being prepared and used, there is little detectable bacterial growth.

Such a system can be constructed for $300 to $500 in 4 to 6 hours. It should serve well in teaching laboratories, small departmental research laboratories, marine stations, and laboratories not blessed with abundant funding to permit a built-in central distilling unit (*3*).

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References and Notes

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2. O. H. Lowry and J. V. Passonneau, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1963), vol. 6, p. 792.
3. To facilitate construction, operation, and care of this distilled-deionized water system, we have prepared detailed directions that will be appended to all requests for reprints.
4. We thank Mr. R. Pearson for assistance in the construction of parts of this system. This project was an outgrowth of research supported by NIH grant GM 12779 and by PHS research career development award GM 5011 to H.M.L.

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Triops (Entomostraca) Eggs Killed Only by Boiling

Abstract. *Temporary rainpools near Khartoum, Sudan, are inhabited by the notostracan crustacean* Triops *which completes its life cycle within 4 weeks. The annual rains fall in late summer, and throughout the winter and early summer the eggs of* Triops *remain in the dried mud or dust where they may be exposed to temperatures up to 80°C. Laboratory experiments show that they can withstand temperatures up to within 1°C of boiling, but are killed in partial vacuum by 70°C, at atmospheric pressure by 100°C, or under pressure by 105°C. Exposure to high temperature seems to be necessary to break the egg diapause.*

Khartoum, capital of the Republic of the Sudan, lies about 15°30′N in the tropical zone at the junction of the Blue Nile with the White Nile; its annual rainfall averages about 12 or 15 cm, almost all falling within 2 months in late summer. Between September and May there is no rain and the skies are generally cloudless. The extensive rainpools that form during the rainy season last about 4 to 6 weeks (depending on the frequency of rainstorms) before drying for the rest of the year. The mud in the pools first dries to a cracked crust before disintegrating to a dust that may reach a temperature of 80°C during the heat of the day between March and May. Even in midwinter in January the dust may be so hot in the afternoon that bare feet are blistered by contact for only 2 or 3 minutes.

For such short-lived pools the fauna is surprisingly diverse; Rzóska (*1*) lists 12 species of entomostracan Crustacea. The most spectacular are the notostracan *Triops granarius* (Lucas), which grows to 40 mm in length, and the anostracans *Streptocephalus proboscideus* Frauenfeldt and *S. vitreus* Brauer which may reach 25 mm. Both the Notostraca and the Anostraca survive the dry period as eggs. In pools between 30° and 33°C *Triops* mature within 16 to 20 days of the first rainfall and establishment of the pools; after 25 days they are all dead, even if the pools persist longer (*1*). There is clearly only one generation a year, and Rzóska suggests that the eggs must be desiccated before they can hatch. In contrast, the Cladocera and Copepoda inhabiting the pools may go through many generations within the brief rainy season, a generation being completed within 2 or 3 days (*1*).

It seems that more than desiccation is required before eggs of *Triops* and *Streptocephalus* can hatch. Samples of mud placed in river water in the laboratory in November and December yield hatches of Cladocera and Copepoda but no signs of Anostraca or Notostraca. Samples collected at the beginning of March, however, show rapid hatches of notostracans and anostracans, even when placed in water at the midwinter temperature of the Nile River—22°C—instead of the normal 30° to 33°C of their pools: *Triops* emerged 2 days after the mud was wetted and grew at the rate of 2 mm/day in water at 22°C, apparently passing through one instar daily, at least until they attained an overall length of 20 mm; *S. proboscideus* appeared on the 3rd day and *S. vitreus* appeared on the 4th day after the mud was wetted. Five days after the first appearance of *S. proboscideus*, 20-mm-long females were carrying egg masses; *S. vitreus* were slower in developing and did not bear eggs until 10 days after hatching.

Since during the summer the mud may reach temperatures of 80°C, it was of interest to know the temperatures that these notostracan and anostracan eggs can withstand. *Triops* was hatched from mud that had been placed dry in an incubator for 1 week at 80°C, and also from mud samples that had been kept for 16 hours in an oven at 98° ± 1°C (longer periods were not tried). Fifteen-minute incubation at 102°C, however, killed any eggs that may have been present in the mud. When mud samples were placed under vacuum such that water boiled at 70°C the eggs were killed by 30-minute exposure to 75°C. Conversely, when mud samples were placed in a pressure vessel and subjected to pressures such that water boiled at 105°C, the eggs withstood 16 hours at 103° ± 1°C but were killed by 15 minutes at 106°C. Eggs of *Triops* hatched from wet mud kept overnight at 50°C but not from wet mud kept at 60°C. Each sample consisted of 100 g of mud (dry weight), and all samples yielded between five and 12 hatchlings if any hatched at all; all conditions were tested with triplicate samples, and the numbers hatching after the various treatments that allowed survival showed no significant differences. In other words, death was an all-or-none effect.

Thus it is apparent that desiccated eggs survive much higher temperatures than do eggs after wetting; indeed they can withstand temperatures within 1°C of the local boiling point of water. But they are killed by the temperature of boiling water whether the temperature is reduced by partial vacuum or raised by pressure. The mechanism of death in desiccated eggs must therefore involve the boiling of any water retained within the eggs: when the water boils, the eggs are killed; if it does not reach boiling point they survive.

Preliminary investigations indicate that not merely is a prolonged period of desiccation required for hatching of eggs both of *Triops* and of *Streptocephalus* in the Sudan; also they require exposure to temperatures exceeding 50°C. This result, however, needs confirmation by another season's work.

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References and Notes

1. J. Rzóska, *Hydrobiologia* **17**, 265 (1961).
2. Work done while I held a Royal Society Leverhulme Visiting Professorship in the University of Khartoum. I thank John Cloudsley-Thompson for facilities provided.

18 April 1968

4-Leucine-Oxytocin: Natriuretic, Diuretic, and Antivasopressin Polypeptide

Abstract. *During water diuresis in anesthetized rats, 4-leucine-oxytocin increased the urine output and the rates of sodium and chloride excretion. The potassium excretion rate was only slightly increased. During vasopressin-suppressed water diuresis, 4-leucine-oxytocin produced similar effects on urine and electrolyte excretions. In addition, it inhibited the vasopressin-induced free-water reabsorption, and it could reverse reabsorption to free-water clearance.*

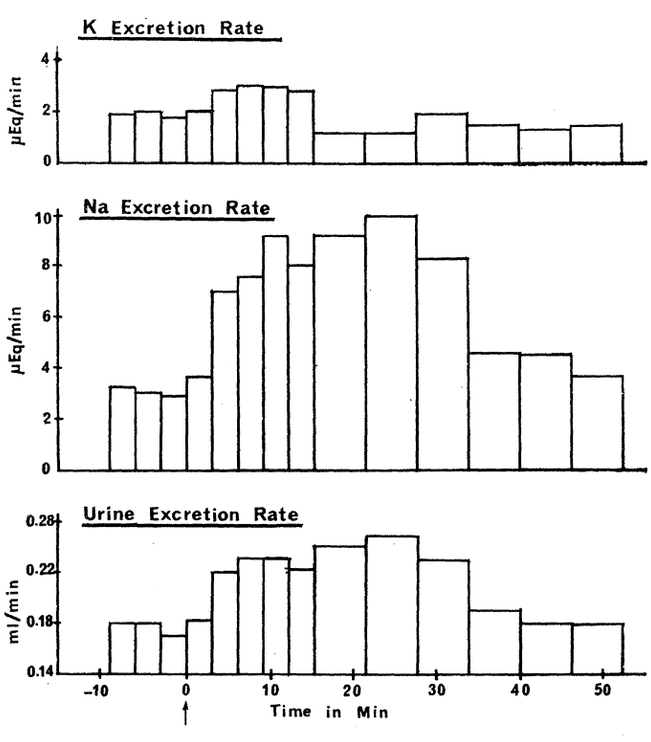
4-Leucine-oxytocin differs from the hormone oxytocin only in that it has a leucine residue in the 4-position instead of the glutamine residue of the oxytocin molecule. This oxytocin analog was synthesized (*1*) as part of a study to determine the molecular requirement for the biological activity of oxytocin.

Bioassays of this analog on rats showed that 4-leucine-oxytocin has approximately $1/50$ of the uterotonic activity of oxytocin. It also has a weak and inconsistent depressor effect on the blood pressure of the rat. It has no antidiuretic activity. On the contrary, it has a potent natriuretic and diuretic effect. Furthermore, it inhibits antidiuretic hormone activity and can reverse the free-water reabsorption induced by vasopressin (ADH) to free-water clearance in rats during vasopressin-suppressed water diuresis. More extensive studies on the renal pharmacology of 4-leucine-oxytocin are being conducted, but we believe that the renal actions of this analog are of sufficient significance and interest that a report at this stage is warranted.

Male Sherman albino rats weighing between 200 and 350 g were used. They were anesthetized with ethanol and prepared for recording and collection of urine as described by Chan (*2*). Water diuresis was induced by an oral water load maintained at a constant 8 percent of the body weight as described by Sawyer (*3*). The hydrating solution was a mixture of 2 percent ethanol, 0.5 percent dextrose, and 0.3 percent NaCl; the ethanol was incorporated to maintain anesthesia.

Urinary and plasma sodium and potassium concentrations were determined by a Baird-Atomic flame photometer, with Li_2SO_4 as an internal standard. Chloride was determined by a Buchler-Cotlove direct-readout chloridometer. Osmolality of urine and plasma was determined by the freezing-point depression method with a Fiske osmometer. The osmolality thus determined is slightly overestimated because of the alcohol content in the samples. This, however, does not greatly affect the calculation for free-water clearance.

In rats during water diuresis, when doses of 4-leucine-oxytocin less than 0.5 μg per 100 g of body weight were injected intravenously, they produced no detectable effect on the rate of urine excretion. This dose is 100 times larger than an effective antidiuretic dose of oxytocin and more than 5000 times larger than that of arginine-vasopressin. Higher doses produced a diuresis which was a consequence of marked natriuresis. The percentage increase in the rate of sodium excretion was greater



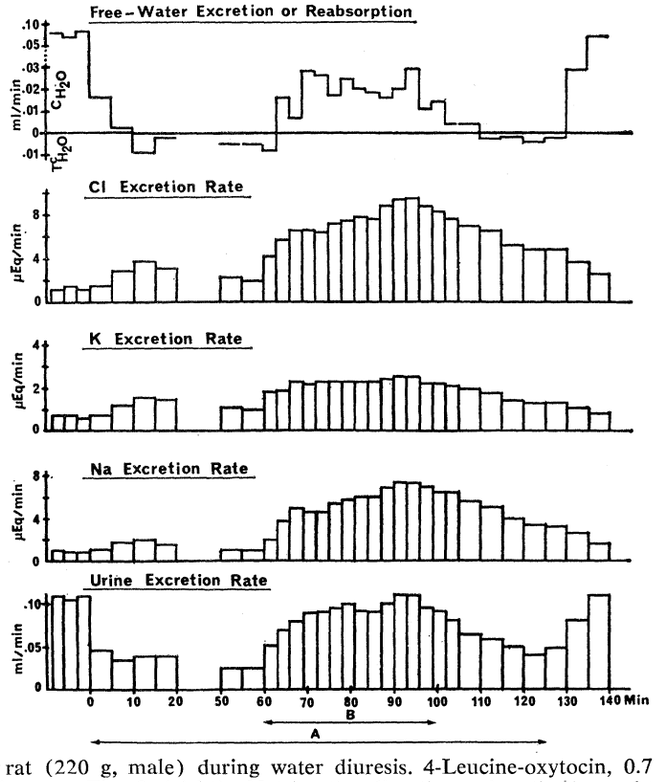


Fig. 1 (left). Natriuretic-diuretic effect of 4-leucine-oxytocin in a rat (220 g, male) during water diuresis. 4-Leucine-oxytocin, 0.7 μg per 100 g of body weight was injected intravenously at zero time. Fig. 2 (right). Natriuretic, diuretic, and antivasopressin effects of 4-leucine-oxytocin in a rat (200 g, male) during vasopressin-suppressed water diuresis. During period *A*, infusion of arginine-vasopressin, priming dose 0.025 milliunit, infusion rate 0.005 milliunit/min. During period *B*, infusion of 4-leucine-oxytocin, priming does 1.0 μg, infusion rate 0.2 μg/min.