centrifuge, as above. The volume of packed cells was noted in each case. It can be seen in the Table that the fluid volumes were very similar in the control group. The Tween 80 treated group showed a wide variation in total fluid. In the control group the packed cell volume was fairly uniform, while the treated cells did show a variation. The average ratio of total fluid to cells, for all the tubes was 0.26 for the control group, and 0.21 for the treated cells. The cell counts were done by combining all the control tubes and mixing these thoroughly and similarly combining and mixing the Tween 80 treated cells. Cell counts were done as above using a red cell diluting pipet and a bright line hemocytometic slide. The results of these counts showed in the pooled control sample $116 \cdot 10^6$ cells per ml. In the pooled Tween 80 treated cells there were $126 \cdot 10^6$ cells per ml. Thus, if the volume used for injection initially, was calculated on the basis of 0.2 ml the number of cells present in the control was $23 \cdot 10^6$ cells per 0.2 ml, and in the treated cells $25 \cdot 10^6$ cells per 0.2 ml. The counts were confirmed with the use of the Coulter cell counter. This also showed that the size class of cells was identical in each case.

Vol. fluid ml	Vol. packed cells ml	Cells/fluid ratio	Cell count Day 0	Day 11
Control				
10.2	2.8			
10.2	2.6			
12.3	3.1			
12.0	3.0			
10.5	3.2			
11.0 Av.	2.9 Av.	0.26	$27 \cdot 10^6$	$23 \cdot 10^6$
Tween 80 treatment				
9.1	2.2			
14.2	3.5			
9.0	2.8			
7.0	2.9			
4.8	1.0			
8.8 Av.	2.5 Av.	0.21	$24 \cdot 10^6$	$25 \cdot 10^6$

Cell counts refer in each case to injected number at day 0, and comparable count in harvested fluid at day 11.

It can be seen that the growth of the Ehrlich ascites carcinoma cells in host mice after Tween 80 treatment appears to be normal. It is not possible to explain the variation in ascites fluid volume of the individual mice after injection with the treated cells. It is probable that this is related in some way with the variation in cellular infiltration into the tissues during early stages of growth of the tumor. It seems evident, however, that treatment of the cells with Tween 80 does not appreciably alter their growth potential. In the earlier findings[2] it was suggested that the cells are able to restore the altered membrane constitution affected by Tween 80 treatment by metabolic processes during the recovery phase in the growth conditions in host mice. These experiments would tend to confirm this.

Zusammenfassung. Der Einfluss von Tween 80 auf das Wachstum des Ehrlich-Lettré-Carcinoms war statistisch nicht zu sichern, wenn die Gewichtszunahme allein gemessen wurde. Individuelle Variation im Flüssigkeits- und Zellgehalt zeigten, dass oberflächenspannungsherabsetzende Substanzen das Carcinomwachstum durch Eigenschaftsänderung der Zellmembran beeinflussen können.

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Transport of Thiamine by the Small Intestine in vitro

In man, the intestinal absorption of thiamine presents a definite obstacle[1], since single oral doses above 2.5–5 mg greatly increase the faecal excretion[2–4]. The results of Da Silva and Ivy[5], in dogs with a chronic Thiry fistula, support this view. In rats, simple diffusion seems to regulate the intestinal absorption of thiamine in vivo[6,7], at least when relatively high doses of vitamin are used. However, Ventura et al.[8] showed comparatively greater absorption of a small than of a high dose of thiamine, and Polin et al.[9,10], employing intestinal loops of chicks in situ and the antithiamine Amprolium, suggested that the thiamine uptake may be an active process superimposed on some passive absorption.

By the everted-intestinal-sac technique, Turner and Hughes[11] were unable to find in vitro any evidence of an uphill transport using $20 \mu M/l$ concentration of thiamine. However, since with several substrates (e.g. basic amino acids[12], pyrimidines[13], *d*-xylose[14]) the demonstration of an uphill intestinal transport could be achieved only by using very low initial concentrations, we applied

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this principle to the study of the thiamine transport in vitro.

Everted sacs (8 cm long) from the upper small intestine of rats (Wistar strain, 100–120 g body weight), prepared according to WISEMAN[15], placed in 100 ml Erlenmeyer flasks with 25 ml of KREBS-HENSELEIT[16], and gassed with 5% CO_2 and 95% O_2, were incubated for 1 h, at 37°C, in a Dubnoff metabolic shaker. The thiamine initial concentration at both sides of the sacs was 0.21 μM/l and the serosal initial volume was 1.4 ml. At the end of the incubation period, each sac was emptied into a 10 ml volumetric flask and the serose was thoroughly rinsed with 0.01 N HCl to the volume of 10 ml.

The thiamine determination was made by a micromodification of the thiochrome method[17] both on the serosal and mucosal final fluids, using a Beckman DU (model G.2400) spectrophotometer with a fluorescence attachment, except in the presence of pyrithiamine, when a Farrand spectrofluorometer was utilized (λ activation, 365 nm; λ emission, 435 nm). Further, in the experiments with 2.4 dinitrophenol (DNP), the fluorimetric determination followed a chromatographic separation of thiamine on Decalso[17].

All the analytical procedures were previously checked on pure solutions of thiamine and thiamine plus DNP or pyrithiamine, showing in every case good recoveries of the vitamin.

As can be seen from the Figure, under our experimental conditions a net transport of thiamine against a concentration gradient could be demonstrated, the serosal concentration of the vitamin increasing up to 2.1 times the initial one. It is noteworthy that in several experiments, where the initial concentration of thiamine was 21 or 2.1 μM/l, no evidence of an uphill transport was found, thus confirming the results of TURNER and HUGHES[11].

The effects of the metabolic inhibitors and of the decrease of the incubation temperature (Table I) greatly substantiate an active transport mechanism, since all depressed the serosal accumulation of thiamine, lowering the Cs/Cm ratio. No modification was found with ouabain.

Of the structural thiamine analogues tested (Table II), only pyrithiamine significantly ($p \ll 0.001$) inhibited the uphill transport of thiamine. Since pyrithiamine, but not oxythiamine, is a potent inhibitor of the thiamine phosphorylase from rat intestine[18], this finding should indicate phosphorylation as the basic mechanism of the intestinal uphill transport of thiamine[19].

Table I. Transport in vitro of thiamine by everted sacs of the small intestine of rats, expressed as ratio of concentration in the serosal and mucosal compartments (Cs/Cm) at the end of 1 h incubation period. Means ± S.E. Initial thiamine concentration 0.21 μM/l; initial Cs/Cm = 1.0

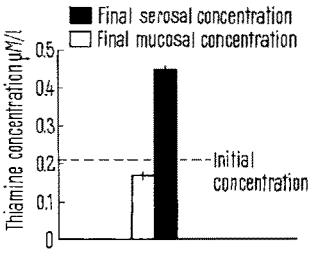
Treatment	Cs/Cm		Inhibition, %
	Controls[a]	Treated	
95% N_2, 5% CO_2, 37°C	(9) 2.30 ± 0.10	(9) 1.28 ± 0.07	44.3
95% O_2, 5% CO_2 27°C	(3) 3.03 ± 0.02	(3) 1.39 ± 0.06	54.1
95% O_2, 5% CO_2, 25°C	(3) 2.42 ± 0.29	(3) 1.05 ± 0.02	56.6
2.4 DNP, 10^{-4} M, 37°C[b]	(8) 2.44 ± 0.19	(8) 1.29 ± 0.09	47.1
Na azide, 10^{-2} M, 37°C[b]	(11) 2.64 ± 0.16	(11) 1.34 ± 0.05	49.2
Ouabain, 10^{-4} M, 37°C[b]	(6) 2.54 ± 0.14	(6) 3.07 ± 0.19	–

[a] Gas phase 95% O_2, 5% CO_2; temperature 37°C. [b] Gas phase 95% O_2, 5% CO_2. In parentheses, number of sacs. DNP = dinitrophenol.

Table II. Effect of some structural analogues of thiamine on its transport in vitro by everted sacs of the small intestine of rats (see Table I). Gas phase 95% O_2, 5% CO_2; temperature 37°C

Structural analogues	Concentration used, μM/l	Cs/Cm		Inhibition, %
		Controls[a]	Treated[b]	
Oxythiamine	0.21	(3) 2.69 ± 0.24	(3) 2.86 ± 0.29	–
Oxythiamine	1.05	(3) 2.65 ± 0.16	(3) 2.47 ± 0.18	6.8
Oxythiamine	2.1	(3) 2.41 ± 0.08	(3) 2.30 ± 0.20	4.6
Pyrithiamine	0.21	(5) 3.18 ± 0.06	(6) 1.84 ± 0.04	42.1
Pyrithiamine	1.05	(5) 3.18 ± 0.06	(6) 1.41 ± 0.07	55.7

[a] Without structural analogue. [b] With structural analogue.



Transport of thiamine against a concentration gradient by the small intestine of rats in vitro. Everted sacs containing 1.4 ml of 0.21 μM/l thiamine solution. Average of 58 sacs ± S. E.

Riassunto. È stato studiato il trasporto in vitro della tiamina con la tecnica dei sacchetti intestinali rovesciati di ratto. Usando una concentrazione di tiamina molto bassa (0.21 μM/l) si dimostra il passaggio della vitamina contro gradiente di concentrazione. L'impiego di inibitori metabolici e di analoghi strutturali della tiamina conferma l'esistenza di un trasporto attivo. In particolare l'inibizione del trasporto da parte della sola piritiamina, noto inibitore della tiaminochinasi intestinale, suggerisce la possibilità che alla base del meccanismo di trasporto della tiamina vi sia la sua fosforilazione.

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