Hormone Action: Oxytocin-induced *in vitro* Milk Release

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HORMONE ACTION IS AN AREA of physiology that presents a challenge to instructors seeking economical procedures for demonstrating principles. The milk-ejecting activity of the neurohormone, oxytocin, on mammary gland tissue is a dynamic example of the mechanism of action of a hormone, yet it is simple and relatively inexpensive to demonstrate.

The main physiological role of oxytocin, a hormone of the posterior pituitary gland of mammals (Linzell 1959), is to stimulate milk release from the mammary gland. Oxytocin is an example of a neurohormone because it is synthesized within neurons in the hypothalamus, an area of the lower part of the brain (diencephalon) responsible for the control of many physiological functions. Oxytocin is transported within these neurosecretory neurons to their neural endings in the posterior pituitary gland. The oxytocin is stored within these axonal nerve endings until stimuli received by the brain cause the release of the hormone from the nerves (fig. 1). It is a peptide hormone (Du Vigneaud 1956) composed of a sequence of amino acids held together by peptide bonds (fig. 2).

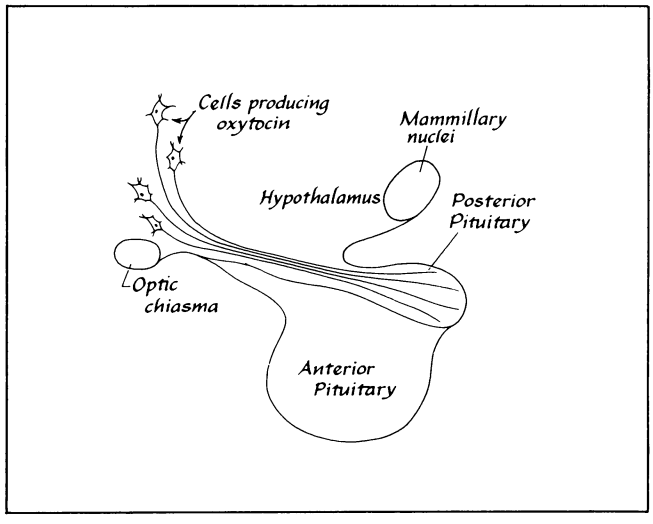

FIGURE 1. Diagram of the pituitary gland emphasizing the relationship between the posterior pituitary (neurohypophysis) and the neurosecretory neurons which originate within the hypothalamus. Oxytocin synthesized within these hypothalamic neurons is transported to and then stored within the terminal axonal endings within the neurohypophysis.

Oxytocin plays an important role in the reproductive physiology of mammals. In addition to causing the release of milk from the mammary glands, it may also cause the uterine muscles that expel the fetus during childbirth to contract. In both cases, the oxytocin initiates contraction of muscle or muscle-like cells (Cowie 1972).

The mammary gland, a distinguishing characteristic of mammals, synthesizes milk for subsequent release during suckling by offspring. Anatomically the mammary gland is composed of clusters of alveoli or microscopic sacs somewhat similar to those found in the lungs. Each alveolus consists of a lumen that stores milk secreted from the surrounding layer of milk-synthesizing cells. The alveolar elements of the gland are held together by connective


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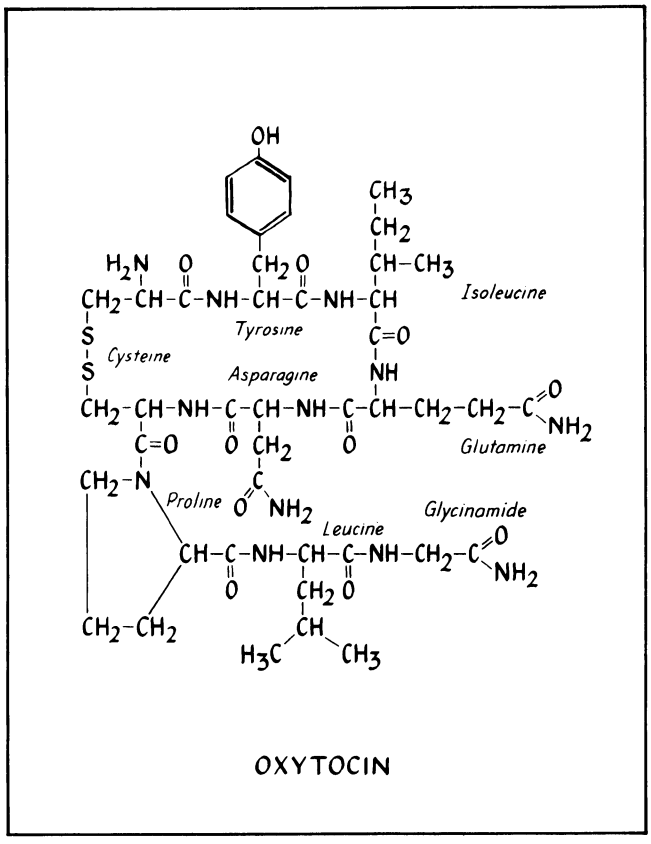

FIGURE 2. The structure of the neurohypophysial hormone, oxytocin.

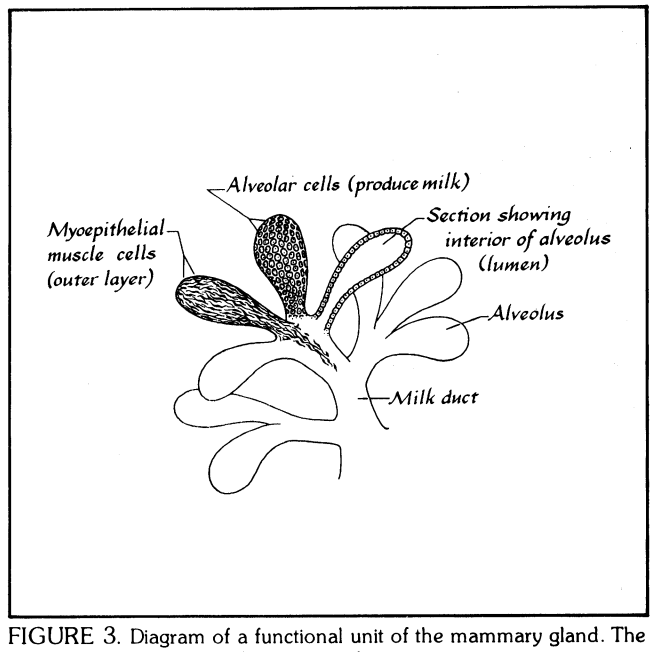

FIGURE 3. Diagram of a functional unit of the mammary gland. The secretory and contractile elements are shown.

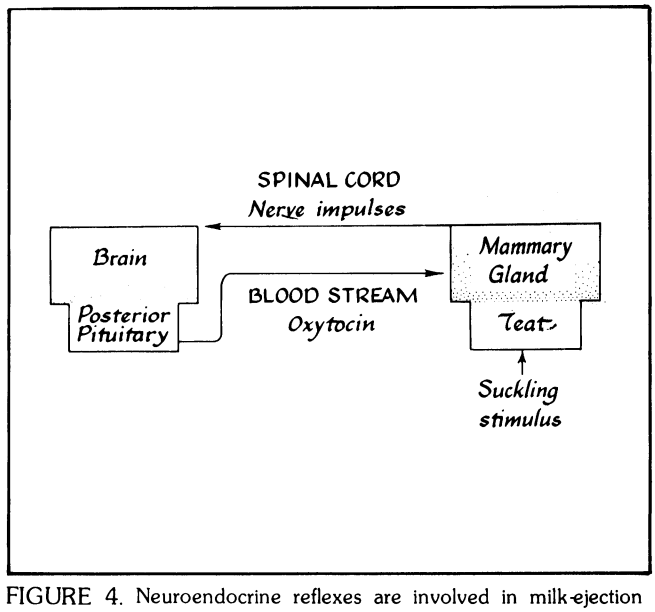

FIGURE 4. Neuroendocrine reflexes are involved in milk-ejection response.

tissue. Over the surface of each alveolus are small muscle-like myoepithelial cells (fig. 3) that contract in response to released oxytocin and expel milk from the lumen of the alveolus into the duct system leading to the exterior opening at the nipple (teat). The release of milk is referred to as milk ejection or milk let-down to distinguish it from milk secretion carried out by alveolar cells.

The lactation process involves a neuroendocrine relationship between the mammary gland and the brain (fig. 4). The act of suckling stimulates nerve impulses that pass from the teat(s) to the brain (Haywood 1975). These signals reach the hypothalamus and stimulate the release of oxytocin from the axons of the neurosecretory neurons in the posterior pituitary. The oxytocin released into the blood reaches the mammary gland and induces contraction of the myoepithelial cells that results in milk ejection (Nickerson, *et al.* 1954).

Preparation of Materials

Mouse Colony. A single adult male mouse housed with 3 to 4 females will impregnate all or most of the females. Two such breeding cages would certainly be sufficient to provide the lactating mothers needed for this experiment. Pups should not be removed until they are haired; they can be removed at almost anytime during the day preceding the experiment. More than one female should be available to ensure that at least one mouse is available. Extra mice can provide additional tissue if it is needed.

Physiological Saline. We use Krebs Ringer bicarbonate (KRB) solution. To prepare this solution, add the designated number of grams of each chemical to distilled water and bring to a total volume of one liter. These chemicals are: NaCl, 6.85; KCl, 0.35; NaH_2CO_3, 2.10; $CaCl_2 \cdot 2H_2O$, 0.28; $MgSO_4 \cdot 7H_2O$, 0.29; $Na_2HPO_4 \cdot H_2O$, 0.16; glucose, 2.0. Any physiological saline containing sodium, potassium, calcium and chloride ions at approximately physiological concentrations will probably suffice.

Hormone Preparation. Oxytocin may be purchased from Sigma Chemical Company (Post Office Box 14508, St. Louis, Missouri 63178) or other companies. A solution with a concentration of 10^{-7} g/ml (equivalent to about 10^{-7} M) will induce maximal *in vitro* milk ejection. The

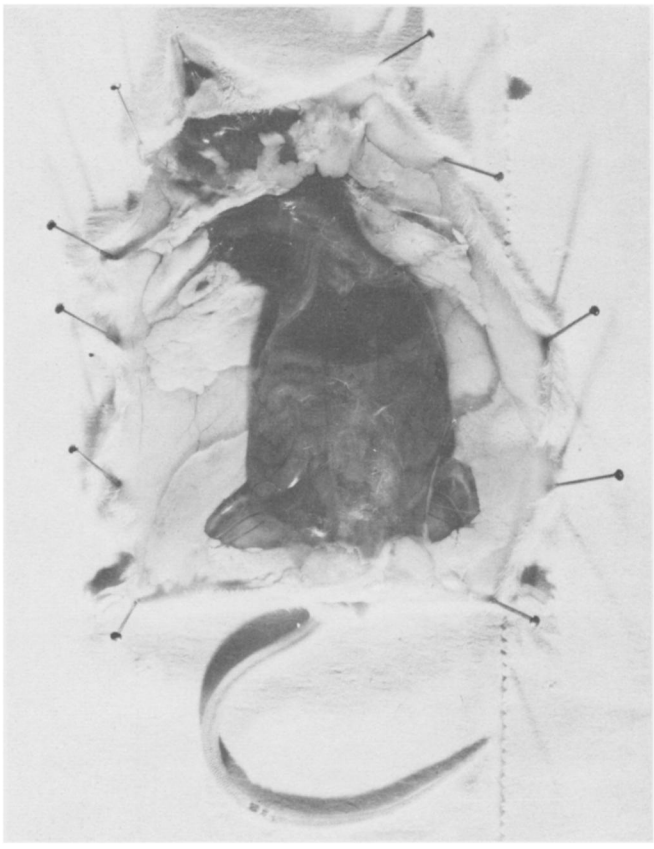

FIGURE 5. Dissection of the lactating mouse to expose mammary gland tissue.

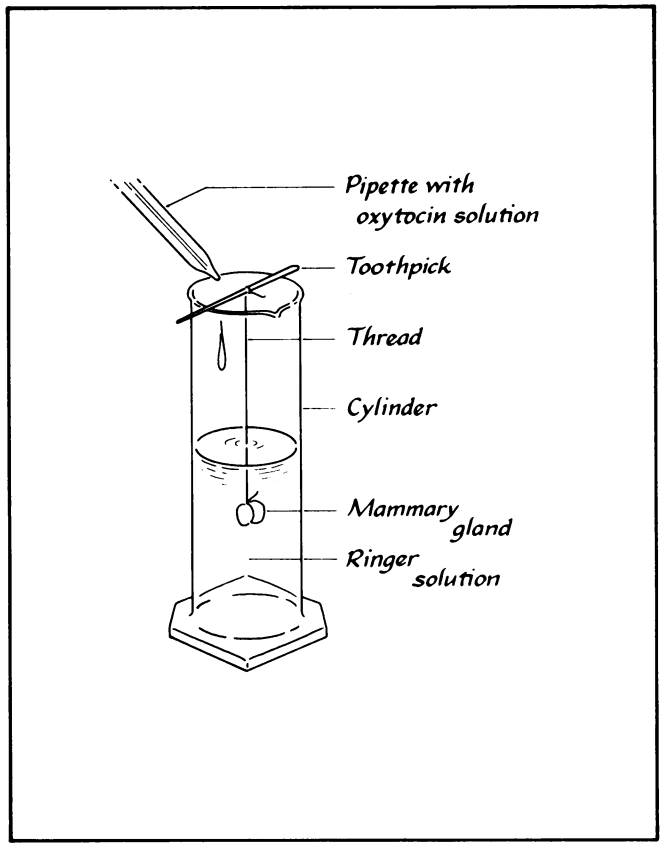

FIGURE 6. Simple method for visualizing milk ejection *in vitro*.

hormone usually is effective in a concentration as low as 10^{-9} g/ml. A convenient preparation is the Sigma aqueous solution of oxytocin (200 USP units per ml). A 10^{-2} to 10^{-4} U/ml solution should induce strong *in vitro* milk-ejecting activity.

Methods

Mammary Tissue Preparation. Obtain lactating mice presently suckling their young. Remove pups (7 days or older) approximately 18 to 24 hours prior to the time the lactating mice are needed for the demonstration. The milk accumulated from the lack of suckling should be visible through the skin. To kill the mouse, quickly etherize it, and dislocate the cervical vertebrae by pressing down on the back of the neck and pulling the body sharply by yanking the tail. An overdose of ether or sodium nembutal can also be used. (Beware of the explosive dangers with ether.)

Make a longitudinal slit through the ventral skin from the genital area up to the chin. Be careful to avoid cutting into the abdominal cavity because this will cause unnecessary bleeding. Gently pull the skin laterally to expose the mammary tissue. Generally, this can be accomplished with almost no bleeding. Pin the skin to the dissecting tray (fig. 5) and keep the tissue moist with physiological saline solution. The mammary glands are composed of four obvious major sections that can be easily removed with little damage. To dramatize anatomical differences in physiological states, expose the mammary glands of a nonlactating mouse or a mouse that has been continuously suckled.

Place the mammary tissue in a beaker of KRB solution as it is removed. Replace the KRB solution a number of times to remove the excess milk released from the glands during manipulations.

Experiment 1. Take one section of gland and gently tie it at an end with a piece of thread and suspend the tissue in a small cylinder or beaker containing about 100 ml of KRB solution (fig. 6). Add a few drops of oxytocin solution to the KRB solution in the cylinder. (If it is desired, the addition of oxytocin can be quantified to determine the concentration of the hormone needed to effect milk ejection. Oxytocin will usually induce *in vitro* milk secretion at concentrations of 10^{-7} to 10^{-9} g/ml.) Within a matter of minutes, jets of milk will issue from the mammary tissue. The milk would normally be released from the orifice of the teat; under these experimental conditions, the normal channels for the passage of milk have been disrupted. The visual aspects of milk ejection may be enhanced or dramatized by holding a piece of black paper behind the container of tissue. For control purposes, another piece of mammary tissue should be suspended in a similar beaker of KRB solution without oxytocin.

Experiment 2. Cut other sections of mammary tissue into pieces approximately 5 mm square with a sharp razor blade. Gently place the pieces of tissue into a beaker of KRB solution. Rinse the tissue several times until the

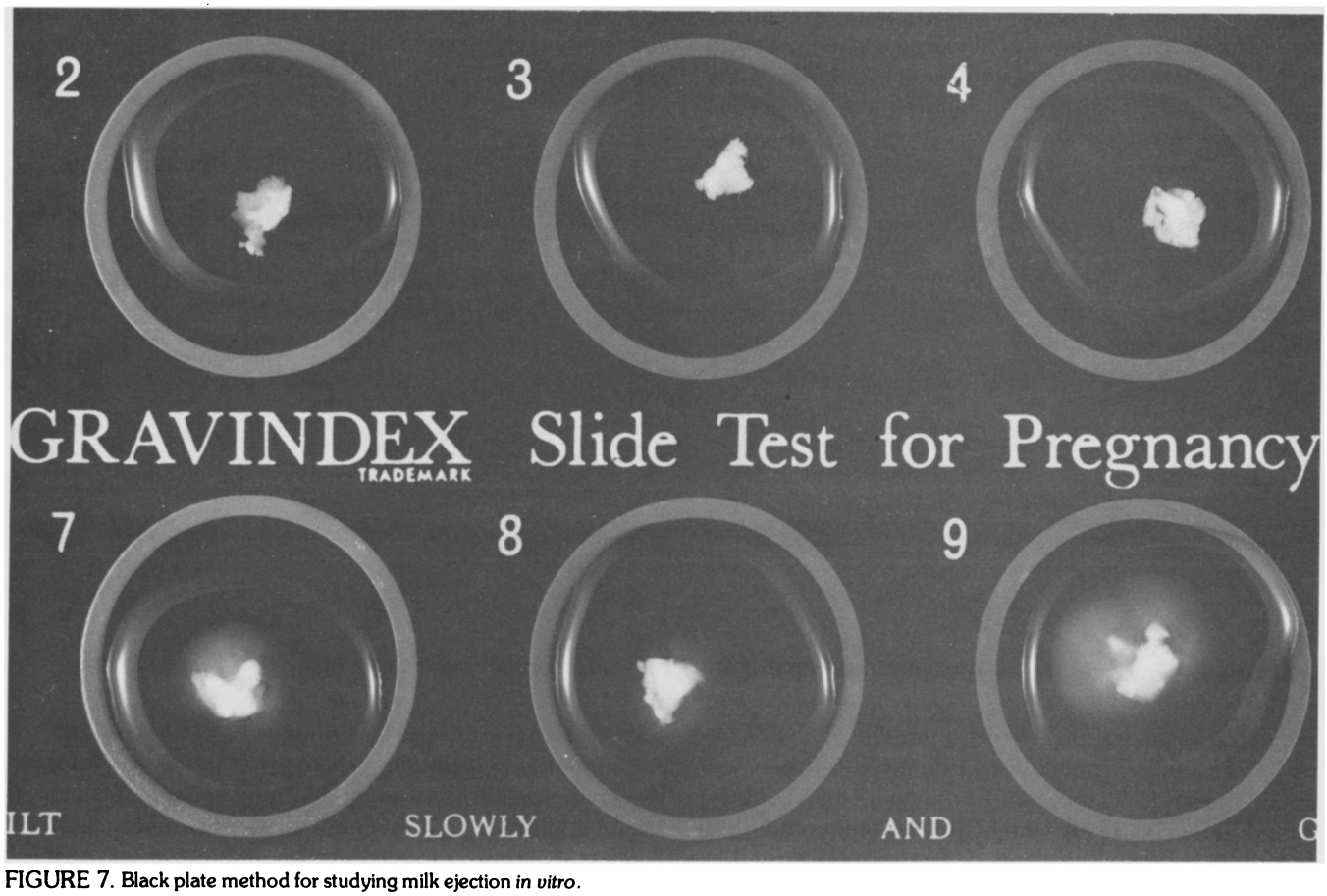

FIGURE 7. Black plate method for studying milk ejection *in vitro*.

intrinsic milk secretion has diminished. Place two rows of clean microscope slides on a piece of black paper. Divide each slide into three equal parts by making a pair of heavy marks with a grease pencil. To one row of slides add one ml of KRB solution to the center section of the slide. The solution will be retained within the section by the grease marks. (The division of the slide can, of course, be made by any other method. We use black glass plates obtained from "Gravindex" pregnancy kits, Ortho Diagnostics, or similar plates obtained from Roche Diagnostics pregnancy kits.) To the other row of slides add one ml of diluted oxytocin solution as in experiment 1. Place a small piece of mammary tissue into the KRB solution on each slide using clean forceps. Again, milk will be ejected from each of the tissues placed in the hormone solution within minutes (fig. 7). A minimal amount of milk will issue from the control tissue during this time.

A single mouse will provide enough tissue for an entire class of about 30 students. Additional information on the methods used in this *in vitro* milk ejecting experiment have been published elsewhere (Hruby and Hadley 1975).

Other Activities

Mouse Anatomy. Because the mice must be killed to provide the mammary gland tissue for the first two experiments, it might be worthwhile to open up the abdominal cavity after removal of the mammary tissue so that students may observe the other endocrine, reproductive, and digestive organs. A male mouse can also be killed to reveal the similarities and differences in reproductive anatomy. Dissection of other pregnant female mice will reveal the distended uteri containing numerous other fetuses. Students can dissect the uteri and examine the young mice under a microscope.

Quantitative Aspects of Hormone Action. Interested students may want to determine the potency of an oxytocin preparation under various experimental (incubation) conditions. In Hruby and Hadley (1975) the methods described are more rigorous than those used in this procedure.

Specificity of Neurohypophysial Hormone Action. Because other neurohypophysial hormones, such as vasopressin, possess a similar chemical structure, they, too, induce milk ejection. To our knowledge, the only other hormone, neurohormone, or neurotransmitter to induce milk ejection is acetylcholine, but this agent is only effective at very high concentrations (10^{-3} to 10^{-5} g/ml). Students may be interested in determining whether other hormones or drugs also cause or affect milk secretion.

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(Concluded on p. 422)

but I do not ever recall an anonymous award of any size being made at a science fair. I am convinced that the type of recognition suitable for fostering student achievement must be determined locally and awards should be assigned with discretion. I can think of no more logical approach to the question because it has such parochial implications. Local science fair directors know their students best.

The 4-H awards different colored ribbons for different accomplishments. Some are first place ribbons, other second place. Some are for the grand champion, some for reserve grand champion placement. Every entrant receives an award—a ribbon. No one goes home feeling her/his efforts were completely in vain, though some realize that they could have done a better job. Likewise, every science fair entrant should be given some acknowledgment of participation. This is the essential minimum.

Who Should Participate?

I cannot leave the subject of science fairs without reference to one more concern. Who should participate? Because sciencing is, in the investigative sense, an activity requiring more than superficial involvement, the science fair experience should be reserved for students who want to do something extra. Students should never be assigned to enter a science fair, unless the assignment is optional with no academic strings attached. I think it is the assigned project that most often appears as a poster, chart, diagram, or model. Such projects, as I have said, only exacerbate the ills of many science fairs.

Limiting the number of entries in fairs through some preliminary screening process may boost their quality. A smaller, higher quality science fair will be a more scientifically and educationally honest activity than the larger fair that is open to all comers. Possibly one of the most grossly unfair occurrences is to give students only a few weeks' notice in which to prepare for the fair. This device may keep participation small, but it also endorses the idea to "hurry and think up something for the science fair." Mediocrity is its direct result; sciencing is also discouraged. Such an approach is not fair.

I do not subscribe to science fairs for only the scientific and academic elite. I do subscribe to high-quality fair administration and participation. High quality need not be synonymous with elitism; it can simply be an acknowledgment that minimal standards, however defined, have been established and used. Participation should be voluntary with no academic reprisals for the student who chooses not to participate. Annual fair dates should be announced one year in advance. If possible, fairs should be held at approximately the same time each year.

In Conclusion . . .

One is obligated to understand what science really is before assuming the responsibility of leading another through the process of sciencing. All science fair directors and teacher-sponsors accept this obligation when they assume a responsibility related to student participation in a science fair. To assume the responsibility without honoring the obligation is not fair to anyone—especially the student.

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Education

The direction in which education starts a man will determine his future life.

Bodily exercise when compulsory, does no harm to the body; but knowledge which is acquired under compulsion obtains no hold on the mind.

Plato