in a rotary evaporator to approximately 100 ml without ever carrying the evaporation to dryness as a precaution against loss of activity. The first 2 tubes of the apparatus were now loaded with the starting material, and again run for 200 transfers. The settling time remained 15 min.

Samples taken from the second run left barely noticeable residues upon evaporation. Contracting activity was usually contained in the range of tubes extending from 11–20, with a clear cut peak at 15. This corresponds to a distribution coefficient of 0.08. The purification of 3-4 l of 1:1 plasma diffusate processed in the first run provided in the second run vasoconstrictor material approximately equivalent in potency to 100–200 µg NOR in 800–900 ml solvent mixture, which was then stored in the frozen state.

(3) *'New'-vasoconstrictine causes the isolated rabbit intestine to contract.* Since on rabbit aorta NOR is not antagonized by a mixture of DBMC and mepyramine, it appeared important to differentiate it from 'new'-vasoconstrictine with such conclusive biological evidence.

Discussion. Comments on the method of preparation. It was found important to use heparinized blood in order to prevent release of serotonin from platelets. Otherwise separation of 'new'-vasoconstrictine might be handicapped by an overwhelmingly large concentration of serotonin.

Meaningful figures on yields cannot be given until a standard preparation of 'new'-vasoconstrictine becomes available. By expressing the amount of 'new'-vasoconstrictine in equivalents of NOR in a bioassay it is tacitly assumed that from one preparation to the other there is no change in the relative sensitivity of the tissue to NOR and to 'new'-vasoconstrictine. This possibility may however not be rigorously correct.

Differentiation from other biologically active substances. As reported in the results, 'new'-vasoconstrictine could be distinguished from serotonin, histamine, or catecholamines. Oxytocin, vasopressin, and bradykinin were also excluded[8], because these substances did not affect the aortic strip at all. Substance P is relatively inactive on the rabbit artery (unpublished). Angiotensin was eliminated earlier on account of biological evidence[8]; and more recently by a preliminary estimate of the molecular weight of 'new'-vasoconstrictine which is half of that of angiotensin. None of the plasma proteins has any contracting activity[10].

Our observations indicate that the bulk of the vasoconstrictor potency of plasma which causes the rabbit artery to contract well beyond 50% of its maximal response, can be attributed to a mixture of serotonin and 'new'-vasoconstrictine[11].

Résumé. La «nouvelle»-vasoconstrictine (SVPx 1962), une hormone vasoconstrictrice du plasma, a été préparée par distribution à contre-courant en quantité suffisante pour l'étude de sa structure chimique et de ses propriétés biologiques.

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After-Potentials Due to an Electrogenic Pump in Molluscan Giant Neurons

A previously undescribed hyperpolarizing wave following the after-potential of every somatic spike was consistently found in 'H' neurons, i.e. neurons showing cholinergic inhibition[1], of the abdominal mass ganglia of the Argentinian land snail *Cryptomphallus aspersa*. Experiments were carried out to determine the underlying mechanism of this wave and the results to be reported suggest that it may be due to an electrogenic pump.

Materials and methods. Isolated ganglia were immersed in a saline solution for mollusc[2] containing: NaCl, 127.8 mM; $MgSO_4$, 3.5 mM; $NaHCO_3$, 3 mM; CaCl, 6 mM. Neuronal somata were impaled with simple or double glass microelectrodes. The methods for recording and stimulation in this preparation have been previously described[3,4].

Results and discussion. The hyperpolarizing wave, hereafter called Ap_2 or second after potential invariably follows each somatic spike and begins about 10 msec after the typical after-potential, hereafter called Ap_1. The hyperpolarization reaches its maximum amplitude (about 4 mV), 75 msec after Ap_1 and the total duration is about 800 msec (Figure 1A).

Ap_1 has the well-known properties of the common after-potential[3,5]; i.e. (a) When the membrane potential is changed by passing hyperpolarizing or depolarizing currents through the cell, the size of Ap_1 varies (see Figure 1). It becomes smaller as the cell is hyperpolarized and beyond a point of reversal potential, it becomes a depolarizing wave. (b) The reversal potential of Ap_1 is related to the external potassium concentration. When this is increased from the normal 4.9 mM to 15 mM, the equilibrium potential is displaced from -60 to -45 mV. On the other hand, at zero potassium concentration it is about -68 mV. (c) Variations of chloride in the solution produce no changes, either in the size or in the reversal potential of Ap_1. These data are in accordance with the potassium permeability theory for the after-potential[3,5] and the reversal potential of Ap_1 may be considered equivalent to the equilibrium potential for potassium (E_K).

Ap_2 behaves very differently from Ap_1. (a) Its size is independent of the membrane potential (see Figure 1)

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and it does not have a reversal potential. It is a hyperpolarizing wave at potentials across the membrane where Ap_1 has already reversed, i.e. at values more negative than E_K and than the chloride equilibrium potential (E_{Cl}), which in these cells is about -50 mV[4]. (b) The voltage and duration of Ap_2 increase with the number of repetitive spikes, which were fired by stimulating the neuronal soma through one of the intracellular electrodes either with short isolated stimuli or with a long depolarizing pulse. As the number of spikes increases, the Ap_2 which follows the last spike in a repetitive series is gradually transformed into a large hyperpolarizing wave of long duration, resembling the post-tetanic hyperpolarization (PTH) of other systems[6–10]. Figure 2 (B, 1) shows the effect of a depolarizing intrasomatic pulse lasting 2.5 sec and which produced 60 spikes. This tetanic stimulation evoked a hyperpolarization, with an amplitude of 10 mV and a duration of 30 sec. (c) The input resistance does not change during the Ap_2 or PTH. The passage of square wave current pulses through the cell, either before or during these phenomena, produces a constant voltage drop. (d) Using 0.3 mM 2.4 dinitrophenol (DNP), a gradual and simultaneous decrease of the Ap_2 and PTH was obtained, with complete disappearance after 45 min. This effect was reversed by washing the preparation with saline for 45 min (see Figure 2). Similar results were obtained with 3 mM sodium azide and by lowering the temperature (4 °C) of the medium. While the cell was in DNP the input resistance did not change. Also, the membrane potential and the potassium equilibrium potential (characterized by the inversion potential of Ap_1) were not influenced by DNP. Ouabain $5 \times 10^{-4} M$ (a value higher than the one used to inhibit the sodium electrogenic pump in *Helix*[11]) does not affect Ap_2 or PTH in our cells.

In the unipolar cells of mollusca an axonic spike may activate other branches of the axon without invading the soma[12]. In our preparation, axonic spikes blocked near the soma, either by hyperpolarizing currents or fatigue[13], are never followed by an Ap_2 (see Figure 3). Furthermore, when there is a partial blockade of the

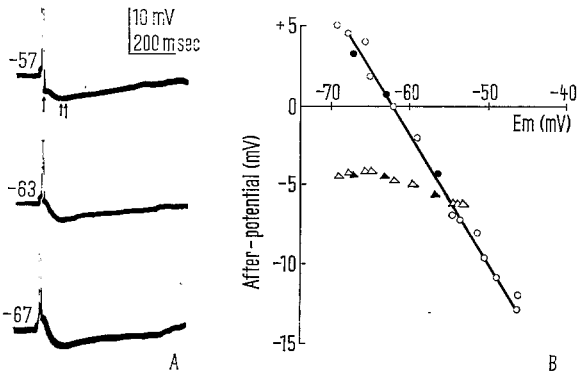

Fig. 1. Variations of the first after-potential (Ap_1) and second after-potential (Ap_2) with the membrane potential. (A) Spikes at 3 membrane potentials: -57 mV; -63 mV and -67 mV. In the upper trace, simple arrow indicates Ap_1; double arrows, Ap_2. Middle trace shows Ap_1 near the inversion potential, followed by Ap_2. In the lower trace, Ap_1 has reversed and is followed by Ap_2. (B) Relationship between the membrane potential, in the abscissa, and size of Ap_1 (circles) and Ap_2 (triangles) in the ordinate. Filled circles and triangles are corresponding values for Ap_1 and Ap_2 at the membrane potentials shown in A. Ap_1 (circles) presents inversion potential. It may be observed that the size of Ap_2 is independent of the membrane potential.

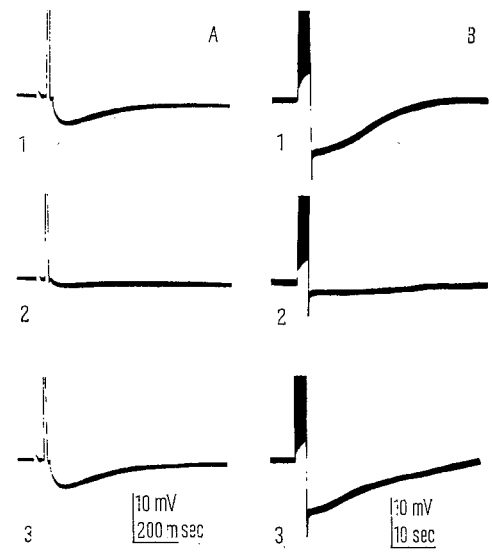

Fig. 2. Abolition of Ap_2 and post-tetanic hyperpolarization (PTH) with 2,4-Dinitrophenol (DNP). (A) 1. Control spike with Ap_1 at the inversion potential, followed by Ap_2. 2. After 30 min in DNP; Ap_2 is almost abolished. 3. Recovery of Ap_2 after washing in fresh saline during 45 min. (B) 1. Train of spikes obtained by a depolarizing pulse of 2.5 sec followed by a post-tetanic hyperpolarization (PTH). 2. After 30 min in DNP, the PTH is greatly reduced. 3. Recovery of the PTH after washing in saline during 45 min.

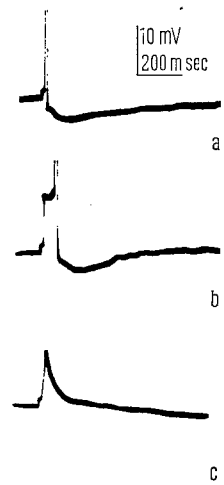

Fig. 3. (a) Somatic spike showing Ap_1 and Ap_2. (b) Same spike showing partial blockade produced by a hyperpolarizing current. The axonic and somatic components of the spike are seen. Ap_2 follows the somatic part of the spike. The delay between Ap_1 and Ap_2 is constant with respect to the control. (c) At a more hyperpolarized potential, there is complete blockade of the somatic spike and only the axonic component is seen. It may be observed that Ap_2 never follows the axonic spike.

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spike, the delay between Ap_1 and Ap_2 is constant with respect to the control without any blockade. If a recurrent synaptic branch is responsible for Ap_2, this wave should be seen even when the axon is discharged without a somatic invasion. Therefore, Ap_2 is related to somatic and not to the axonic spike and is not synaptic in origin.

These findings on the Ap_2 and PTH exclude the interpretation that they are caused by an increase in permeability, as was proposed for PTH in other systems[8]. Ap_2 has no reversal potential and it is a hyperpolarizing wave at values that are beyond E_{Cl} or E_K; furthermore, there is no change in conductance either during Ap_2 or PTH.

We conclude that Ap_2 and PTH are caused by an active pump, stimulated, probably, by the positive charges which penetrate the soma during the spike. Both are inhibited by DNP, sodium azide, or low temperature, the difference between them being mainly quantitative, since by increasing the number of spikes Ap_2 is gradually transformed into PTH.

To explain the PTH in mammalian C fibers, a neutral pump has been proposed[6]. The hyperpolarization would be caused by the removal of potassium from the extracellular fluid by the pump. In our system, the values of Ap_2 and PTH are more negative than those of the membrane potential in a medium with zero potassium. This does not agree with the neutral pump theory, and hence, we do not support it for 'H' neurons. Other authors favor an electrogenic pump to explain PTH in preparations studied by them[7, 9, 10].

We believe that the mechanism of Ap_2 and PTH in our system is an electrogenic active pump, extruding positive charges which have penetrated during the spike, without a tight coupling with other charges. An active entrance of chloride cannot explain Ap_2 or PTH, since a total replacement of this ion by sulphate or acetate did not reduce the amplitude of these phenomena. Since somatic spikes in 'H' cells are mainly calcium dependent[14], and they can be obtained in sodium-free media concurrently with Ap_2 or PTH, the extruded ion could be calcium. Further work is proceeding to clarify this point. The fact that ouabain does not affect Ap_2 nor PTH is an interesting finding since SCHATZMANN[15] found that the calcium pump in erythrocytes is also insensitive to ouabain[16].

Resumen. En un grupo de neuronas centrales de caracol, cada potencial de acción es seguido invariablemente por una onda hiperpolarizante. Esta onda no es debida a un aumento de permeabilidad de la membrana; aumenta en duración y voltage a medida que se aumenta el número de potenciales de acción que la producen y es eliminada reversiblemente por inhibidores metabólicos. Se la interpreta como producida por una bomba electrogénica que extruye las cargas que penetraron al soma neuronal durante el potencial de acción.

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Activity and Isolated Phytoestrogen of Shrub Palmetto Fruits (*Serenoa repens* Small), a New Estrogenic Plant

Very few estrogenic compounds have been isolated from plant materials due to the exhaustive techniques and small yield of the active principle. Among them, β-sitosterol possesses a considerable estrogenic potency when isolated from *Glycyrrhiza glabra*[1] and rice polish[2] as well as when the commercial product was crystallized from methanol and tested[3]. HÄNSEL et al.[4,5] detected a relatively high concentration of free (18.9 mg/100 ml) and bound (22.7 mg/100 ml) sitosterols in dried shrub palmetto fruits (family: Palmae) which is of some medical value. This big sitosterol-content of the fruits stimulated the plant investigation and its active principle in this work.

The dry fruits (*Serenoa repens* Small., formerly called *Sabal serrulatum* Schult) were crushed and Soxhlet-extracted with 90% methanol for 10 h. Evaporation of the alcohol under reduced pressure left 5.34 gm/100 ml of a yellow brownish residue which possessed no estrogenic activity when 10 mg was injected s.c. in 10 immature female mice according to EVANS et al.[6]. As higher doses always posed the problem of separation from the neutral oil, the other part of the extract was subjected to partial purification[7]. Subcutaneous doses of 2.5 mg and 5.0 mg daily given 3 days of this partially purified extract induced increases of 20.72 and 32.95 per 100 mg of the control uterus respectively, establishing a significant estrogenic activity.

Then, β-sitosterol was isolated chromatographically[8] to be recrystallized from methanol in the form of needles melting at 137–138 °C

$C_{29}H_{50}O$ (414.40)	found:	C 83.61%	H 11.93%
	calculation:	C 83.99%	H 12.15%

Further confirmation was afforded by the Rf value (0.87) on silica gel plates developed in chloroform/acetone (8/2) and paper chromatograms developed in ethyl acetate/chloroform/paraffin oil (65/25/10). Also, the UV- and IR-

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