could in turn be triggered by a microapocrine releasing activity at the axon level, similar to that described in the neurosecretory system of several other species[10–12].

Resumen. El tamaño y el número de neuronas vesiculadas en los núcleos neurosecretores del perro están significativamente correlacionados con el largo axonal. Despues de la sección del tallo hipofisiario, las células vesiculadas degeneran más rapidamente que las no-vesiculadas. Estos resultados permiten suponer que las vesículas neurosecretoras se originan como resultado de mecanismos de secreción neuroapocrina en los axones.

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Instituto de Investigaciones Médicas M. y M. Ferreyra, Casilla de Correo 389, Córdoba (Rep. Argentina), 15 November 1971.

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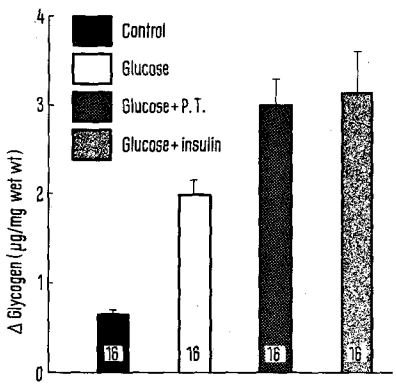
Effect of a Potent Hypolipemic Agent on Glycogen Metabolism

The presence in blood of high levels of cholesterol, non esterified fatty acids (NEFA) and tryglycerides is a common feature of several diseases. Besides the metabolic disturbance that this abnormal levels reflect, they also interfere with other metabolic functions, i.e. the high levels of serum NEFA impair glucose uptake in muscle[1]. Therefore, pharmacologists and internists are continuously searching for compounds able to drop down the abnormally high levels of circulating lipids. In 1968, Pereira et al.[2,3] described a new compound, 5-(3-pyridyl) tetrazole (3-PT) with a chemical structure similar to the nicotinic acid one. Like this acid, 3-PT has a potent in vivo hypolipemic effect. In the present experiment we have tested the effect of 3-PT upon carbohydrate metabolism in muscle and compared it with the insulin one, using an in vitro experimental model.

Material and methods. Female mice of the C3H-S strain, 9 weeks old, were used throughout the experiments. These animals were provided by the Instituto de Embriología, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. They were caged in groups of 10 in a room ad hoc at a temperature of 25 ± 1°C with water and food ad libitum and illumination (fluorescent light 40 W) from 06.00 to 18.00 h alternating with 12 h darkness.

In the present experimental design, lots of 18 animals each were killed by cervical dislocation and decapitation at 16.00 h. In each animal the diaphragm was quickly and carefully dissected, washed with cool buffer in a Petri dish and middly excized. One hemidiaphragm was kept as a non-incubated control while the corresponding pair was treated as it will be described: Following a 20-min preincubation period in a flask with a 4°C medium, the hemidiaphragms were transferred and incubated in a second flask containing a medium at 37°C for 90 min in a Dubnoff shaker. In both periods, the flasks were continuously gassed with 95%O_2–5% CO_2. The preincubation medium contained bovine albumin (100 mg/100 ml) and glucose (300 mg/100 ml) in Krebs-Ringer-Bicarbonate (KRB) with the addition of glutamate, fumarate and pyruvate. The 37°C incubation medium had the same composition, but in some cases, either (3-PT) or cristalline insulin was added in a concentration of 1.25 mg/100 mg and 1 mU/ml, respectively. In other cases a combination of both compounds was simultaneously studied. At the end of the incubation period both the non-incubated as well as the incubated hemidiaphragms were treated for glycogen extraction and determination, according to Seifer's method[4]. The results were expressed as the quotient obtained substracting the glycogen value achieved in the control and non-incubated hemidiaphragm from the one attained in the paired incubated one.



Each bar represents average ± S.E.M. In circles, number of cases. P between control and glucose < 0.001. P between glucose and 3-PT < 0.005. P between 3-PT and insulin N.S.

Results. The Figure shows the results obtained expressed as μg of glycogen per mg of wet weight tissue. The incubated hemidiaphragms present significantly larger glycogen values when compared with the non-incubated ones ($P < 0.001$). Otherwise, the addition of either insulin or 3-PT produces a further increase above the one elicited by glucose alone ($P < 0.005$). When insulin and 3-PT were simultaneously tested, the tissue behaved as if it were in the presence of a single compound (data not shown). On the other hand, the 2 compounds – in the concentration employed – produce similar changes in the tissue glycogen content of the incubated hemidiaphragms.

Discussion. Muscle glycogen increases when the tissue is incubated in the presence of high levels of glucose[5]. Furthermore, this in vitro synthesis of glycogen can be enhanced by the addition of insulin to the incubation medium. Our results indicate that 3-PT in a concentration

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of 1.25 mg/100 ml was able to stimulate glycogen synthesis in the incubated tissue with a potency equivalent to 1 mU of cristalline insulin. Conversely, this compound was unable to modify the stimulatory effect of insulin upon the above-mentioned process (data not shown). No data are available yet about the effect of 3-PT on carbohydrate metabolism. Studies are in progress trying to explain the mechanism through which the drug exerts its glycogenic effect. This compound also inhibits the release of NEFA from adipose tissue elicited by norepinephrine in vitro[3]. The miniumum effective drug concentration employed in those experiments was below that used in the present ones ($10^{-3}M$ and 8.1×10^{-2}, respectively). Experiments performed in human beings have demonstrated that the administration of this compound significantly lowered the NEFA serum levels in normal fasted volunteers[2,3,6].

The present results clearly show that the 3-PT works directly on muscle carbohydrate metabolism. Thus, this compound could play an important role in the treatment of clinical diseases in which both conditions, high levels of circulating NEFA and impaired carbohydrate metabolism, are present, such as the case of diabetes. This possiblity warrants further research on the applicability of this drug.

Resumen. Mediante la incubación de hemidiafragmas de ratón, se estudió el efecto de un potente hipolipemiante sobre la síntesis de glucógeno in vitro. Este compuesto, 3-PT, produjo un franco incremento de la síntesis de glucógeno, comparable al obtenido con 1 mU de insulina.

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Y-Organ of the Crab, *Varuna litterata* (Fabricius)

GABE[1,2] observed a pair of glands in the maxillary or antennary segments of malacostracans and named them 'Y-organs'. Since his discovery, the morphology of this organ has been reported in a few crustaceans[3,4]. Surgical ablation and replacement therapy have confirmed that the Y-organ hormone controls various physiological activities of the crustaceans.

In the crab, *Varuna litterata*, the Y-organ on either side is situated ventral to the adductor muscle in the antennary segment. It is a compactly ovoid structure (Figure 1) measuring 0.4–0.5 mm in diameter and is surrounded by a sinus. For the histological observations on Y-organ, paraffin sections of the tissue fixed in Bouin's fluid were cut at 6–8 μm and stained with Gomori's chrom-alum-haematoxylin phloxin (CHP) and Heidenhain's azan methods[5].

The Y-organ is composed of closely packed subspherical cells distinguishable into 2 types: small and large. The small cells are more abundant, with a distinctly staining, roughly spherical nucleus. The nucleus is characterized by a single, centrally placed or peripherally situated nucleolus. The chromatin material, in the form of small particles, is seen lining the inner surface of the nuclear membrane. The cell outline, as also the cytoplasm, are indistinct (Figure 2). The large cells are scarce and when stained with CHP show phloxinophilic nuclei and basophilic cytoplasm. The evenly distributed cytoplasmic granules are coarse and basophilic in nature. The nucleus possesses a central nucleolus and the chromatin material is uniformly

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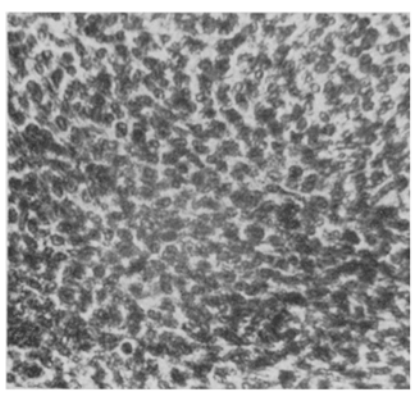

Fig. 1. Transverse section of the Y-organ of *V. litterata*, showing closely packed cells.

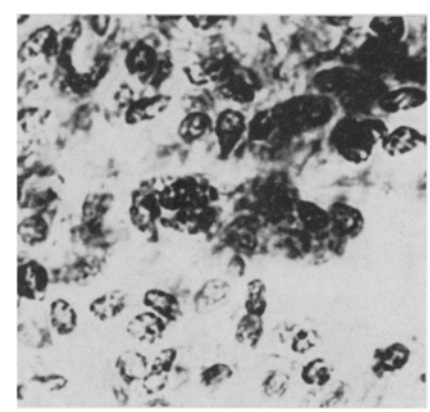

Fig. 2. Transverse section of the Y-organ of *V. litterata*, showing 2 types of cell.