosphatase was the dominant metabolic enzyme when [IP₃] was greater than 5 μM irrespective of [Ca²⁺]. The regions that occur physiologically are not known since the intracellular range of [IP₃] yet to be defined.

IP₃ was metabolized very slowly with a half-life of approximately 30 min. The k value of the 5-phosphatase for IP₄ was similar to that for IP₃. The reported Kₘ of the 5-phosphatase for IP₄ in other species is approximately 1 μM (7, 51). When the initial [IP₃] was 10 μM, the metabolism of IP₄ followed first order kinetics. Therefore, it is unlikely that the Kₘ of the 5-phosphatase for IP₃ in Xenopus oocytes was as low as 1 μM. When [Ca²⁺] was elevated, phosphorylation of IP₃ to IP₄ was much faster than degradation of IP₄. Consequently, repeated or persistent stimulation of IP₃ production in the presence of high nanomolar to micromolar [Ca²⁺], could result in IP₄ concentra-

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#### Table I

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Metabolism of Inositol 1,4,5-Trisphosphate and Inositol 1,3,4,5-Tetrakisphosphate by the Oocytes of *Xenopus laevis*

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