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Coupling of cell metabolism and active transport in glucose absorption by the intestine

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The absorption of glucose by the intestine is a process of active transport through membranes, enzymaticaly controled. The energy necessary must be furnished by the cell metabolism. The intimate mechanism coupling this metabolism to the active transport is still unknown.

We have attempted to study this problem testing the effect of known metabolic inhibitors on the intestinal absorption of glucose. If an inhibitor of a metabolic process inhibits this absorption, it is very probable that this process is directely or indirectely implicated in the mechanism of absorption.

The absorption experiments have been performed following the SOLS and PONZ method in rats [1]. The intestinal loop, 20 to 30 cm. long, was isolated «in situ» by two canules that permit its repletion, washing and further repletion at a constant pressure. In this way, it is possible to compare the rate of absorption of identic or different solutions in the same intestinal loop of a given animal. Usually there were performed four succesive absorptions, each one of thirty minutes, with glucose solutions 5.4 %. In the second and fourth absorptions the inhibitors were present in the sugar solutions to be absorbed. Those inhibitions accompanied by toxic manifestations were not considered.

Table I shows the results with 19 inhibitors listed in order of activity. It gives the minimal effective concentrations with

1. SOLS, A. and PONZ, F., R. esp. Fisiol. 3, 207, 1047.

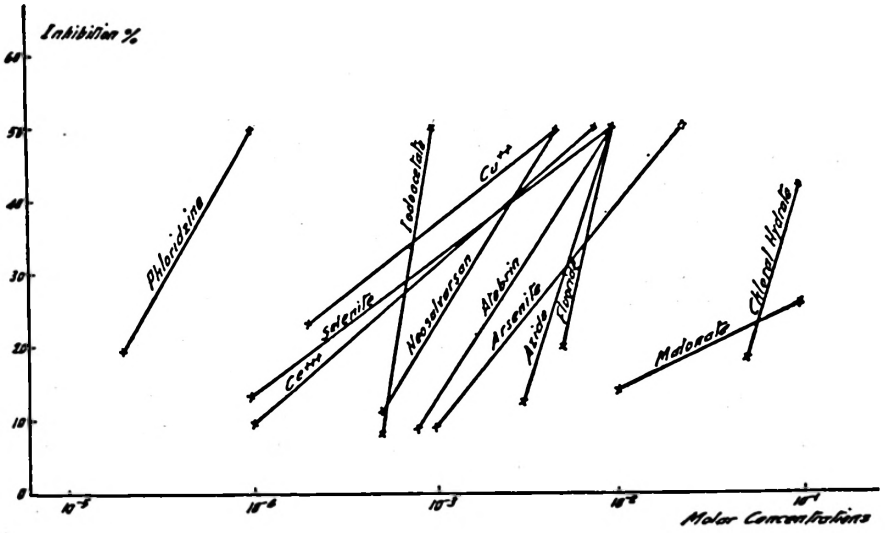
TABLE I

Effect of inhibitors on glucose absorption by rat intestine.
Method of succesive absorptions after SOLS *and* PONZ

Inhibitor	Umbral concent. (M)	Umbral inhibit. %	50 % inhibit (M)	Reversibility by washing	Inhib. increase by repeating absorption with inhibitor
Phloridzine	2×10^{-5}	19.6	10^{-4}	Total	—
Selenite	10^{-4}	13.6	10^{-2}	Part.	+
Uranyl	?	?	2×10^{-4}	—	+
Ce+++	10^{-4}	9.4	8×10^{-3}	Part.	+
Cu++	2×10^{-4}	23.3	5×10^{-3}	—	+
Iodoacetate	5×10^{-4}	8.1	10^{-3}	—	+
Neosalvarsan	5×10^{-4}	11.0	5×10^{-3}	—	+
TEM	7.5×10^{-4}	14.1	?	—	+
Atebrin	8×10^{-4}	9.0	10^{-2}	—	+
Arsenite	10^{-3}	9.0	2.4×10^{-2}	Part.	+
Sodium azide	3×10^{-3}	15.0	10^{-2}	—	+
Fluoride	5×10^{-3}	20.0	10^{-2}	—	+
Malonate	10^{-2}	14.0	10^{-1} (26 %)	—	+
Chloral hyd.	5×10^{-2}	18.0	10^{-1} (42 %)	Part.	+ letal
Phenyl acetate	Non effective up to 4×10^{-2}				
Methadone	» » 1.5×10^{-1} (Toxic)				
Sulfanilamide	» » 5×10^{-2}				
Sulfoguanidine	» » 10^{-3} (Sat.)				
Sodium cyanide	» » 5×10^{-3} (Toxic)				

its corresponding inhibitions, and the concentrations producing a 50 % inhibition. Malonate and chloral hydrate did not succeed in inhibiting at 50 %. Malonate is difficultly absorbed. The ineffective inhibitors lacked action even using the maximum concentrations, limited by toxic effects or insolubility.

If the solution with inhibitor is eliminated and the intestine is well washed with saline, and a new glucose solution without inhibitor is put for absorption, it is seen that the absorption is



Effect of inhibitors on glucose absorption by rat intestine.

Fig. 1

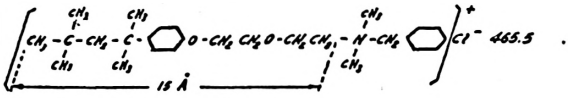
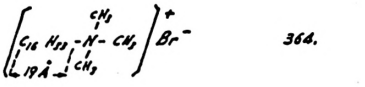
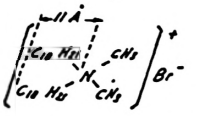
normal only in the case of phloridzine. All the other inhibitors produce inhibitions only partially or not reversible by whasshing. This shows that the inhibitors have been fixed by the mucosa. If another absorption is practiced again with the same inhibitor one can see if the inhibition is higher than the first time; if this ocurres, it is also shown that the inhibitor has been fixed. Again it is only phloridzine that does not inhibit more the second time than the first. Therefore, phloridzine is not fixed by the mucosa.

Figure 1 shows the same data and perhaps allows one to see the different activity of the different inhibitors.

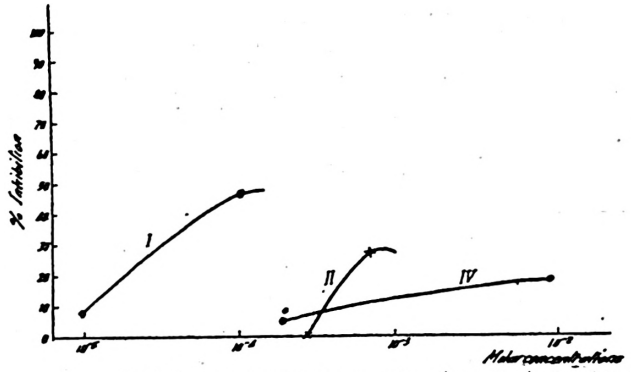
We have also tested the action of four quaternary ammonium germicide compounds, which we had already studied in experiments in yeast [2]. The compound III, that was the least

2. PONZ, F. and PARES, R. Comm. to III. Internat. Cong. Bioch., *R. esp. Fisiol.*, **11**, 253, 1955.

Tested Ammonium Quaternary Compounds

Compound	Chemical Name	Formula	W. M.
I	Diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride		465.5
II	Alkyl-dimethyl-benzyl-ammonium chloride		353.5
III	Cetyl-trimethyl-ammonium-bromide		364.
IV	Didecyl-dimethyl-ammonium bromide		406.

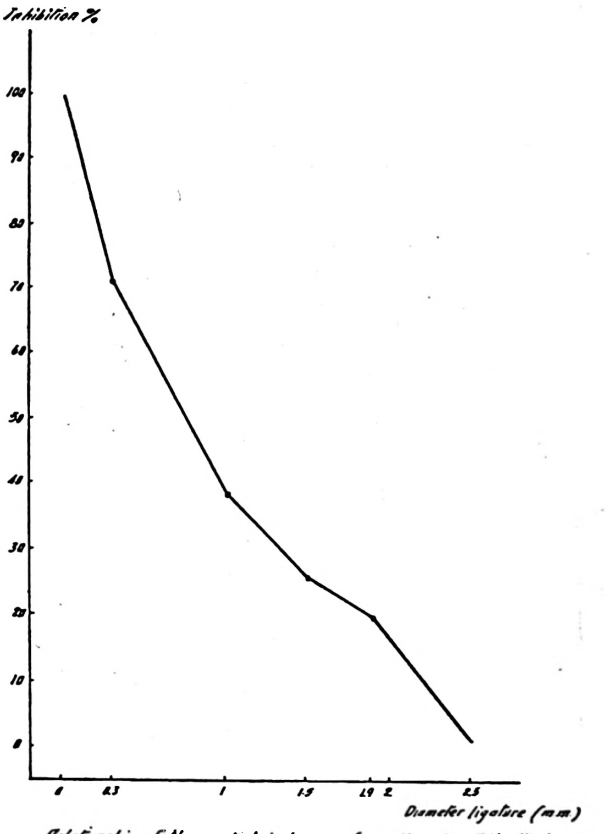
active on yeast, was found to be ineffective in inhibiting the intestinal absorption of glucosa at concentrations not letal. The other three are less active in the intestine than in yeast. Figure 2 shows that the compounds I and II are the most affective inhibitors ; both with bencyl groups and an aliphatic chain about 15 Angstroms in length. At the minimal effective concentrations, compound I should be fixed entirely by the membrane and so its inhibitory effect can be attribuited to an action at the cell surface.



Effect of ammonium quaternary compounds on glucose absorption by rat intestine

Fig 2

The inhibition provoked by all the tested substances is due to a local action on the mucosa absorbing glucose and not to a general intoxication. This is demonstrated by comparing the



Relationship of the partial ischaemia (mm diameter of the ligature) to the inhibition of the glucose absorption

Fig. 3

effects in experiments in which the inhibitor was absorbed from a different intestinal loop. Inhibitor concentrations that produce very high inhibitions of absorption were without effect when administred by another intestinal loop.

Another part of this work is devoted to studing the influence of the oxygen supply of the mucosa on the absorption.

The partial ligature of the blood vessels corresponding to the intestinal loop absorbing glucose provokes inhibitions according to the degree of the ischaemia (Fig. 3). Freed the ligatures, the absorption capacity is slowly restored.

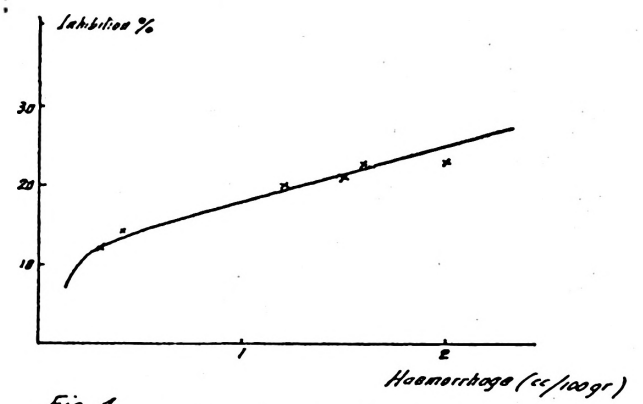


Fig 4 Effect of the haemorrhage (cc/100gr) on the glucose absorption

The haemorrhage, from 0.3 ml. per 100 g. of corporal weight inhibits the absorption (Fig. 4) proportionaly to the importance of the bleeding (Munk'technique).

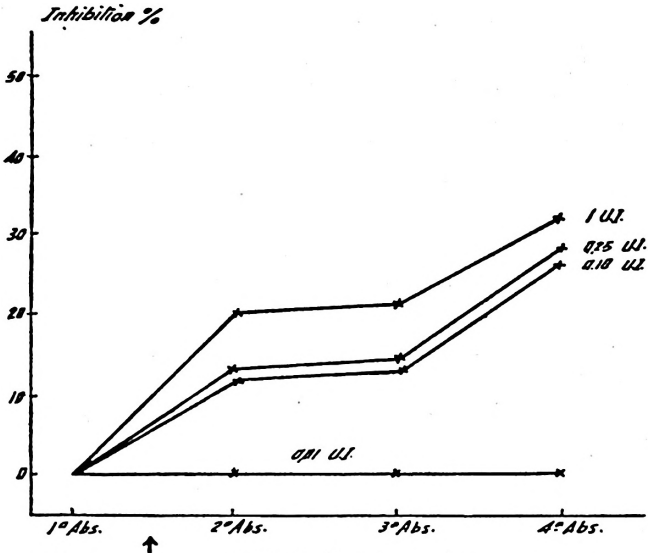


Fig. 5 Effect of subcutaneously injected Pituitrin on the intestinal absorption of glucose.

Pituitrin (Fig. 5) subcutaneously injected, provokes a prolonged inhibition of the glucose absortion, that increases with the dosage.

Histamine subcutaneously injected (Fig. 6), also inhibits the absorption persistenly and increasingly.

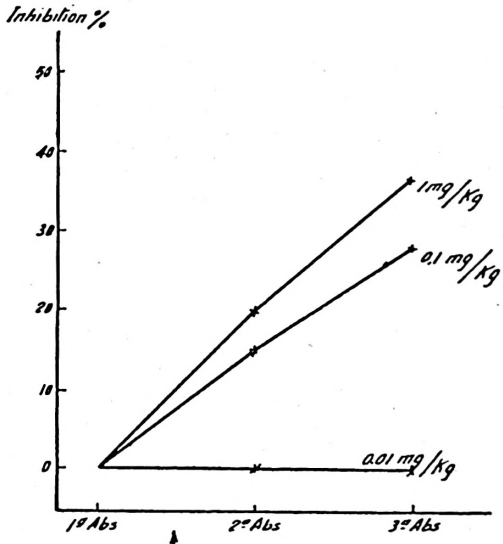
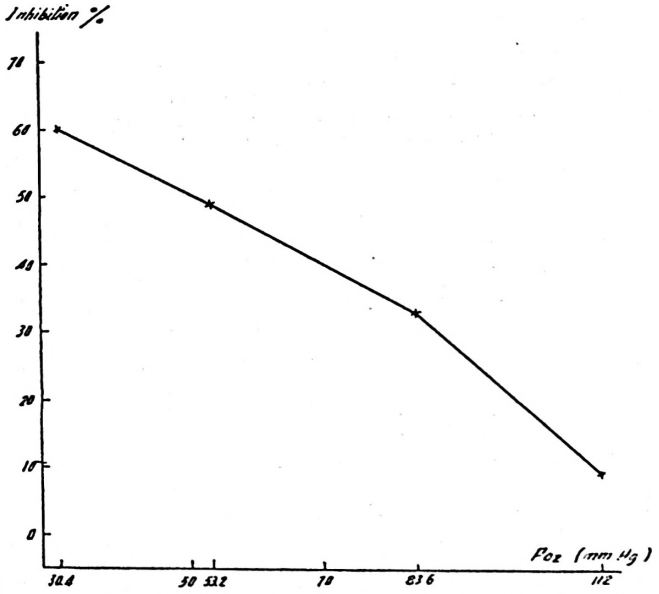


Fig 6 Inhibition of the intestinal absorption of glucose by subcutaneously injected histamine.

Anoxic anoxia is also effective. If the animals are obliged to respire an atmosphere oxygen poor, it can be demonstrated very clearly the influence on the glucose absorption. Fig. 7



Relationship of the oxygen partial pressures in artificial atmosphere to the inhibition of the glucose absorption

Fig. 7

shows how from oxygen partial pressures of 112 mm. Hg. the capacity of glucose absorption decreases reaching inhibitions of 60 % with partial pressures of 30.4 mm. Hg. Upon returning the rats to normal air, the absorption improves and after 20 minutes becomes normal.

Inhibition of the glucose absorption by decreasing oxygen in air. Respiration in closed space (250 cm)

Fig 8

The same thing is seen in Fig. 8. The rats respired now by means of a tracheal canule from a container of 250 ml. The atmosphere becomes more and more poor in oxygen, while the CO_2 is absorbed whit soda lime. During a series of succesive absorptions, the rate of glucose absorption decreases progresively. After fifteen or thirty minutes breathing in a normal atmosphere, the absorption is restored.

All these preceeding experiments carry along with them, a decrease of the oxygen supply to the mucosa : 1) by diminishing the minute volume circulating through the intestine (partial ischaemia, vasopresin, posthaemorrhagic reflex vasoconstriction, histaminic capillary paralysis) ; 2) or by diminishing of haemoglobin (haemorrhage) ; 3) or by diminishing

the partial pressure of oxygen of the inspired air. The conclusion of this, is that the oxygen is an important factor in the transport of glucose accross the membrane.

We can now see in the Fig. 9, a scheme of the possible interpretation of the results. The glucose is actively transported across the membrane by an unknown process requiring energy.

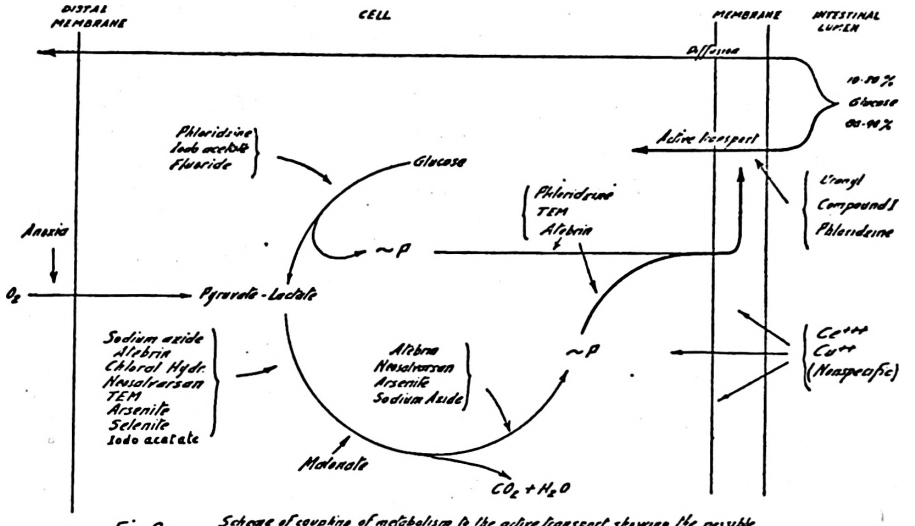


Fig. 9 Scheme of coupling of metabolism to the active transport, showing the possible points of action of the tested inhibitors.

The phosphorylation of the penetrating sugars has not been proven, but it is very probable that energy transferences from sources of energy rich phosphates (perhaps ATP) are involved. Cell metabolism permits the reposition of these energy donors. The most common way must be exogenous or endogenous glucose utilization. Its efficiency will be of course much higher if the sugar is oxidized to CO_2 and H_2O, than if this is not possible. The diminution of the oxygen supply to the mucosa will produce therefore an inhibition of the absorption by impeding the oxidative metabolism.

The action of the tested inhibitors is also understendable. Unfortunately, they are not very specific. In the scheme the most probable points of action have been indicated, according to the enzymatic process most affected by them. Malonate, a competitive, inhibitor of succinic-dehydrogenase, must act on the oxidative fase. Phloridzine, iodoacetate, and fluoride are well known inhibitors of glycolisis. Sodium azide, Atebrin,

Chroral-hydrate, Neosalvarsan, Triethylen-melamine, Arsenite, Selenite, and iodoacetate, inhibit the pyruvate oxidation by blocking the O_2 utilization, or oxidative decarboxylation, or Coenzim A, etc. Atebrin, Neosalvarsan, arsenite and sodium azide also inhibit oxidative phosphorylation. Phloridzine, TEM, and atebrin, are known inhibitors of phosphate transferences.

Four tested compounds act in the cell surface itself: Uranyl, quaternary ammonium compounds I and II, and, perhaps, phloridzine. Uranyl is not penetrating and probably ows its effect to blocking energy rich phosphate [3,4]. Compounds I and II remain strongly adsorbed by the membrane. The fact that the inhibition by phloridzine is so easily reversible by washing, not being fixed by the mucosa, suggests a preferent or exclusive action in the membrane.

Cupric and ceric ions can act inspecificaly by constituting complexes with enzymatic or non enzymatic proteins, in the membrane or cytoplasm.

3. ROTHSTEIN, A. and LARRABEE, C., J. cell. comp. Physiol. 32, 247, 1948.
4. ROTHSTEIN, A. and MEIER, R., J. cell. comp. Physiol. 38, 245, 1948.