Effect of cold acclimation on norepinephrine (NE) and serotonin (5HT) contents in interscapular brown adipose tissue (IBAT) and NE and epinephrine (E) contents in adrenals of hypophysectomized rats

	28°C Sham-operated (7)	Hypophysectomized (7)	15°C Sham-operated (7)	Hypophysectomized (5)
a) Interscapular brown adipose tissue				
NE ng/IBAT	167 ± 14	135 ± 9	259 ± 18*	201 ± 15*
NE ng/g	557 ± 24	735 ± 90	548 ± 36	733 ± 36
5 HT ng/IBAT	67 ± 7	54 ± 4	172 ± 23*	151 ± 20*
5 HT ng/g	219 ± 14	293 ± 40	359 ± 36*	519 ± 20*, ×
b) Adrenals				
NE µg/2 adrenals	3.95 ± 0.80	3.32 ± 0.46	3.68 ± 0.98	3.24 ± 1.10
E µg/2 adrenals	28.83 ± 0.96	9.89 ± 0.45×	29.93 ± 1.61	15.24 ± 2.58*, ×

Results are presented as means ± SEM; * significant effect of cold acclimation in SO and H rats; × significant effect of hypophysectomy in rats kept at either 28°C or 15°C; ( ) in brackets: number of animals.

The total and relative values of 5 HT were higher in cold acclimated rats. These results are in close agreement with those previously obtained[14]. Hypophysectomy did not modify the content of BAT in 5 HT; however, the relative level (ng/g) was higher (45%) in H15, than in SO15 rats. It can be concluded that hypophysectomy does not modify the NE and 5HT contents in IBAT and does not impair the cold acclimation-induced increases in these two amine levels.

Adrenals (table). In cold acclimated control rats, although a significant hypertrophy of adrenals was induced, no increases in NE and E contents were observed. Hypophysectomy did not change NE stores in adrenals. However, E content was considerably reduced, 3 times in H28 but only twice in H15; it was significantly more important in this last group than in the former. As indicated by histological studies, the atrophy of adrenals after hypophysectomy is mainly due to atrophy of the zona fasciculata; the medulla is smaller than in normal animals but presents a functional aspect. The fact that hypophysectomy did not affect the NE content but led to a reduction of E content might be explained by a decrease in enzymatic activity which promotes the methylation of NE in E. The activity of phenylethanolamine-N-methyl transferase, an enzyme that synthesizes E from NE in the adrenal medulla, is markedly depressed following hypophysectomy[15,16].

From these results, it may be postulated that the cold-induced stimulation of the sympatho-adrenal system is little modified by hypophysectomy.

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Lack of influence of vitamin D deficiency on insulin release from the isolated pancreatic islets of rats

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Summary. Pancreatic islets were isolated from young (100 g) and adult (390 g), normal and vitamin D deficient male Sprague-Dawley rats. The release of insulin from leucine-stimulated or glucose-stimulated islet was not altered by vitamin D deficiency. The in vitro addition of either 25-hydroxy- or 1,25-dihydroxyvitamin-D had no effect on insulin release from either normal or vitamin D deficient islets. We conclude that the earlier report (Normal et al., Science *209* (1980) 823–825) on vitamin D deficiency depressing insulin secretion from the perfused pancreas must be related to the vitamin's effect on insulin synthesis and not the islet's release of insulin.
Key words. Vitamin D deficiency, insulin release in; vitamin D deficiency, isolated pancreatic islet activity in; Sprague-Dawley rats, vitamin D deficiency in.

Recently Norman et al. reported that vitamin D deficiency depressed insulin secretion from the isolated perfused rat pancreas stimulated by the secretogogues, arginine[1] and glucose[2]. In support, Clark et al.[3] showed that administration of 1,25-dihydroxyvitamin-D to vitamin D deficient rats increased the serum level of insulin.

To test whether vitamin D is required for the synthesis and/or release of insulin from the pancreas, we have focused on the release aspects of vitamin D treatment. 25-Hydroxy- and 1,25-dihydroxyvitamin-D were added to isolated pancreatic islets from young and adult, normal and vitamin D deficient rats, and the amount of insulin released upon stimulation by leucine or glucose measured by radioimmunoassay.

Materials and methods. Individual islets were isolated from the pancreas of young (100 g) and adult (390 g) normal and vitamin D deficient male Sprague-Dawley rats as previously reported[4].

Table 1. Effects of 25-hydroxyvitamin-D on insulin release from leucine-stimulated normal and vitamin D deficient pancreatic islets incubated 90 min at 37 °C

Treatment	Insulin release (μunit/islet)
Normal	
Control, 0 ng/ml	59 ± 3[a]
25-Hydroxyvitamin-D, 10 ng/ml	56 ± 2
25-Hydroxyvitamin-D, 40 ng/ml	63 ± 3
25-Hydroxyvitamin-D, 100 ng/ml	57 ± 2
Vitamin D deficient	
Control, 0 ng/ml	54 ± 2
25-Hydroxyvitamin-D, 10 ng/ml	43 ± 2
25-Hydroxyvitamin-D, 40 ng/ml	48 ± 2
25-Hydroxyvitamin-D, 100 ng/ml	45 ± 2

[a] Mean ± SE of the mean of 4 observations in duplicate.

Table 2. Effects of 1,25-dihydroxyvitamin-D on insulin release from glucose-stimulated normal and vitamin D deficient pancreatic islets incubated 60 min at 37 °C

Treatment	Insulin release (μunit/islet)
Normal	
Control, 0 ng/ml	46 ± 2[a]
1,25-Dihydroxyvitamin-D, 100 ng/ml	42 ± 3
Vitamin D deficient	
Control, 0 ng/ml	44 ± 3
1,25-Dihydroxyvitamin-D, 100 ng/ml	44 ± 4

[a] Mean ± SE of the mean of 6 observations in duplicate.

Young vitamin D deficient rats were purchased from Harlan Sprague-Dawley, Indianapolis, IN. Adult vitamin D deficient rats were obtained from Dr H. F. DeLuca, University of Wisconsin, Madison, WI. Animals were killed immediately upon arrival and the pancreas removed for islets isolation.

Ten intact islets, capable of insulin release, were handpicked twice, placed in ice-cold Eagles' solution and stored on ice for 30 min prior to addition of the secretagogues (10 mM L-leucine and 10 mM L-glutamine or 200 mg/dl glucose) and the test compounds. 25-Hydroxy-vitamin-D and 1,25-dihydroxyvitamin-D were gifts from Dr J. C. Babcock, The UpJohn Co., Kalamazoo, MI, and Dr M. R. Uskokovic, Hoffmann-La Roche, Nutley, NJ, respectively. The compounds were dissolved in 95% ethanol and subsequently diluted in the Eagles' incubation medium to give a final ethanol concentration of 0.1% which did not interfere with insulin release from the islets (data not shown). The islets and test compounds were incubated 60–90 min at 37 °C in a Dubinoff shaker. The reaction was stopped and the insulin released was measured as previously reported[4].

A two-tailed Student t-test was used to ascertain levels of significance[5]. Probability values of 0.05 or less were considered significant.

Results and discussion. Table 1 shows the effect of vitamin D deficiency on insulin release from leucine-stimulated pancreatic islets of young Sprague-Dawley rats (100–150 g). Although there were observable differences between the two treatments (vitamin D deficient rats had less fat, showed a more friable skin, and a more easily digested pancreas by collagenase), no differences were observed for the release of insulin from the isolated pancreatic islets. No differences were found in insulin release upon the addition of 25-hydroxyvitamin-D to the islets of normal or vitamin D deficient rats. Similar results were recorded for glucose-stimulated pancreatic islets of adult (200–400 g) vitamin D deficient and normal rats (table 2). No differences in insulin release from glucose stimulation were found between the two treatments. The addition of 1,25-dihydroxyvitamin-D to the islets of either group did not affect insulin release; it remained the same.

Therefore, it may be concluded that vitamin D deficiency, in these tests, did not alter the release of insulin from isolated pancreatic islets nor was there a direct in vitro effect of 25-hydroxyvitamin-D or of 1,25-hydroxyvitamin-D on the release of insulin by the isolated pancreatic islets. We thus conclude that the previous reports of vitamin D effect on insulin secretion[1–3] must be related to synthesis and not to release from the pancreatic islet.

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Anaerobic biodegradation of m-, o-, p-hydroxycinnamic acid by an adapted microbial consortium

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Summary. The degradation of ortho-, meta- and para-hydroxycinnamic acid by an acclimated consortium (or 'mixed bacterial culture') was investigated. The biodegradation was evaluated by monitoring substrate disappearance. The relative rates of consumption were in the order: para > meta > > ortho. Only in the catabolism of meta- and para-hydroxycinnamic acid, the demolition of the side chain by the loss of a C_1 unit is involved. The utilization of para-hydroxycinnamic acid by the consortium occurred rapidly and completely within a 38-day incubation.

Key words. Anaerobic metabolism; acclimated consortium; hydroxycinnamic acid isomers.

In the context of studies on the anaerobic degradation of phenolic acids, which are widespread in nature, Andreoni et al.[1] described a consortium (or 'mixed bacterial culture') that grew on caffeic acid as the only carbon and energy source. It was also able to utilize ferulic acid, cinnamic acid, protochatecuic acid, vanillic acid, m- and p-hydroxycinnamic acid. Nali et al.[2] gave evidence that three reactions are involved in the early stages of the anaerobic catabolism of some phenylpropenoic acids; the