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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spectra were made, as well as analysis of the acetyl derivative of the compound.

This isolated β-sitosterol was estrogenically active when gradual doses were injected in groups of 10 immature female mice (NMRI-Han). The uterine response to increasing levels of β-sitosterol indicated no relation between its dose and the estrogenic activity (Table). Based upon the mouse-uterine-weight test, 2.0 µg represented the minimum daily dose per mouse when injected s.c. Under the same conditions, a dose response curve was constructed with 17-β-estradiol (Schering) from which the estrogenic potency of the partially purified extract and the isolated active principle was estimated. As expressed in terms of estradiol equivalents, the extract and β-sitosterol were 9.68×10^{-5} and 7.71×10^{-2} respectively.

The relatively high potency of β-sitosterol is very marked compared with the other identified phytoestrogens. BICKOFF et al.[9] mentioned that coumestrol is 30 times as active as genistein which is 4.53×10^{-5} estradiol equivalents[10]. According to WONG and FLUX[11], the relative potencies of genistein, biochanin A and diadzin are 1.5:1.0:0.4, a result which differed from that of CHENG et al.[12] giving the greatest activity in diadzin, an equal activity in biochanin A and genistein, and least in formononetin. In this connection, a study of the literature emphasizes a great difference in opinion regarding estrogenic potency of the individual phytoestrogens. But this is not astonishing if we consider the variables, e.g. location, temperature, plant variety, experimental animals, estrogenic test, etc., which affect estrogenicity in plants[13,14].

Estrogenic activity of shrub palmetto fruits and its isolated active principle

Group No.	Daily dose (µg)	Average body wt. (g)	Uterine wt. as % of body wt. (mg/100 ml)	P value
		Control		
1	oil	9.2 ± 0.275	69.5 ± 2.94	–
		Crude extract		
2	10,000	10.2 ± 0.304	65.5 ± 2.05	≃ 0.20[a]
		Partially purified extract		
3	2500	10.6 ± 0.331	83.9 ± 1.86	≃ 0.0027
4	5000	10.5 ± 0.396	92.4 ± 1.81	< 0.0002
		β-sitosterol		
5	1.0	11.2 ± 0.320	73.0 ± 3.75	≃ 0.735[a]
6	2.0	12.2 ± 0.256	85.1 ± 4.12	≃ 0.01
7	5.0	11.2 ± 0.357	80.8 ± 2.29	≃ 0.01
8	10.0	11.5 ± 0.392	83.2 ± 3.85	≃ 0.02
9	25.0	12.5 ± 0.354	82.0 ± 1.47	< 0.005
10	50.0	9.9 ± 0.314	81.2 ± 2.58	≃ 0.01

The mean value (± S.E.) of 10 animals in each group. [a] Insignificant difference between experimental and control mice.

Zusammenfassung. 21 Tage alten weiblichen Mäusen wurde während dreier Tage durch s.c. Injektionen ein Extrakt von Sabalfrüchten (*Serenoa repens* Small, früher *Sabal serrulatum* Schult) verabreicht. Es wurde eine hohe Östrogenaktivität festgestellt, welche durch β-Sitosterol hervorgerufen wird, welches in dieser Pflanze in relativ hoher Konzentration vorhanden ist.

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Transmission of Rauscher Leukemia in Mice

Rauscher Leukemia Virus (RLV) produces a rapid erythroid leukemia and develops lymphoid leukemia in mice surviving the abnormal erythrocytopoiesis[1-4]. The experiments presented in this communication are concerned with the transmission of RLV in mice.

The stock of RLV was obtained from spleen extracts from leukemic mice supplied by Dr. F. J. RAUSCHER of the National Cancer Institute, Bethesda, Maryland, and from plasma from leukemic Ha/ICR swiss mice in our laboratory. The experimental procedures employed in these experiments are similar to those previously described in our studies with Friend virus[5-7].

Table I presents data showing our attempt to transmit RAUSCHER leukemia (RL) to newborn Ha/ICR swiss by various body fluids, tissue extracts, and feces from RAUSCHER virus-infected female and male Ha/ICR swiss mice. All specimens were collected from mice when viremia was at its peak, during the first month after infection.

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