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Inhibitory Effect of Captan in the Small Intestine Absorption Capacity of the Mouse

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Captan, N-(trichloromethyl)-thio cyclohex-4-n-1,2-dicarboximide has been used as a protective, non-systemic fungicide for the treatment of foliar and seed-borne diseases showing low toxicity to animals in laboratory tests (Gaines and Linder, 1986). On the other hand, there are evidences indicating this fungicide has a mutagenic and teratogenic action on different animal species (Bridges, 1975; Carere et al., 1978; Martin and Lewis, 1979).

Little is known about the action of captan on physiological parameters of animals who have been exposed to it. Among them the small intestine, important from the view point of absorption of nutrients needed for growth and maintenance has proved to be very sensitive when exposed to environmental pollutants. The transport mechanism in epithelial cells may be affected by relative low doses of such chemicals (Iturri and Wolff, 1982; Madge, 1976; Iturri and Peña, 1986; Miller, 1981; Wapnir et al., 1977).

Therefore this investigation was designed to evaluate the effect of captan on the intestinal absorption capacity of the mouse by measuring the transport of D-glucose and L-tyrosine through the intestinal epithelium in *in vitro* and *in vivo* preparations.

MATERIALS AND METHODS

Experiments were carried out in adult female mice (CF_1 strain) obtained from Instituto de Salud Pûblica, Chile. They were fed a commercial diet *ad libitum* with free access to water. All animals ranging in weight from 25-30 g were fasted 24 h prior to the experiment.

In *in vitro* experiments animals were killed by atlas dislocation. The mid portion of the small intestine (3-4 cm long) from each animal was excised, washed with ice-cold 0.15 M NaCl and everted forming an intestinal sac according to Wilson and Wiseman (1954). Details of the preparation have been described in a previous work (Iturri and Wolff, 1982). Alternate intestinal pieces according to their position relative to the stomach were used as control or

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experimental material in order to minimize the transport variability of the segments in succesive experiments. Captan dissolved in ethanol 95% was diluted in a Krebs-Henseleit solution to reach a final concentration of 10^{-4} - 10^{-7} M inside the sac.The active transport of D-glucose (10 mM) and L-tyrosine (2 mM) was evaluated by measuring the increase in concentration of both compounds inside the sac after 60 min of incubation. D-glucose was determined using the method of Somogyi-Nelson (Nelson, 1944) and L-tyrosine by the method of Lowry et al. (1951). The sacs were weighed and the area determined at the end of the experimental period.

In *in vivo* experiments animals were fasted 24 hr prior to the experiment and anesthetized with i.p. injection of urethane (0.12 g/100 g body weight). A laparotomy (3-4 cm) was performed to locate and isolate the mid portion of the jejunum between two polyethylene cannulae. An inlet cannula was inserted and tied to the intestine and connected to a perfusion pump (Harvard Apparatus, Mod 600-900). The outlet cannula placed 4-5 cm apart from the inlet cannula was used to collect the perfusate. Before starting the perfusion, the intestinal lumen was flushed with 0.15 M NaCl. Intestinal perfusate was a Krebs-Henseleit solution maintained at 30°C containing D-glucose (10 mM) and L-tyrosine (2 mM) with or without captan dissolved in the solution to give a final concentration equal to 10^{-4} - 10^{-7} M. The transport of D-glucose and L-tyrosine was evaluated measuring the change in concentration of both compounds in the perfusate for a 30 min period during 2 hours, being the perfusion rate of 0.1 ml/min. D-glucose was determined by the Somogyi-Nelson (1952) method and L-tyrosine by the method of Lowry et al. (1951).

Results are expressed as means ± SEM. Statistical analysis was done in an IBM computer, model PC XT using an ANOVA test and Duncan's test for multiple comparison between means (Sokal and Rohlf, 1969) being the significance accepted with values of probability less than 0.05.

RESULTS AND DISCUSSION

Figure 1 show the effect of captan (10^{-7} - 10^{-4} M) on the active transport of D-Glucose (10 mM) and L-tyrosine (2 mM) in everted sacs of mouse small intestine. Results are expressed as moles x $10^{-8} \times g^{-1} \cdot h^{-1}$. In all cases the inhibition of the active transport of both compounds increased as the concentration of the fungicide increased. This inhibition was statistically significant ($P < 0.01$) in the case of tyrosine with the two highest concentrations of captan used (10^{-5} - 10^{-4} M) compared with values from control group. The glucose absorption capacity even though showed to be inhibited in a concentration dependent manner was not statistically significant probably due to a high variability observed within groups. However, the decrease showed by experimental groups compared with values from control allow us to assume that there is an inhibition produced by captan. The results obtained in the present study show that captan at concentrations as low as 10^{-5} M may inhibit epithelial transport of glucose and

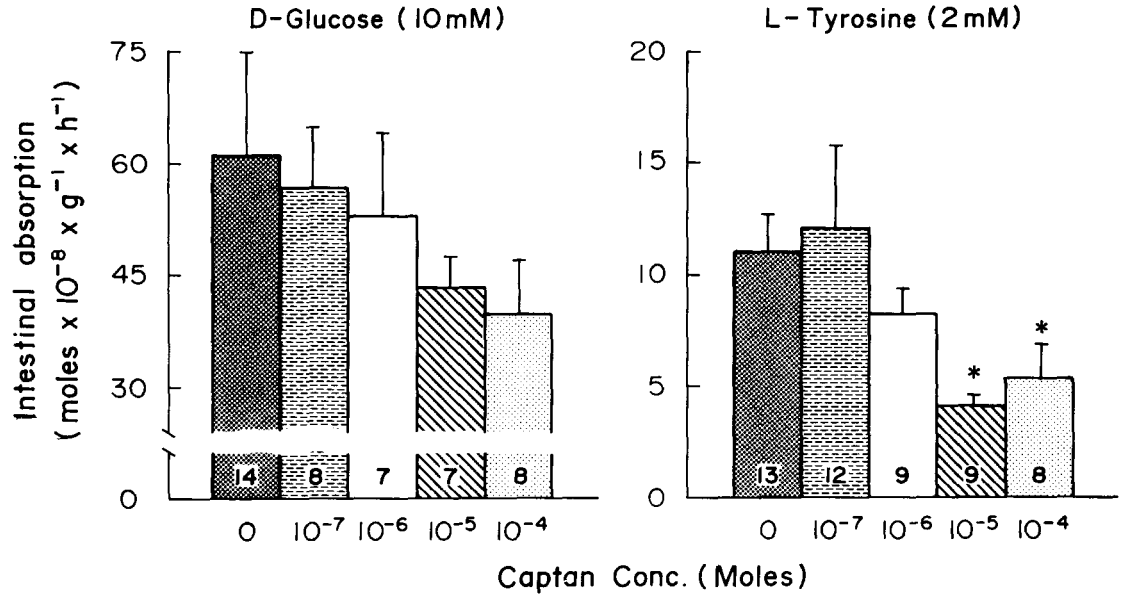

Figure 1. The effect of captan on the active transport of D-glucose and L-tyrosine in evented sacs of mouse small intestine. Results are expressed as moles x 10^{-8} x g^{-1} x h^{-1}. Vertical lines (top of bars) represent ± SE of the means. Number inside bars represent number of experiments. *$P < 0.01$

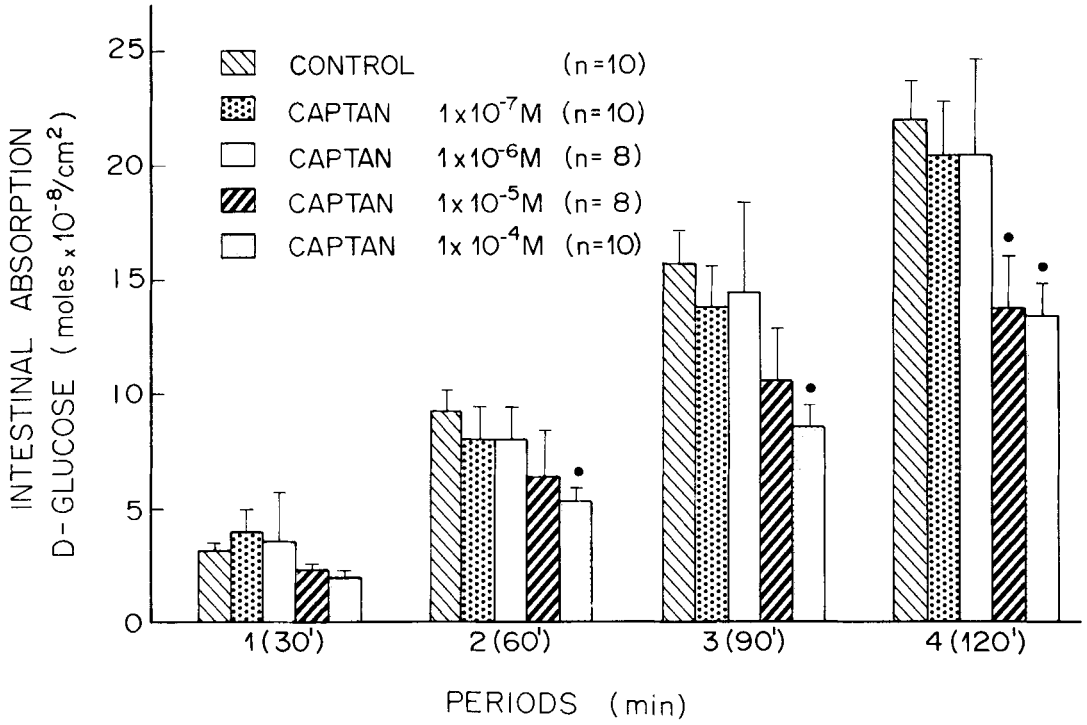

Figure 2. The effect of captan on the mouse intestinal absorption of D-glucose during a 30 min perfusion period during 2 hours. The perfusion rate was 0.1 ml/min. Results are expressed as moles x $10^{-8}/cm^2$. Vertical lines (top of bars) represent ± Se of the means. (n) = number of experiments.
•P < 0.01

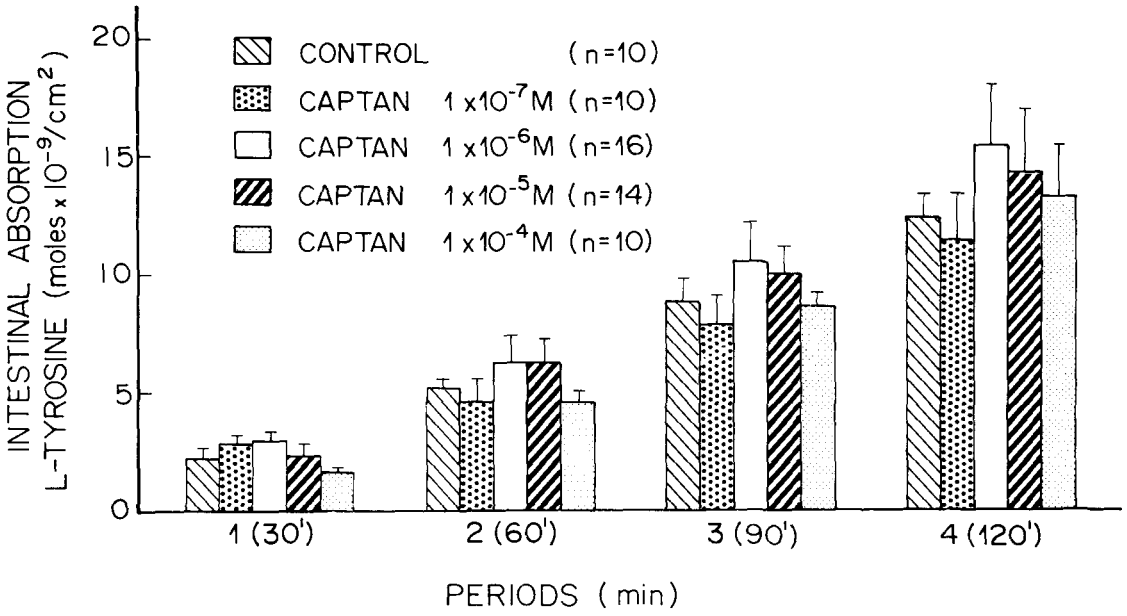

Figure 3. The effect of captan on the mouse intestinal absorption of L-tyrosine during 30 min perfusion period during 2 hours. The perfusion rate was 0.1 ml/min. Results are expressed as moles x 10^{-9}/cm^2. Vertical lines (top of bars) represent ± SE of the means. (n) = number of experiments.

tyrosine in everted sacs of mouse small intestine. As was pointed out previously (Iturri and Wolff, 1982) nothing can be said from the results obtained with this experimental procedure about the effect of this fungicide on the passive transepithelial transport for these non-electrolytes.

Figures 2 and 3 show the effect of captan on the intestinal uptake of D-glucose and L-tyrosine during a 30 min perfusion period during two hours. Fig. 2 shows that the absorption capacity for glucose uptake decreases as the concentration of captan increases. This fact is more noticeable in periods 3 (90 min) and 4 (120 min) with a captan concentration equal to 10^{-5} and 10^{-4} M ($P < 0.01$). This inhibition by captan is equal to 39.2% (10^{-5} M) and 44.4% (10^{-4} M) at 90 min and slightly diminishes at 120 min being 37.3% (10^{-5} M) and 38.6% (10^{-4} M). The effect on tyrosine transport was not significant with the concentrations used (Fig. 3).

It has been established how sensitive the intestinal epithelium might be in nutrient uptake when toxic substances are present (Iturri and Wolff, 1982; Iturri and Peña, 1986; Miller,1981; Madge 1976; Wolff and Bull, 1982; Iturri et al., 1989). There are no data related to the effect of captan on the intestinal absorption capacity of mammalian species.

The exact mechanism by which captan induces inhibition of intestinal uptake *in vitro* and *in vivo* is not clear from this study. At the present stage, however we assume that captan may be interfering on 1) membrane lipids of epithelial cell, 2) the $(Na^+ + K^+)$-ATPase activity from the basolateral membrane of epithelial cell or 3) the protein molecule with carrier capacity located in the brush border membrane of the epithelial cell. In this respect there are some experimental evidences showing that chemicals may decrease intestinal absorption capacity affecting a single or combination of these mechanisms (Musch et al., 1990; Iturri et al., 1989; Chauncey et al., 1988; Wolff and Bull, 1982; Miller, 1981). Finally the present findings are relevant in assessing the potential value of how apparent non toxic substances may interfere with intestinal nutrient absorption, an important process needed for growth and maintenance of the individual.

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