

Clinical aspects of allergic disease

Detection of the major urinary metabolite of prostaglandin D₂ in the circulation: Demonstration of elevated levels in patients with disorders of systemic mast cell activation

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The symptoms and hemodynamic alterations that accompany episodes of systemic mast cell activation have been largely attributed to excessive prostaglandin(PG)D₂ release. Quantification of the major urinary metabolite of PGD₂ has been invaluable in elucidating a role for PGD₂ in these clinical entities and in the biochemical evaluation of systemic mastocytosis. With the use of a modified mass spectrometric assay for the major urinary metabolite of PGD₂, this metabolite was detected in plasma from 10 normal volunteers (3.5 ± 1.4 pg/ml). Ingestion of niacin, which induces endogenous release of PGD₂, increased plasma levels of this metabolite 6.3 to 33 times above the upper limit of normal by 2 hours. Thereafter, levels declined gradually but remained elevated for up to 6 to 8 hours. In contrast, circulating levels of 9α,11β-PGF₂, the initial metabolite of PGD₂, peaked by 30 minutes and returned to baseline by 2 hours. The clinical utility of measuring the major urinary metabolite in the circulation was demonstrated by detection of markedly increased levels in plasma and serum from patients with systemic mastocytosis and a patient with a severe type I allergic reaction. Thus in the biochemical evaluation of episodes of systemic mast cell activation and endeavors to further elucidate the role of PGD₂ in human disease, there are kinetic advantages of measuring the major urinary metabolite of PGD₂ in the circulation. One particular advantage is the evaluation of clinical events, which only in retrospect are suspected to be associated with excessive release of PGD₂, yet plasma or serum was obtained proximate to the event. (J ALLERGY CLIN IMMUNOL 1994;93:817-24.)

Key words: Prostaglandin D₂, mastocytosis, anaphylaxis, nicotinic acid, prostaglandin metabolism

Prostaglandin (PG)D₂ has been found to be an important mediator in human disease and is the principal cyclooxygenase product of mast cells.¹ PGD₂ is overproduced in patients with systemic mastocytosis, and the severe vasodilation and hemodynamic alterations that accompany episodes

Abbreviations used

PG: Prostaglandin
PGD-M: 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid

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of systemic mast cell activation in these patients have been attributed in large part to the release of excessive quantities of this prostaglandin.² Further, PGD₂ has been causally implicated in the adverse effects of certain pharmacologic agents. For example, the intense cutaneous vasodilation, which accompanies ingestion of pharmacologic doses of niacin administered to reduce serum cholesterol, has been attributed to a property of niacin to selectively release large quantities of PGD₂.³ Interestingly, the release of PGD₂ after ingestion of niacin is not accompanied by a concomitant release of histamine, suggesting that the

cell that is activated by niacin to release PGD_2 is not the mast cell.

Because of rapid metabolism and artifactual generation of prostaglandins by formed elements in blood during sampling of blood and isolation of plasma, it is well recognized that measurement of the metabolites rather than the parent compound is the most reliable approach to assess endogenous production of prostaglandins.⁴ The predominant pathway of metabolism of PGD_2 has been shown to initially involve transformation of PGD_2 by the 11-keto-reductase enzyme to $9\alpha,11\beta\text{-PGF}_2$, which is then further degraded by β - and ω -oxidation and other processes.⁵ The major metabolite of PGD_2 excreted into the urine has been identified as $9\alpha,11\beta$ -dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (PGD-M).⁶ To provide a reliable means to assess endogenous production of PGD_2 , we developed a stable isotope dilution mass spectrometric assay for PGD-M.⁷ Quantification of the urinary excretion of this metabolite has proven invaluable in establishing a role for PGD_2 in disorders of systemic mastocyte activation and other clinical entities. In addition, although quantification of histamine metabolites has been considered the most sensitive biochemical indicator of systemic involvement in patients with mastocytosis,^{8,9} we have recently found that urinary levels of PGD-M are increased to an even greater degree than histamine metabolites in patients with systemic mastocytosis and are often elevated when histamine metabolite levels fall within the normal range (manuscript in preparation).

Quantification of PGD-M in urine to assess endogenous release of PGD_2 is convenient because urine samples can be collected for analysis outside of the clinical setting. Not infrequently, however, patients seek medical attention during or proximate to an event, which only in retrospect is considered to be associated with release of PGD_2 (e.g., systemic mast cell activation). In such situations serum or plasma has often been obtained and saved for routine analyses, whereas urine has not. Under these circumstances, the ability to measure a relatively long-lived and stable circulating metabolite of PGD_2 in plasma or serum would allow determination of excessive PGD_2 release to be made retrospectively.

After endogenous prostaglandin release, early proximal metabolites have been shown to appear quickly in the circulation but are also cleared rapidly.¹⁰ On the basis of previous studies by Granstrom et al.,¹¹ which demonstrated that the

major urinary metabolite of $\text{PGF}_{2\alpha}$ has a much longer half-life in the circulation compared with the more proximal metabolite of $\text{PGF}_{2\alpha}$, 15-keto-13,14-dihydro- $\text{PGF}_{2\alpha}$, we reasoned that the major urinary metabolite of PGD_2 (PGD-M) may also be a relatively long-lived circulating metabolite. Therefore we attempted to determine whether PGD-M could be detected in plasma and serum from normal volunteers and carried out studies to explore the kinetics of its appearance and disappearance in the circulation. For these latter studies, endogenous release of PGD_2 was induced by the ingestion of niacin, which evokes a rapid and marked release of PGD_2 over a limited period of time, approximately 15 to 60 minutes, similar to what occurs during spontaneous episodes of IgE-independent systemic mastocyte activation in patients with mastocytosis and IgE-dependent anaphylaxis in allergic individuals. The kinetics of the appearance and disappearance of PGD-M in the circulation were also correlated with the appearance and disappearance of the initial early metabolite of PGD_2 that is formed, $9\alpha,11\beta\text{-PGF}_2$.

METHODS

Materials

Niacin (nicotinic acid) tablets (500 mg) were obtained from the Vanderbilt University Hospital Pharmacy. Dye mixture no. 1 for thin-layer chromatography was obtained from Analtech, Inc. (Newark, Del.). All other chemicals and reagents were obtained from sources previously described.⁷ Unlabeled PGD-M was chemically synthesized and then converted to the [$^{18}\text{O}_4$]-derivative for use as an internal standard by the method of Murphy et al.,¹² which involves successive steps of methylation and alkaline hydrolysis with Li^{18}OH .^{12,13} Preparation of [$^2\text{H}_7$] $9\alpha,11\beta\text{-PGF}_2$ was as previously described.¹⁴

Assay methods for PGD-M and $9\alpha,11\beta\text{-PGF}_2$

$9\alpha,11\beta\text{-PGF}_2$ and PGD-M were quantified by gas chromatography/negative ion chemical ionization-mass spectrometry as described previously.^{7,14} Minor modifications of the assay described for measurement of PGD-M in urine were incorporated to permit its detection in plasma. Briefly, 2 to 4 ml of plasma or serum was acidified to pH 3 with 1 mol/L HCl, and then 0.88 ng of the [$^{18}\text{O}_4$] PGD-M internal standard was added. The sample was allowed to stand at reduced pH and room temperature for 30 minutes to allow quantitative cyclization of the lower side chain to a hemiketal, lactone. The plasma or serum was extracted with a C-18 Sep-Pak cartridge (Waters Chromatography Div., Millipore, Milford, Mass.) as described for urine; however, elution through a silica Sep-Pak, which had been routinely used in the assay for PGD-M in urine, was omitted. Omission

sion of this step yielded both enhanced recovery and purification of PGD-M in plasma and serum. The upper carboxyl was then methylated, followed by methoximation of the keto group at C-15 by treatment with a solution of 3% methoxyamine HCl in water (300 μ l) and acetonitrile (75 μ l). Borate buffer (1.5 ml, pH 9.1) was then added, and neutral lipids were extracted with 2 ml of ethyl acetate. The aqueous layer was then acidified to pH 3 with 1 mol/L HCl, and PGD-M was extracted with 2 ml of methylene chloride. The lower carboxyl was then converted to a pentafluorobenzyl ester, and the partially derivatized PGD-M was purified by thin-layer chromatography as described for urine.¹⁵ After conversion to a trimethylsilyl ether derivative, quantification of PGD-M was accomplished by selected ion monitoring of the negative ions formed by loss of the pentafluorobenzyl moiety: mass-to-charge ratio was 514 for endogenous PGD-M and 522 for the [¹⁸O₄] internal standard.

Human subjects

In the studies described below involving patients or human subjects, informed consent and the approval of the Vanderbilt Institutional Review Board were obtained. Mastocytosis in these patients was diagnosed by evidence of increased proliferation of mast cells in the bone marrow and increased urinary excretion of histamine metabolites. Two of the three patients also exhibited urticaria pigmentosa, which was confirmed by skin biopsy.

RESULTS

Determination of PGD-M in plasma

With the use of the modified assay for urinary PGD-M, PGD-M could be reliably detected in relatively small volumes (2 to 4 ml) of plasma and serum from normal volunteers. The levels of PGD-M in plasma from 10 normal volunteers (five men and five women) was found to be 3.5 ± 1.4 pg/ml (mean $\pm$ SD). Levels measured in the male subjects (3.7 ± 1.0 pg/ml) were not significantly different from levels measured in female subjects (3.2 ± 1.8 pg/ml).

Biochemical kinetics of PGD-M and 9 α ,11 β -PGF₂ in the circulation after ingestion of niacin

The time course of appearance and disappearance of PGD-M and the initial metabolite of PGD₂, 9 α ,11 β -PGF₂, were compared in three volunteers after ingestion of 500 mg of niacin to induce endogenous release of PGD₂. The biochemical kinetics of PGD-M in the circulation were also correlated with the excretion of PGD-M into the urine. After ingestion of niacin, plasma levels of 9 α ,11 β -PGF₂ increased rapidly. Peak

levels of 54, 154, and 208 pg/ml occurred between 30 and 45 minutes and returned to baseline levels of less than 3 pg/ml by 2 hours (Fig. 1, A). Levels of PGD-M in plasma also increased dramatically after ingestion of niacin. In contrast, the increases of PGD-M in the circulation compared with those of 9 α ,11 β -PGF₂ were delayed and remained elevated for a substantially longer period (Fig. 1, B). At 1 hour after ingestion of niacin, levels of PGD-M had increased to between 3.2 and 8.4 times above baseline and reached a maximum of 12 to 14 times above baseline at 1 to 3 hours. Levels thereafter declined slowly but were still elevated by as much as 2.6 to 4.3 times above baseline in two individuals 7 to 7.5 hours after ingestion of niacin. The time course and magnitude of the increases in excretion of PGD-M into the urine were found to be very similar to the increases in levels of PGD-M in the circulation (Fig. 2).

Comparison of levels of PGD-M in plasma and serum and stability of PGD-M during storage

In clinical situations in which a diagnosis is only considered in retrospect, either serum or plasma, but not both, may be available for analysis. In addition, these samples may have been stored for variable lengths of time under a variety of conditions. Initially, we compared the levels of PGD-M measured in both plasma and serum and examined the stability of PGD-M in plasma and serum during storage at -20° C. Plasma and serum were obtained from a normal volunteer before and 2.5 hours after ingestion of 500 mg of niacin to induce an increase in levels of PGD-M in the circulation. An aliquot of both plasma and serum at baseline and after niacin administration were assayed immediately in triplicate for PGD-M, and aliquots of plasma and serum after niacin administration were then stored at -20° C and assayed 8 weeks later. No significant difference was found between levels of PGD-M measured in plasma and serum, and the levels measured were unaffected by storage at -20° C for 8 weeks (Table I). The latter finding is consistent with the previous demonstration that PGD-M is stable for at least 1 year in urine that has been stored at -20° C.⁷ We also wished to determine whether PGD-M would be stable in a plasma sample stored at room temperature for up to 48 hours. Once again, blood was taken from an individual 2.5 hours after the ingestion of niacin, and the plasma was separated from the blood elements by centrifugation. One

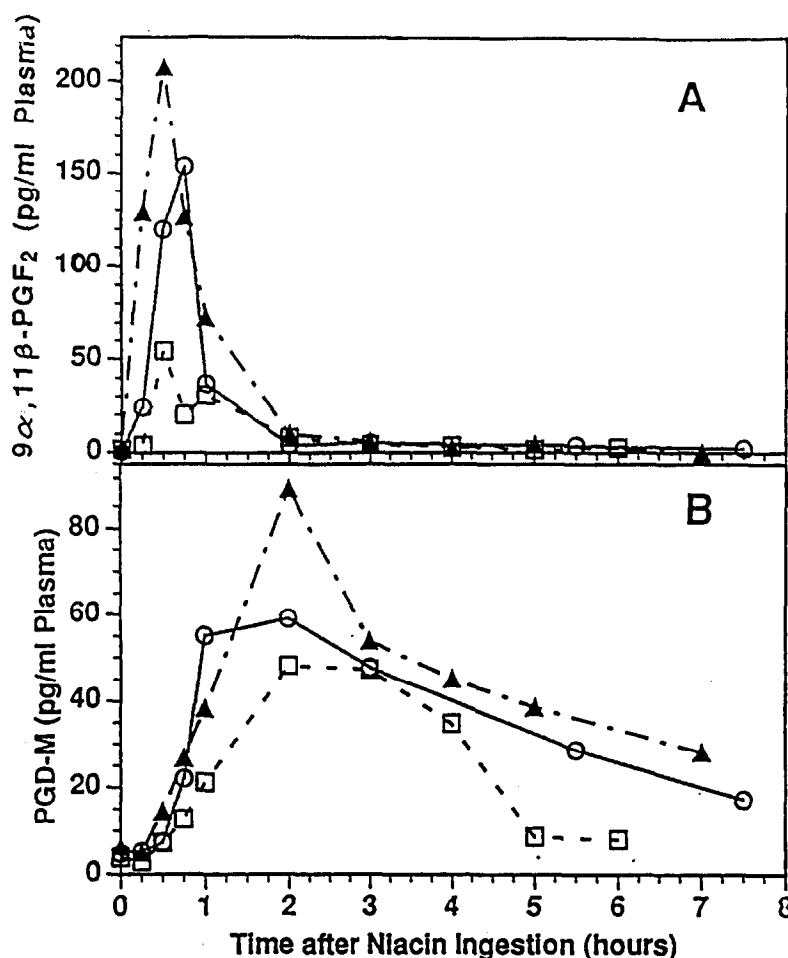


FIG. 1. Plasma $9\alpha,11\beta\text{-PGF}_2$ (A) and PGD-M (B) levels at various times after ingestion of niacin (500 mg) in three normal volunteers (subject 1, squares; subject 2, circles; subject 3, triangles).

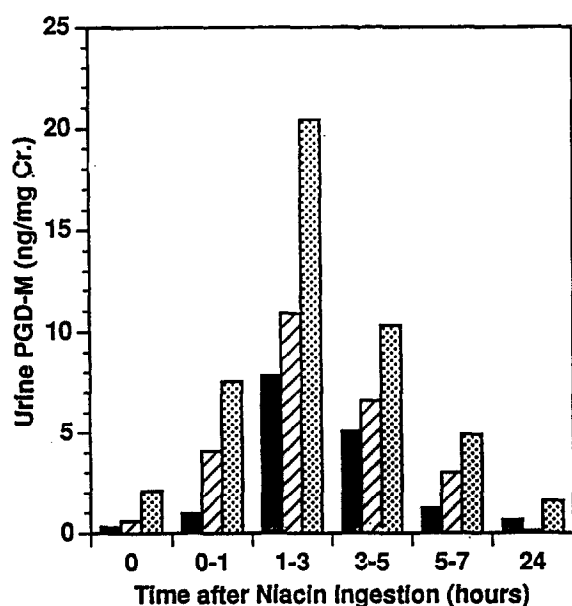


FIG. 2. Urinary excretion of PGD-M after ingestion of niacin (500 mg). Serial urine samples were collected concurrently with the plasma sampling of the three volunteers shown in Fig. 1 (subject 1, solid bars; subject 2, striped bars; subject 3, speckled bars). Cr., Creatinine.

milliliter aliquots ($n = 3$) of the plasma were assayed immediately after plasma separation, and then additional aliquots were assayed after 24 hours and 48 hours at room temperature. Plasma PGD-M levels were 20 ± 2 pg/ml at time 0, 18 ± 3 at 24 hours, and 19 ± 3 at 48 hours. Thus the stability of PGD-M in plasma allows even samples kept at room temperature for up to 48 hours to be used for diagnostic purposes.

Assessment of the clinical utility of measuring circulating levels of PGD-M in the evaluation of disorders of systemic mastocyte activation

PGD₂ is produced in abundance by mast cells.¹ The data presented previously in individuals after administration of niacin indicated the potential clinical utility of measuring levels of PGD-M in the circulation in situations associated with a release of PGD₂. In the case of niacin, however, the release of PGD₂ is not accompanied by a release of histamine, suggesting that the cells activated by niacin to release PGD₂ are not mast

TABLE I. Comparison of plasma and serum levels of PGD-M at baseline and 2.5 hours after administration of niacin in fresh samples and after storage at -20°C for 8 weeks

	PGD-M (pg/ml)	
	Fresh	Frozen
Baseline		
Plasma	3.6 ± 0.4	ND
Serum	3.7 ± 0.3	ND
After niacin administration		
Plasma	106 ± 15	119 ± 16
Serum	111 ± 6	126 ± 17

ND, Not done.

TABLE II. Measurement of PGD₂ metabolites in disorders associated with mast cell activation

Prostanoid (nl range, mean $\pm$ 1 SD)	Patient 1* (mastocytosis)	Patient 2* (mastocytosis)	Patient 3† (mastocytosis)	Patient 4‡ (anaphylaxis)
Plasma PGD-M (pg/ml) (nl = 3.5 ± 1.4 pg/ml)	31/18/15	530	3800/9400/50,000	430
Urinary PGD-M (ng/ml creatinine) (nl = 1.08 ± 0.36 ng/mg creatinine)	70/80/66	135		
Plasma 9 α , 11 β -PGF ₂ (pg/ml) (nl < 3 pg/ml)		143		5

nl, Normal value.

*Measured during quiescent phases of disease.

†Measured during severe episodes of mast cell activation.

‡Measured 90 minutes after onset of urticaria and bronchospasm and 15 to 20 minutes after administration of corticosteroids and epinephrine.

cells.³ Therefore we examined the feasibility of measuring PGD-M in the circulation to detect episodes of systemic mast cell activation and its utility in the biochemical evaluation of patients with systemic mastocytosis (Table II).

In two patients with histologically documented systemic mastocytosis, levels of circulating PGD-M and urinary PGD-M were measured at quiescent times when the patients were not experiencing episodes of mastocyte activation (patients 1 and 2, Table II). In both patients levels of PGD-M in the circulation and urine were significantly elevated, and in patient 2 the levels exceeded 100 times the upper limit of normal. Levels of the initial metabolite of PGD₂, 9 α ,11 β -PGF₂, were also measured in patient 2 and were substantially increased. Patient 3 was a patient with systemic mastocytosis who was evaluated at Vanderbilt University Hospital 12 years ago and died at that time of refractory shock during an episode of unusually severe mastocyte activation. Samples of serum and plasma, which had been obtained from this patient during the fatal episode of mastocyte activation and during two other milder but severe episodes of mast cell

activation, had been stored at -20°C and were analyzed for PGD-M. The results of these analyses were most dramatic, with findings of levels of PGD-M in the circulation of 775 and 1900 times above the upper limit of normal during the two milder episodes and levels of more than 10,000 times above normal during the fatal episode of mast cell activation.

Finally, we had the opportunity to measure levels of PGD-M and 9 α ,11 β -PGF₂ in plasma that was obtained from a patient with no history of allergic reactions who presented to a local emergency room with acute onset of urticaria and bronchospasm of unknown cause. Blood was obtained for analysis approximately 90 minutes after the onset of symptoms and 15 to 20 minutes after treatment of the patient with corticosteroids and epinephrine. The level of 9 α ,11 β -PGF₂ in normal individuals is generally at or below the limits of detection (<3 pg/ml) and was 5 pg/ml in this patient. Interestingly, the level of PGD-M in plasma was increased markedly (86 times above the upper limit of normal). The finding of a nearly normal level of 9 α ,11 β -PGF₂ but a markedly el-

evated level of PGD-M may be explained by the fact that this patient was treated with epinephrine. Epinephrine has been shown to directly inhibit activation and mediator release from mast cells.¹⁶ Thus treatment with epinephrine may have inhibited further release of PGD₂ after which levels of 9 α ,11 β -PGF₂ returned rapidly to normal because of its relatively rapid metabolic clearance rate. However, levels of PGD-M remained elevated because, as demonstrated previously after ingestion of niacin, PGD-M is cleared much more slowly from the circulation. Nonetheless, this exemplifies the potential value of measuring levels of PGD-M rather than 9 α ,11 β -PGF₂ in the circulation in the evaluation of disorders associated with systemic mast cell activation.

DISCUSSION

The use of biochemical markers for the diagnosis of disease is fraught with difficulties. Initially, overproduction of these markers needs to be identified as being associated with a pathologic process. Sensitive and specific techniques must be developed for their detection in biologic fluids, and the specificity of the relationship of the mediator to the particular pathologic phenomenon must be established. In fact, almost no biochemical marker to date completely fulfills such criteria. In addition, the clinical utility of a marker can be affected by the temporal relationship between the appearance of biochemical marker and the pathologic process. Several biochemical markers have been used to implicate activation of mast cells in disease states and in the biochemical diagnosis of the disease of abnormal proliferation of mast cells, systemic mastocytosis. Histamine is stored in mast cell granules and is released after activation of mast cells, and therefore measurement of histamine in plasma and urine has been used extensively to assess endogenous mastocyte activation. However, elevated levels of this mediator can also result from activation of basophils *in vivo* or arise as artifacts from *ex vivo* activation of basophils during blood sampling and plasma or serum isolation. Dietary intake of histamine can be another source of increased levels of histamine in plasma and urine.¹⁷ The most dramatic example of this phenomenon is scombroid fish poisoning.¹⁸ Artificially elevated urinary histamine levels can also result from the decarboxylation of histidine by bacteria colonizing the genitourinary tract of some individuals, especially women.^{8, 9} Finally, another limitation of measuring histamine is that increased levels of histamine in the circulation are

short-lived after an episode of mast cell degranulation (<1 hour) as a result of the rapid metabolism.¹⁹ Quantification of longer-lived metabolites of histamine can be advantageous in this regard, and it also eliminates the problems of artifactual generation of histamine by genitourinary tract bacteria or by release of histamine from basophils during blood sampling.^{8, 9} However, this approach does not allow determination of the source of histamine (i.e., endogenous vs exogenous or mast cells vs basophils).

Tryptase is another component of mast cell granules, and increased levels of tryptase can be detected in the circulation after an episode of mast cell degranulation.^{19, 20} Because the tryptase content of basophils is only a small fraction of that contained in mast cells, measurement of tryptase is believed to be more specific than measurement of histamine as an indicator of mast cell activation. Another advantage of measuring plasma tryptase levels is that the time course of the appearance and disappearance of tryptase in the circulation, like that of PGD-M, extends over several hours.¹⁹ This kinetic advantage, shared by both tryptase and PGD-M, is related to the slow clearance of these molecules from the circulation and can be of considerable clinical value. For example, patients often arrive at a medical facility proximate to but after resolution of an event, which may be thought to involve mast cell activation. Perhaps more commonly, a diagnosis of mastocyte activation may only be considered some time after the patient's initial presentation. In either situation plasma or serum obtained for routine tests, but not urine, may have been kept and would be available for measurements of PGD-M and/or tryptase. A potential disadvantage of tryptase measurement in implicating mast cell activation is that current methods do not allow detection of tryptase in normal individuals; thus, tryptase measurement may be insensitive for the detection of mild episodes of mast cell activation.^{19, 20} However, the ability to measure PGD-M in normal individuals allows definition of a normal range, thus permitting the detection of mild episodes of mast cell activation.

In the biochemical evaluation of disorders suspected to involve mast cell activation, it is important to recognize the specific advantages and disadvantages of measuring various mast cell secretory products. Evaluation of clinical events, which suggest mast cell activation with only a single biochemical marker, may provide misleading results, whereas results obtained from the use

of a combination of biochemical markers may be interpreted with greater confidence. Our own experience exemplifies the value of using such a combined approach. For example, in individuals with scombroid fish poisoning, the finding of normal levels of PGD-M but markedly increased urinary excretion of histamine and N-methylhistamine suggested that the increased levels of histamine were derived from ingestion of the large quantities of histamine that are present in spoiled scombroid fish rather than from activation of mast cells by substances contained in the fish.¹⁸ Because neuropeptides, such as substance P and neuropeptide Y and perhaps additional unknown substances, can release histamine from mast cells without causing production of PGD₂,^{21, 22} measurement of circulating levels of tryptase would have been of considerable value in confirming that the source of increased histamine levels in these individuals was derived from the spoiled fish rather than from endogenous activation of mast cells. Another example of the value of using a combination of biochemical markers is the finding that ingestion of niacin, which results in intense flushing similar to that which occurs during episodes of systemic mastocyte activation, is associated with a release of large quantities of PGD₂, which is not accompanied by a release of histamine.³ Because no situation has yet been described in which mast cell activation is associated with a selective release of PGD₂ without a concomitant release of histamine, these findings suggested that the cell that is activated by niacin to release PGD₂ is not the mast cell. Although clinical syndromes of mast cell activation that are associated with a selective release of either histamine (e.g., by neuropeptides) or PGD₂ from mast cells have not yet been identified, the use of multiple biochemical markers of mastocyte activation in conjunction with other measurements (e.g., serum neuropeptide levels) may permit the identification of such disorders, if indeed they exist.

In summary, the ability to measure circulating levels of PGD-M can provide a valuable tool for assessment of endogenous release of PGD₂, regardless of the cell of origin. Its chemical stability and relatively slow clearance from the circulation allows an assessment of a release of PGD₂ during clinical events by measuring levels of PGD-M in samples of serum or plasma that may have been obtained and stored proximate to but after resolution of the event. We have documented the increased release of PGD₂ in anaphylaxis for the

first time by measurement of PGD-M levels in plasma obtained after treatment of allergic reaction. When combined with measurements of other mast cell secretory products, quantification of PGD-M in plasma can provide a valuable addition to the biochemical evaluation of disorders of systemic mast cell activation. Although the mass spectrometric method of assay for measurement of PGD-M described herein is highly accurate and sensitive, the method is not highly efficient and requires expensive instrumentation. This may prevent PGD-M quantitation from becoming generally available for routine clinical evaluations. However, because of the recognized clinical utility of measuring PGD-M both in plasma and urine, efforts are currently underway to develop a more facile and inexpensive immunoassay method for the measurement of PGD-M, which could be widely used for both investigational purposes and routine clinical assessment.

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