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The Effect of Dicumarol on the Active Transport, Diffusion and Oxygen Consumption of Intestinal Sacs *

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This work includes the investigation of the effect of dicumarol on the active transport, diffusion and oxygen uptake of intestinal sacs. The results indicate that dicumarol, 10^{-5} M and 5×10^{-5} M, when added to the mucosal fluid, inhibits the active transport of galactose from the mucosal to the serosal fluids and inhibits oxygen uptake in the tissue.

If dicumarol were initially present only in the serosal fluid, it had no influence on the galactose transfer.

It was observed that dicumarol directly hastens the arabinose transference from the mucosal to the serosal fluid (arabinose being initialy present only in the mucosal fluid).

The urea transfer is basically constant, independent of the presence or absence of a 10^{-5} M dicumarol concentration in the mucosal fluid.

The active transport of galactose from solutions without dicumarol, in inhibited following a 30 minutes preincubation with 5×10^{-5} M dicumarol.

In a previous investigation conducted by this Department, using the *in vivo* successive absorption technique (5), it has been verified that dicumarol inhibits the intestinal absorption of glucose whereas it increases that of arabinose. A similar effect has been verified (6) in the case of diafragma. Based on these results, it was decided to investigate by means of the *in vitro* technique: 1. The influence of dicumarol on the active transport of D-galactose through intestinal sacs. 2. The influence of dicumarol on the transference of L-arabinose and urea which otherwise are not actively transported. 3. The oxygen consumption by intestinal sacs.

Materials and Methods

White Wistar rats weighing 150-200 g were used. These rats were starved for

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24 hours prior to the experiment. Everted sacs, 3 cm long, were prepared from the middle portion of the small intestine in accordance with the *in vitro* technique of WILSON and WISEMAN (8). The sacs filled with 0.3 ml of serosal fluid were placed in 20 ml Warburg, flasks, each containing 4 ml of mucosal fluid. The mucosal and serosal fluids were taken from a 1.000 ml prepared saline solution, composed of 5.746 g of NaCl; 0.354 g of KCl; 0.162 g of KH_2PO_4; 0.294 g of $MgSO_4 \cdot 7H_2O$; 1.971 g of $Na_2HPO_4 \cdot 2H_2O$; 0.488 g of $NaH_2PO_4 \cdot 2H_2O$; and recently boiled distilled water.

The mucosal and serosal fluid initially contained 5 mM of D-galactose. In the case of L-arabinose and urea the concentration in the mucosal fluid only was 50 mM and 10 mM respectively.

The Warburg flasks were maintained in a water bath at 37 $\pm$ 0.01° C and shaken at 90 oscillations/minute at an amplitude of 3 cm. At the end of each experiment, the oxygen consumption and the D-galactose, L-arabinose and urea concentrations in the mucosal and serosal fluids as well as in the intestinal wall were measured.

The oxygen consumption results are expressed in micromoles per 100 mg of wet tissue. The wet to dry weight ratio was constant, corresponding to 83.0 $\pm$ 0.6 percent of water. The net transfer of galactose, arabinose and urea from the mucosal to the serosal fluids is expressed in micromoles per 100 mg of wet tissue. The concentration index (Sf/Si). ie., the ratio of the final to initial galactose concentrations in the serosal fluid, is also included as an indication of the active transport. The sugar and urea concentrations were determined utilizing the SOMOGYI (7) and MAC FATE (4) methods respectively.

Results

INFLUENCE OF DICUMAROL ON THE ACTIVE TRANSPORT OF GALACTOSE. — The results in Table I indicate that dicumarol, when added to the mucosal fluid, inhibits the active transport of galactose from the mucosal to the serosal fluids.

Table I. *Effect of dicumarol on the active transport of galactose (5mM) and on the oxygen consumption of everted intestinal sacs.* Absorption time 30 minutes. The values are accompanied by the average standard error.

N.° exp.	Dicumarol M	Galactose transfer μM/100 mg	Active transport Sf/Si	O_2 uptake μM/100 mg
25	—	1.05 $\pm$ 0.13	2.34 $\pm$ 0.13	3.60 $\pm$ 0.12
9	10^{-5}	0.54 $\pm$ 0.13	1.78 $\pm$ 0.06	3.39 $\pm$ 0.15
11	5×10^{-5}	0.02 $\pm$ 0.01	1.02 $\pm$ 0.01	2.43 $\pm$ 0.12

It was observed, in 30 minute control experiments (ie.. those in which dicumarol is not present), that galactose, at an initial 5 mM concentration both in the mucosal and serosal fluids, is actively transported to the serosal fluid with a resulting concentration index Sf/Si = 2.34. Thus a 2.34 fold concentration gradient was produced together with a net galactose transference of 1.05 μM/100 mg or wet tissue. When a 10^{-5} M dicumarol concentration was present in the mucosal fluid, this transference is reduced to 0.54 μM/100 mg with a resulting concentration index Sf/Si = 1.78. At a 5×10^{-5} M dicumarol concentration, the active transport of galactose essentially ceases. The resulting concentration index was 1.02. The differences in the results for these three cases are very significant.

In other experiments, 10^{-5} M and 5×10^{-5} M dicumarol concentration were initially present only in the serosal fluid. Under these conditions, it had no influence neither on the galactose transfer nor on the concentration index.

INFLUENCE OF DICUMAROL ON THE ARABINOSE TRANSFERENCE. — It is known that arabinose is not actively transported by

the intestine. For this reason dicumarol was added to the mucosal fluid in order to determine its influence, if any, on the arabinose transference. It was observed that dicumarol directly hastens the arabinose transfer from the mucosal to the serosal fluid (arabinose being initially present only in the mucosal fluid).

Table II indicates that, in control experiments lasting 30 minutes. arabinose, at a concentration of 50 mM in the mucosal fluid, is transfered to the serosal fluid of the order of 0.86 ± 0.09 μM/100 mg of wet tissue. This same transfer is of the order of 1.46 ± 0.07 μM/100 mg when a 5×10^{-5} M dicumarol concentration is present. There is thus a transfer increase of approximately 70 %.

INFLUENCE OF DICUMAROL ON THE UREA TRANSPORT. — It has been seen that dicumarol inhibits the active galactose transport and increases the arabinose difusion under the conditions indicated above. It was thus decided to determine if dicumarol has a specific influence on arabinose, or whether the behavior of arabinose could be explained by an unspecified increase in the intestinal permeability. For this reason the influence of dicumarol on the transfer of urea, which initially present only in the mucosal liquid at a 10 mM concentration, was studied. It can be seen in Table III that the urea transfered is basically constant, independent of the presence or absence of a 10^{-5} M dicumarol concentration in the mucosal fluid.

Table II. *Effect of dicumarol on the diffusion of arabinose across the intestinal wall.* Concentration of arabinose and dicumarol 50 mM and 5×10^{-5} M respectively in the mucosal side.

Incubation at 37° C during 30 min. The values are acompanied by the average standard error.

N.° exp.	Dicumarol M	Arabinose in serosal μM/100 mg	O_2 uptake μM/100 mg
20	—	0.86 ± 0.09	3.03 ± 0.06
20	5×10^{-5}	1.46 ± 0.07	2.48 ± 0.06

Table III. *Effect of dicumarol on the diffusion of urea (10 mM) across the intestinal wall.*

Concentration of urea and dicumarol 10 mM and 5×10^{-5} M respectively on the mucosal side. Incubation at 37° C during 1 hour.

N.° exp.	Dicumarol M	Urea in serosal μM/100 mg
8	—	0.80 ± 0.12
10	5×10^{-5}	0.77 ± 0.05

INCUBATION INFLUENCE. — Dicumarol's influence on the intestinal absorption of glucose had been previously studied in *in vivo* experiments. When placed in the interior of the intestinal loop, a glucose inhibition resulted which persisted even after ample washing of the intestinal loop intended to illiminate any residual dicumarol penetration into the epithelial cells or else its stable fixation at the membrane level. On the other hand, this *in vivo* persistence was not observed in the case of arabinose (5). In view of these results, it was decided to investigate whether an initial incubation of the intestinal sacs containing 5×10^{-5} M dicumarol would have any influence on the galactose transport. It was found that 10 to 30 minute preincubations produce appreciable effects. The active galactose transport from solutions without dicumarol is inhibited by 70 % following a 30 minute preincubation with 5×10^{-5} M dicumarol.

INFLUENCE ON THE OXYGEN CONSUMPTION. — A decreased oxygen consumption by the intestines resulted for the various dicumarol concentrations used in these experiments. When control intestines were filled with 5 mM galactose or 50 mM arabinose, the oxygen consumption was

3.60 ± 0.12 μM/100 mg and 3.03 ± 0.06 μM/100 mg respectively. When filled together with 5×10^{-5} M dicumarol the said consumption was 2.43 ± 0.12 and 2.48 ± 0.06. Thus there was a decrease of 32.5 % and 18.15 % in each case.

A decreased oxygen consumption on the order of 31 % was also produced after a 10 to 30 minute preincubation with 5×10^{-5} M dicumarol.

Discussion

Based on the results of these experiments it is evident that the presence of dicumarol in the mucosal fluid inhibits the oxygen consumption in everted rat intestinal loops. This inhibition seems to indicate that dicumarol penetrates into the cells by way of the mucosal membrane and produces its inhibitive metabolic action thereby decreasing the energetic disponabilities fo the cells. In addition, the active galactose transport is also inhibited. 10^{-5} M dicumarol concentrations in the mucosal fluid produce a galactose disminution of almost 50 %, whereas at a concentration of 5×10^{-5} M said inhibition is almost 100 %.

The oxygen consumption disminutions observed are not completely proportional to those obtained for the galactose transport. Although the results do not reject a direct influence of dicumarol on the transport mechanism, it seems more reasonable that the galactose active transport inhibition is due to a lack of neccessary energy, in view of the fact of the small oxygen consumption as well as the uncoupling action of the oxidative phosphorylation (2).

The possibility of a competitive inhibition is discarded since, in previous experiments in this laboratory using hamster intestine (1), it has been observed that dicumarol produces a non-competitive inhibition on the galactose transport. In addition, a 10 minute tissue preincubation in a 5×10^{-5} M dicumarol solution causes a smaller oxygen consumption and less sugar transport. This persistent effect could be due to the dicumarol penetration into the interior of the cells during the preincubation and perhaps as well to its relatively stable fixation on the mucosal membrane. This phenomenon occurs both in *in vivo* (5) as well as in *in vitro* experiments, and is partially though not completely reversible after intestinal washing.

Both the oxygen consumption and the active galactose transport showed no alteration in those experiments in which dicumarol was present in the serosal fluid. It would seem that it either did not penetrate into the cells or else penetrated in insufficient quantity to produce inhibition.

Dicumarol clearly facilitates the arabinose diffusion from the mucosal to the serosal fluids. For a 5×10^{-5} M dicumarol concentration in the mucosal solution, there was a 70 % net passage increase of arabinose, which initially was present only in the experiments with galactose. This surprising *in vivo* effect would seem to indicate that dicumarol acts at the membrane level thus aiding the passage, one whose transference does not require energy uncoupling. When the transference of urea, initially present only in the mucosal fluid, is measured, its transport to the serosal fluid is not modified by the dicumarol. and thus is contrary to what one would expect from an indiscriminate permeability increase.

Utilizing dietyl-etyl-bestrol (uncoupler of oxidative phosphorilization), the transfer of arabinose and xylose give similar results (3).

After preincubation in dicumarol microscope studies of the membrane did not show any alterations.

The different behavior of dicumarol in beth cases is understandable considering the molecular differences between arabinose and urea both with respect to their

dimensions as well as to the cast coefficient lipids/water and other factors which could effect its penetration.

In order to explain dicumarol's influence on the arabinose transference, it would be necessary to conduct further experiments with other sugars which are not actively transported, since the diffusion of some sugars is increased in anaerobiotic conditions (9).

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