scintillation counter was used for determining the radioactivity.

24 daily doses (20 mg/kg) of unlabelled guanoxan were given orally to a male beagle dog (11.5 kg). These were followed by a similar dose of radioactive guanoxan (3.24 μc). The urine was then collected over a period of 4 days, concentrated in vacuo and extracted with methanol. Radioactivity measurements at this point indicated that the recovery of ^{14}C-labelled metabolites was in the order of 52–53% of the administered radioactive dose.

The fractionation of the ^{14}C-labelled metabolites was carried out by two different methods simultaneously: (a) absorption on Amberlite IRC 50 resin, buffered to pH 6.5 with disodium hydrogen phosphate, then elution of the metabolites with water, 0.5 *N* HCl and 1.2 *N* HCl in methanol successively; (b) preparative thick paper descending chromatography using *n*-propanol-aqueous molar ammonium carbonate-concentrated ammonia (0.88) (70:30:2) as the developing solvent.

The identification and characterization of the metabolites were achieved by direct comparison of the Rf values with those of standards using chromatographic, electrophoretic and combined bidimensional techniques, and also by the preparation of solid derivatives (reineckates, picrates) and by comparison of these with authentic derivatives.

Fractionation by method (a) gave the following labelled metabolites: arginine, 4.3%; creatine-guanidinoacetic acid, 12%; creatinine-urea, 19%; hydroxylated guanoxan, 4%; guanoxan 13.6%. Fractionation according to (b) afforded the same metabolites, but better separation was achieved between creatinine and urea: arginine, 5%; creatine-guanidinoacetic acid, 9.5%; creatinine, 15.5%; urea, 3.6%; hydroxylated guanoxan, 4.5%; and guanoxan, 15%.

These results suggest that the guanidino group of guanoxan underwent transamidination in the presence of ornithine and glycine, to give arginine and guanidino acetic acid respectively, the latter giving rise to creatine and creatinine. Hydrolysis of the guanidino group to urea does not appear to be very extensive judging by the small amount of labelled urea found (3–4%). It is assumed that desimidation does not take place because the metabolic product, *N*-[2(1,4-benzodioxanyl)methyl]urea, was not detected even in traces. The position of the hydroxylation in the aromatic ring was not established fully, but the chromatographic behaviour of a phenolic metabolite was identical to that of synthetic 2-guanidinomethyl-7-hydroxy-1,4-benzodioxan sulphate, m.p. 263–264.5°.

The latter was prepared from 2-chloromethyl-7-hydroxy-1,4-benzodioxan[13] by conversion to the azide (b.p. 158–164°/0.6 mm) followed by hydrogenation to the 2-aminomethyl-7-hydroxy-1:4-benzodioxan (hydrogen maleate, m.p. 184–185°) and guanylation with 1-amidino-3,5-dimethylpyrazole sulphate.

The metabolic fate of the benzodioxan ring system will be discussed in the future.

Resumen. El sulfato de 2-guanidinometil-1:4-benzodioxano (guanidino ^{14}C) se sintetizó a partir del 2-aminometil-1:4-benzodioxano y el sulfato de S-metiltiouronio (uronio ^{14}C). Un 52–53% de la dosis radioactiva administrada a los perros se recuperó en la orina en la forma de los siguientes compuestos radioactivos: arginina, creatina, acido guanidino acético, creatinina, urea, 2-guanidinometil-1:4-benzodioxano y un derivado hidroxilado (fenólico) del anterior. Se sintetizó tambien el sulfato de 2-guanidinometil-7-hidroxi-1:4-benzodioxano.

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Phosphorylation of Thiamine in the Intestinal Wall During Absorption in vivo

Several experiments in vitro[1–3] have shown that intestinal tissue is able to phosphorylate thiamine. The possibility that thiamine is phosphorylated during its intestinal absorption has been suggested by some authors[4,5] and denied by others[6]. However, a clear experimental evidence of the relationship between thiamine phosphorylation and its intestinal absorption has never been produced, although Machida[7] found some thiamine phosphates in the wall of isolated intestinal tracts of the rat after incubation with thiamine.

Recently Ventura and Rindi[8] were able to show an uphill transport of thiamine by the everted intestinal sacs of the rat in vitro, and put forward the hypothesis that the underlying mechanism of transport could be thiamine phosphorylation.

Here we will refer to some results we obtained in an in vivo study of thiamine phosphorylation during the intestinal absorption of equivalent amounts of thiamine hydrochloride and thiamine-propyl-disulphide (TPS), a well-known thiamine derivative rapidly absorbed and completely transformed into thiamine by the intestinal mucosa[9–11].

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Thiamine phosphate content of rat intestinal wall during thiamine absorption. Mean ± S.E.

Compound administered	Dose[a] mg	No. of rats	Content[c], μg/g wet tissue			P[b]
			TMP	TDP	TMP + TDP	
Saline (controls)	–	5	0.25 ± 0.03	0.91 ± 0.11	1.16 ± 0.09	
T	0.1	3	0.31 ± 0.02	1.69 ± 0.38	2.00 ± 0.36	0.05 ± 0.02
TPS	0.1	5	0.47 ± 0.17	1.36 ± 0.16	1.83 ± 0.19	0.05 ± 0.02
T	1.0	3	0.14 ± 0.01	2.47 ± 0.47	2.61 ± 0.34	0.01 ± 0.001
TPS	1.0	6	0.65 ± 0.23	4.15 ± 0.50	4.80 ± 0.44	< 0.001

T = thiamine; TMP = thiamine monophosphate; TDP = thiaminediphosphate; TPS = thiamine propyldisulphide. [a]Dissolved in 5 ml of saline. [b]Calculated for TMP + TDP, with Student's t test, in comparison with the controls. [c]Expressed as thiamine chloride hydrochloride.

The intestinal absorption was studied by a modification[9,12] of the Cori technique on the small intestine in situ. The rats were sacrificed 2 h after the introduction into the intestine of the compound used, dissolved in 5 ml of saline, since we found in other experiments that, at that time, the amount of thiamine phosphate was the greatest[11]. The whole intestine was then rapidly taken, emptied, blotted on filter paper and finally homogenized for 3 min by an Ultra-Turrax mod. 45/6 homogenizer in 5% trichloracetic acid. The final pH was 0.80–0.85.

When we used thiamine hydrochloride, the thiamine phosphate content was calculated by the difference between total thiamine and free thiamine, both determined by the thiochrome method[13], following the modifications suggested by MICKELSEN et al.[14] in order to lower the blanks.

When we used TPS, an analytical procedure (to be published in extenso[15]) was devised, which allowed the simultaneous determination of free and phosphorylated thiamine as well as of TPS by the thiochrome method of MICKELSEN et al.[14].

Recovery experiments, adding to intestinal tissue known amounts of thiamine, TPS and/or thiamine diphosphate, always gave reliable results; no transformation of TPS was ever noticed.

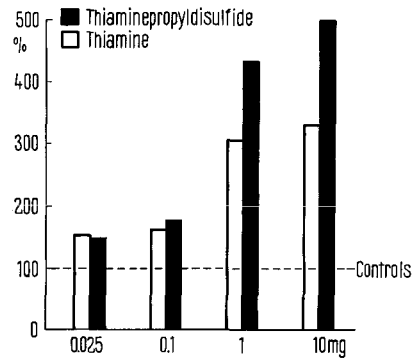
The phosphorylated thiamine content of the intestinal wall, expressed as % of the control content determined after introduction into the rat bowel of 5 ml of saline alone, during absorption of different amounts of thiamine hydrochloride of TPS is shown in the Figure.

The analysis of variance of the results of the determinations showed a significant difference among the doses: between the two compounds, the difference was significant only for the doses of 1 and 10 mg.

A confirmation of these results came from some experiments where the specific method of RINDI and DE GIUSEPPE[16] was used to determine separately thiamine monophosphate and thiamine diphosphate in the intestinal wall during absorption of 0.1 and 1 mg of thiamine hydrochloride or equivalent amounts of TPS. The results (Table), which are in good accordance with those shown in the Figure, specify that the increase of the phosphorylated thiamine content in the walls was essentially due to thiamine diphosphate. Here again, the increase during absorption of 1 mg of TPS was significantly greater than that during absorption of 1 mg of thiamine hydrochloride ($p < 0.001$).

In conclusion, during the intestinal absorption of thiamine in vivo, a significant increase of phosphorylated thiamine (thiamine diphosphate) in the intestinal wall was demonstrated, both with an indirect and with a direct specific analytical procedure, 2 h after the introduction of equivalent amounts of thiamine hydrochloride or TPS. The increase after administration of 1 and 10 mg of TPS was higher than after the equivalent quantity of thiamine.

Some in vitro experiments with labelled thiamine are now in progress to investigate further the relationship between thiamine phosphorylation and thiamine intestinal absorption.



Thiamine phosphorylated contents of rat intestinal wall, expressed as percentage of the control content, during absorption of different amounts of thiamine hydrochloride or thiamine propyldisulphide.

Riassunto. 2 h dopo l'introduzione di tiamina cloridrato o di tiaminpropildisolfuro nell'intestino tenue di ratto in vivo si trova un aumento significativo di tiamina fosforilata nella parete intestinale, essenzialmente a carico del tiamindifosfato.

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