median reduction was by a factor of 12, n = 5 experiments). Acyloxyacyl hydrolysis thus appeared to produce a greater reduction, by approximately a factor of 10, in LPS tissue toxicity than in immunostimulatory activity.

These results confirm that acyloxyacyl groups are important for tissue toxicity, as expressed in the Shwartzman test; presumably, they provide the necessary stimulus to critical target cells. Although acyloxyacyl groups also add to the immunostimulatory activity of LPS, as measured by the B-cell mitogenicity test, overall this contribution seems quantitatively less important than their contribution to toxicity. In previous studies of the biological role of acyloxyacyl groups, isolated lipid A and its analogs were used. The present results suggest that acyloxyacyl groups are also critical determinants of the bioactivity of the lipid A that occurs in nature, linked to a saccharide chain in LPS (*12*).

To our knowledge, this is the first description of an enzymatic activity that modifies the bioactivities of LPS. Antibody-opsonized LPS undergo deacylation by neutrophils and macrophages (*4, 13*); a plausible hypothesis places acyloxyacyl hydrolases in an acid intracellular compartment (for example, lysosomes), where deacylation of bacterial LPS may accompany the digestion of other bacterial components (*14*). Enzymatically deacylated LPS, released from phagocytic cells by exocytosis (*15*) or cell death, might then interact with target cells such as B lymphocytes. However, the complete intracellular fate of LPS is not known; our studies have all dealt with purified LPS, not the LPS in bacteria per se; and other enzymes may also process LPS. For example, murine macrophages can remove 3-hydroxytetradecanoyl residues from LPS (*13*); this deacylation may completely inactivate the molecules.

We suggest that a major function of neutrophil acyloxyacyl hydrolases is to detoxify LPS. Maneuvers that increase enzyme activity in vivo thus might prevent or ameliorate some of the toxic manifestations of Gram-negative bacterial diseases. It is also possible that enzymatically detoxified LPS may stimulate potentially beneficial components of the inflammatory response. Preservation of some of the immunostimulatory potency of detoxified LPS might allow animals to derive immune priming (*16*) during health and enhancement of antibody formation during infections. Acyloxyacyl hydrolases thus may have a biological role that parallels one proposed function of lysozyme, another enzyme in phagocytic cells that cleaves a toxic bacterial cell wall polymer (peptidoglycan) into products (muramyl peptides) that may have immunostimulatory activities (*17*).

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20 May 1986; accepted 28 July 1986

Inhibin-Mediated Feedback Control of Follicle-Stimulating Hormone Secretion in the Female Rat

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The secretion of follicle-stimulating hormone (FSH) by the anterior pituitary gland is regulated by the interaction of hypothalamic and gonadal hormones. Recently, proteins termed inhibins that selectively suppress FSH secretion have been purified and characterized from the gonadal fluids of several species. Antibodies to a synthetic peptide encompassing the amino terminal 25 residues of the recently characterized porcine inhibin were used to develop a specific radioimmunoassay (RIA) for inhibin and to neutralize endogenous inhibin during the estrous cycle of the rat. The administration of 20 international units of pregnant mare serum gonadotropin (PMSG) stimulated the secretion of inhibin in intact immature female rats, whereas ovariectomy caused an abrupt decrease in plasma inhibin concentrations that were not prevented by the injection of PMSG. The infusion of a polyclonal antiserum to inhibin, from 12 noon on proestrus to 1 a.m. on the morning of estrus, as well as its acute intravenous injection during diestrus I or II, caused an increase in plasma FSH (but not luteinizing hormone) concentrations. These results support the hypothesis of a feedback loop between the release of ovarian inhibin and FSH in the female rat.

ALTHOUGH GONADOTROPIN-RELEASING hormone (GnRH) and sex steroids play a major role in controlling the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland (*1, 2*), data from many studies indicate the presence of an additional regulatory protein referred to as an inhibin. This water-soluble, nonsteroidal protein is of gonadal origin and selectively inhibits the release of FSH (*3, 4*). Several groups (*5–10*) have reported the isolation of proteins that may account for inhibin activity from porcine and bovine follicular fluid and have described partial amino terminal sequences of these proteins, which have two

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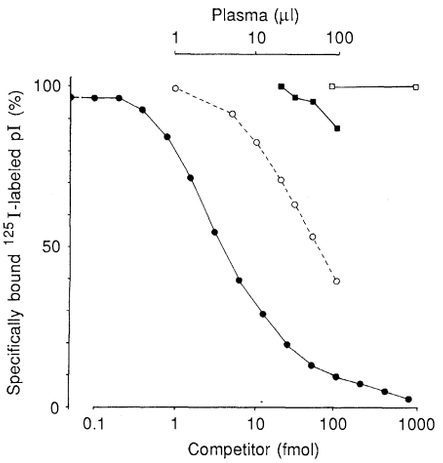
Fig. 1 (left). Displacement of ^{125}I-labeled pInhibin-α-(1-25)-Gly-Tyr (pI) binding to inhibin antiserum by synthetic pInhibin-α-(1-25)-Gly-Tyr (●), plasma from female rats (○), or gonadectomized animals (■); (□) represents LH, FSH, GnRH, PMSG, human chorionic gonadotropin (hCG) and transforming growth factor-β (TGF-β). Inhibin was measured in plasma by specific RIA with the use of a rabbit antiserum raised to a synthetic fragment of porcine inhibin α subunit, pInhibin-α-(1-25)-Gly-Tyr, coupled by bisdiazotized benzidine to human α globulin. The ^{125}I-labeled pInhibin-α-(1-25)-Gly-Tyr was purified by high-performance liquid chromatography. The ability of multiple doses of samples of rat plasma to compete with binding to the antibody was compared to that of standard α-inhibin(1-25)-Gly-Tyr. The displacement curve obtained with increasing volumes of plasma was parallel to that obtained with the peptide standard. Proteins such as FSH, LH, GnRH, PMSG, hCG, and TGF-β were not able to displace antibody-bound tracer in this assay and plasma from gonadectomized rats was ≥1000% less active than control plasma. Because no pure rat inhibin is available, we could not estimate absolute levels of inhibin in the rat. Therefore, relative inhibin concentrations are expressed as percentage of values in control rats. Antibody-bound radiotracer was separated from free radiotracer by precipitation of first antibody with sheep antiserum to rabbit γ globulins. Intra- and inter-coefficients of variance: 3.63% (n = 5) and 4.00% (n = 9), respectively.



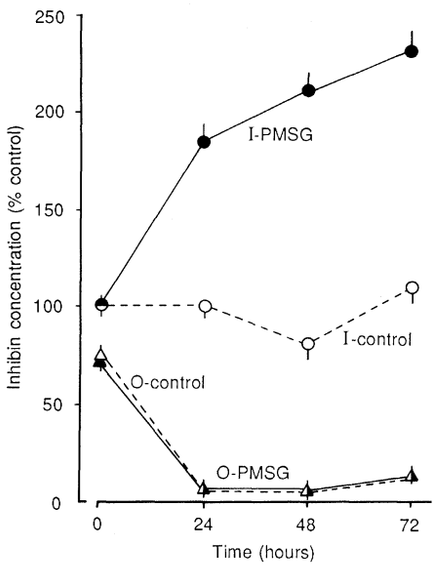


Fig. 2 (right). Effect of the intravenous injection of PMSG on inhibin secretion. 25-day-old Sprague-Dawley rats were either sham-operated (I), or ovariectomized (O) 1 minute before the intravenous injection of the treatment, under ether anesthesia. In each group, eight rats received the vehicle (control) and eight rats were injected with 20 IU PMSG. Blood samples were obtained immediately before the injection, as well as 24, 48 and 72 hours later under light ether anesthesia. The RIA was as in Fig. 1. Each point represents the mean ± SEM of eight rats. Plasma FSH measured at the 72-hour point was intact control rats, 12.6 ± 1.09 ng/ml; intact rats given PMSG, 4.7 ± 0.50 ng/ml (difference = 7.9 ± 0.86 ng/ml; n = 8; $P \leq 0.01$ versus intact control); ovariectomized control, 27.4 ± 3.86 ng/ml; ovariectomized PMSG, 27.9 ± 1.95 ng/ml (difference = 0.50 ± 0.04 ng/ml; n = 8; $P > 0.05$ versus ovariectomized control).

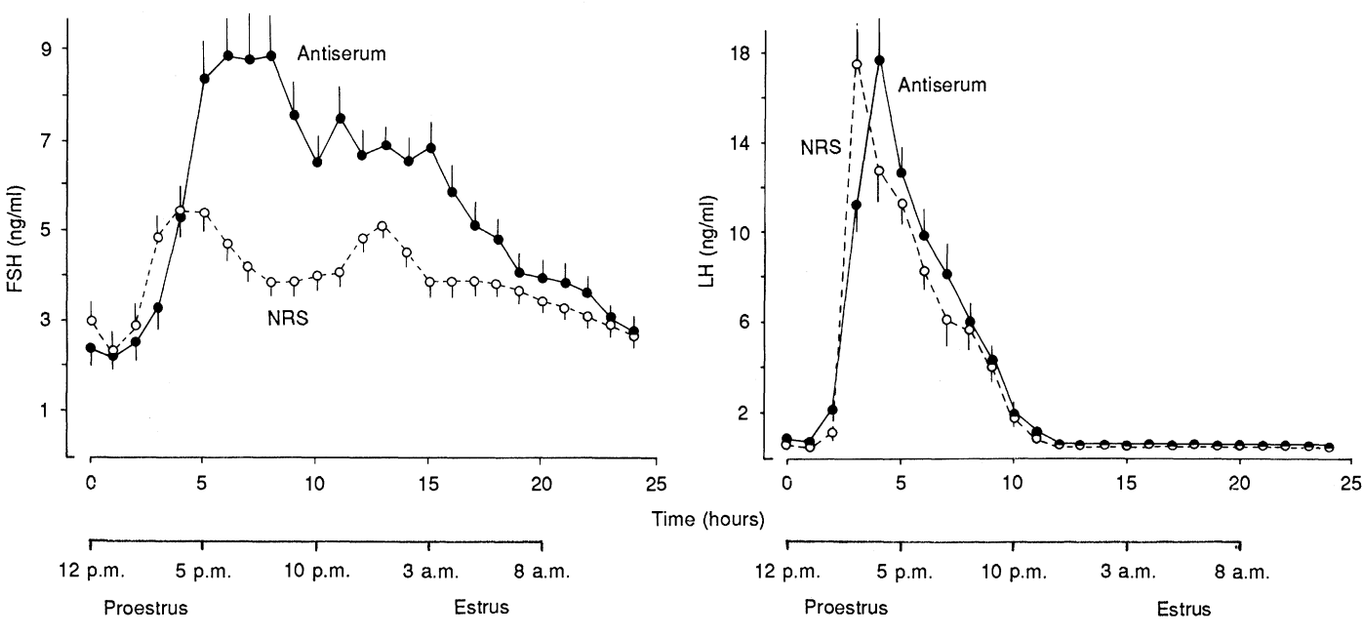


Fig. 3. Effect of the intravenous infusion of normal rabbit serum or inhibin antiserum on plasma FSH and LH levels during proestrus and estrus. Adult Sprague-Dawley female rats that had shown at least four consecutive 4-day estrous cycles were used. On the morning of proestrus, they were equipped with indwelling venous catheters in the jugular and femoral vein. At 12 noon, a first blood sample was obtained through the jugular cannula, and the infusion of NRS (○) or antiserum to inhibin (●) was immediately started in the femoral vein at the rate of 0.2 ml per hour, with a Harvard 2265 Multiple Syringe Pump (Harvard Apparatus Company, Inc., South Natick, MA). The infusion lasted 15 hours (solid horizontal bar). Blood samples were then obtained every hour and, immediately afterwards, the animals were given an injection of an equivalent volume of reconstituted red blood cells. These cells were obtained from whole blood of donor female rats (taken at random stages of the estrous cycle), which was mixed with citrate-phosphate-dextrose (15% volume to volume), filtered on Whatman 1 paper, centrifuged, washed with saline, and mixed with charcoal-extracted Plasmanate (Cutter Biological Products). Lights were off from 7 p.m. until 5 a.m., during which time the room was equipped with a red light. The rats were provided with free access to rat food, water, and oranges. FSH and LH levels were measured by an RIA with materials provided by the NIADDK distribution program (standards = LH- and FSH-RP2). FSH values were measured in duplicate samples of individual plasmas; LH values were measured in duplicate samples of plasmas pooled from two rats per point. The FSH RIA was modified to accommodate the large amount of circulating globulins present in the plasma by the addition of 100 μl of sheep antiserum to rabbit γ globulins diluted 1:4 as a second antibody; in the LH RIA, separation of antigen-antibody complexes was achieved by the addition of 100 μl of staphylococcal protein-A (Pansorbin, Calbiochem-Behring Corp., La Jolla, CA). Each point represents the mean ± SEM of nine to ten rats. No SEM is shown when it is smaller than the point.

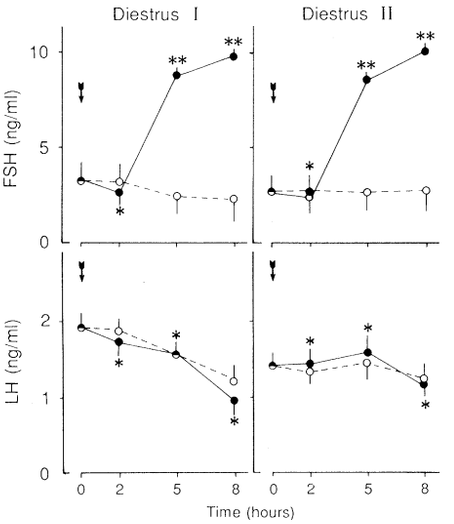


Fig. 4. Effect of an intravenous bolus of normal rabbit serum or antiserum to inhibin on plasma FSH and LH levels during diestrus I (left panel) or diestrus II (right panel). One milliliter of NRS (○) or inhibin antiserum (●) was administered immediately after the first blood sample was obtained, at 9 a.m. (indicated by arrow). Bleeding was performed under light ether anesthesia. Each point represents the mean ± SEM of six female rats. The results were analyzed by analysis of variance. *$P > 0.05$; **, $P \leq 0.01$. For details of FSH and LH RIA's, see legend to Fig. 3.

subunits (α and β). The sequences of the precursors of porcine, bovine, and human inhibin α subunits and β subunits (*11–14*) have been deduced from complementary DNA sequences. Inhibin has been shown to inhibit the release of basal FSH, but not LH, in vivo and in vitro (*3, 4*); and, using a variety of bioassays, several investigators have reported that circulating concentrations of inhibin are inversely related to plasma FSH levels (*15–18*).

There are several examples of a dissociation between the secretion of LH and FSH, such as the secondary FSH surge observed in the rat during the morning of estrus (*19–24*), that cannot be explained by modulation by either GnRH or sex steroids. Although the administration of antiserum or antagonists to GnRH blocks the primary proestrus surge of FSH, the suppression of the secondary rise in plasma FSH levels can only be achieved through the injection of inhibin preparations (*19, 22*). These results suggested that inhibin can indeed modulate FSH secretion during the estrous cycle, but do not establish whether endogenous inhibin has a physiological role. With knowledge of the sequence of inhibins from various species (*7–14*), it is now possible to prepare antibodies to study the role of endogenous inhibin and monitor its circulating levels.

The concept of a reciprocal relation between plasma FSH and inhibin concentration, which implies that FSH induces increases in inhibin secretion, was based on bioassay measurements of inhibin concentrations (*25, 26*). Such measurement reflected the net FSH secretion–modulating activity of a biological fluid such as plasma, or ovarian follicular fluid [which is now known to contain both inhibins and FSH-releasing β-β dimers of inhibin (*27, 28*)] rather than the levels of a specific protein. Using a specific radioimmunoassay (RIA) (Fig. 1), we have monitored immunoreactive inhibin concentration in immature female rats injected with pregnant mare serum gonadotropin (PMSG), a molecule that has long-acting FSH activity (*29, 30*). The intravenous injection of 20 international units (IU) of PMSG to intact animals caused a 241 ± 5% increase in plasma inhibin ($n = 8$) 72 hours after administration of PMSG (Fig. 2). The gonads are the main source of the circulating inhibin released by PMSG because in ovariectomized rats injected in the same way there was a rapid and marked decrease in basal inhibin levels to 9.9 ± 0.1% of control values, and no stimulatory effect of PMSG was observed. These results extend an earlier report in which amounts of inhibin had been estimated by bioassays (*31*) and emphasize the feedback effect between circulating concentration of FSH and inhibin.

Using an antiserum that recognizes specifically the amino terminus of the α subunit (*8–13*) of inhibin, we tested the hypothesis that endogenous inhibin at least partially modulates plasma FSH values during the estrous cycle. In a first experiment, adult cycling female rats received an infusion of normal rabbit serum (NRS) or rabbit antiserum to inhibin, and FSH and LH release were monitored over a 24-hour period (12 noon of proestrus to 12 noon of estrus). As expected (Fig. 3), NRS-injected rats showed two peaks of plasma FSH values (at 4 to 5 p.m. on proestrus and 12 a.m. on estrus) and a single peak of plasma LH levels (at 4 p.m.) on proestrus. Antibody neutralization of endogenous inhibin caused a sustained increase in plasma FSH (but not LH), which started to rise 3 hours after the beginning of the infusion and, as analyzed by the multiple comparison test of Duncan, remained statistically ($P \leq 0.01$) different from control values throughout the rest of the treatment. The fact that, in the rats receiving the antibody, the highest FSH levels were observed during the period of the expected proestrus gonadotropin surge probably reflects the action of GnRH unopposed by endogenous inhibin. When the infusion stopped after 15 hours on the morning of estrus, plasma FSH levels started to decrease; assuming a linear decline in the final stages of the two curves, they would reach equality at approximately 25 hours. In a second experiment, regularly cycling female rats received one acute intravenous injection of NRS of inhibin antiserum at 9 a.m. during diestrus I or II (Fig. 4). The antiserum markedly elevated plasma FSH levels over values measured in NRS-injected animals without altering LH secretion. Our results suggest that endogenous inhibin is an important modulator of FSH secretion during the estrous cycle of the female rat, and that its secretion by the gonads is at least partially controlled by FSH.

Note added in proof: Since this report was submitted, two groups have reported changes in immunoreactive inhibin following administration of FSH. McLachlan *et al.* have detected inhibin in the plasma of women given FSH, but not untreated subjects (*32*). Hsueh *et al.* have found that cultured granulosa cells secreted inhibin when exposed to FSH (*33*).

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34. Supported by NIH grant HD13527 and by contract B85.28A/ICCR from The Population Council. Research conducted in part by the Clayton Foundation for Research, California Division. C.R. and W.V. are Clayton Foundation investigators. We thank G. Morgan, G. Berg, L. Spaulding, S. Cappel, J. Vaughan, R. Galyean, J. Dykert, and R. Kaiser for technical assistance, and R. Hensley, B. Connor, and B. Baasch-Reed for manuscript preparation.

1 May 1986; accepted 12 August 1986

Miocene Characid Fishes from Colombia: Evolutionary Stasis and Extirpation

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Fossil fishes from the Miocene La Venta fauna of the Magdalena River Valley, Colombia, are identified as *Colossoma macropomum* (Characidae), a living species from the Orinoco and Amazon basins. The fossils document a long and conservative history for a species that is highly specialized for feeding on streamside plants. The phylogenetically advanced position of *Colossoma* in the subfamily Serrasalminae implies that six related genera and other higher characid taxa originated well before 15 million years ago. This discovery also corroborates neontological evidence for a vicariance event that contributed species from Miocene Orinoco-Amazon faunas to the original Magdalena region fauna. The fossils suggest a formerly diverse Magdalena fauna that has suffered local extinction, perhaps associated with late Cenozoic tectonism. This new evidence may help explain the depauperate nature of the modern Magdalena River.

"IT IS QUITE OUT OF THE QUESTION to transport all of these genera over the present barrier formed by the Cordilleras of Bogotá . . ." [C. H. Eigenmann, 1920 (*1*)]. The tremendous richness of the South American fish fauna is concentrated in the Amazon and Orinoco rivers. Beyond these basins, into northwestern Colombia and then lower Central America, the number of fish species falls sharply. This diversity pattern exists in spite of suitable habitat in faunistically depauperate rivers and requires a historical explanation. Such explanation has been frustrated by incomplete phylogenetic and distributional data for living fishes and a very poor paleontological record (*2*).

The Magdalena River, lying between the Andean Cordilleras Oriental (Bogotá) and Central, drains about 241,000 km^2 of central Colombia. The modern Magdalena fish fauna comprises about 150 species, far fewer than estimates for the adjacent Orinoco and Amazon faunas (*3*). On the basis of the high proportion of shared taxa, Eigenmann (*1*) proposed that the Andean Cordillera Oriental is younger than the lowland Magdalena region, thus allowing for direct capture of widespread Orinoco and Amazon fishes when the Magdalena Basin formed as the mountains rose. If this is so, why are there not more Orinoco and Amazon elements in the Magdalena fauna? An alternative hypothesis considered by Eigenmann involves chance dispersal of species into the Magdalena from elsewhere. The dispersal of freshwater fishes is severely constrained by the evolution of landform and watersheds. Fishes may not have had enough opportunities and time to reach relatively young or remote waters. If Eigenmann's vicariance hypothesis is correct, we would expect greater faunistic similarity in the past, before local extinction and divergence obscured the similarity between faunas that were once one.

Paleontological investigations in Colombia have recovered jaw elements and teeth of a large characid fish from Miocene deposits in the Magdalena Basin. This material is indistinguishable from the extant *Colossoma macropomum*. Today this species occurs in the Orinoco and Amazon systems: it is absent from the Magdalena. Given their age and extralimital locality, these fossils bear noteworthy implications for the history of fishes in South America.

The specimens come from the upper Magdalena River Valley northwest of Villavieja, about 3°15′N, 75°13′W, Huila Department, Colombia. Stratigraphically, the fossils occur in clays, called the "Monkey Unit" (*4*), of the Villavieja Formation (Honda Group). These fishes are part of the La Venta fauna (*5*) that is approximately 15 million years old. The precise dating of this fauna is problematic (*6*), but variation in age estimates does not affect our conclusions. The strata are composed of continental deposits derived from the Cordillera Central. The area presently incorporated into the Magdalena Valley was then a piedmont fluvial plain sloping to the east and southeast and running parallel to the Cordillera Central (*7*). There was no major uplift in the Cordillera Oriental during Honda time although local blocks along the range may have been uplifted.

We report the following fossil specimens now deposited in the Museo Geológico of the Colombian Instituto Nacional de Investigaciones Geológica-Mineras (INGEOMINAS) at Bogotá: (i) partial mandible (Fig. 1A), including fragments of both jaws

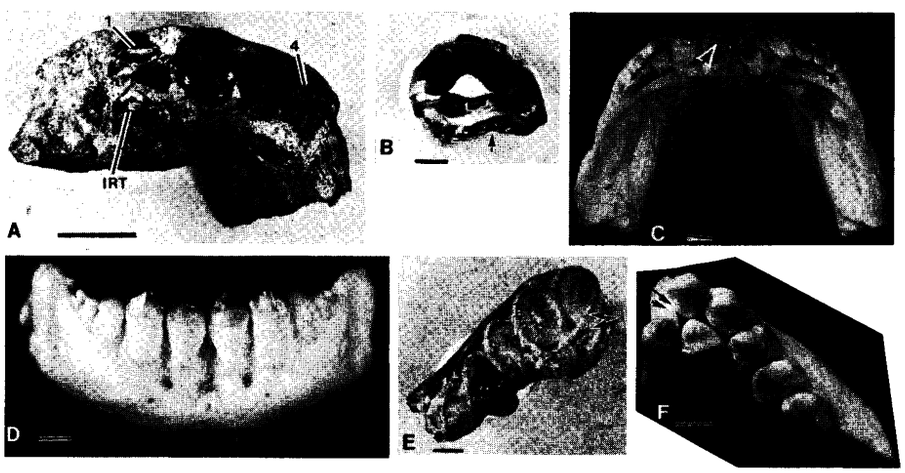

Fig. 1. Jawbones and teeth of *Colossoma macropomum*. (A) Fossil right mandible, dorsal view. Scale bar, 10 mm. (B) Isolated first outer row tooth. Scale bar, 2 mm. In (B) and (C), arrows point to a notch on the posterior side of the outer tooth for reception of the inner row tooth. (C) Modern mandible, dorsal view. (D) Same as (C), anterior view. Scale bars for (C) and (D), 5 mm. (E) Fossil right premaxilla, ventral view. Scale bar, 3 mm. In (E) and (F), arrows indicate facing margins of symphyseal outer and inner row teeth. (F) Modern left premaxilla, ventral view. Scale bar, 5 mm. Abbreviations: 1, first outer row tooth base; 4, fourth outer row tooth base; and IRT, inner row tooth base.

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