Table 4. Effect of vitamin C on response of spleen lymphoid cells to con A*

Experiment	Treatment	cpm Unstimulated	con A	Δ cpm	Δ cpm mean ± S.E.M.	Significance
			2 weeks			
I	Vitamin C	827 ± 49	12866 ± 264	12039	11692 ± 283	
		609 ± 42	11955 ± 127	11346		
						$p < 0.025$
	Control	121 ± 5.5	1447 ± 52	1326		
		426 ± 17	5778 ± 178	5352	3403 ± 1163	
		340 ± 8.5	3873 ± 340	3533		
			4 weeks			
II	Vitamin C	399 ± 6.4	41353 ± 731	40954		
		1250 ± 31	53984 ± 521	52734	42490 ± 4784	
		682 ± 40	34465 ± 712	33783		
						$p < 0.05$
	Control	613 ± 18	30277 ± 342	29664		
		341 ± 25	21460 ± 335	21119	21068 ± 4311	
		436 ± 27	12856 ± 342	12420		
			8 weeks			
III	Vitamin C	1427 ± 8.5	56724 ± 1544	55297		
		879 ± 29	57079 ± 399	56200	47666 ± 7003	
		456 ± 20	31957 ± 261	31501		
	Control	387 ± 16	6179 ± 198	5792		$p < 0.05$
		485 ± 48	8996 ± 90	8511	12975 ± 5089	
		573 ± 21	25197 ± 45	24624		

*BALB/c mice were placed on a vitamin C regimen (250 mg% in drinking water) at 2.5 months of age. At 2 weeks (males) and at 4 and 8 weeks (females) spleen lymphoid cells were harvested for con A stimulation. Values presented are of assays for individual mice carried out in groups of 2–3 vitamin C and control animals. The different time points represent 3 separate experiments. Assays of ^{3}H-thymidine incorporation were performed in triplicate in each case.

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probably responsible for effecting CMI[11]. For example, entrapment of macrophages by such lymphokines as macrophage inhibitory factor (MIF) and heightened activation by macrophage activation factor (MAF) result in more ready ingestion and degradation of infectious agents. Thus, the cell-mediated immune response is considered to provide a mechanism for enabling the infected host to rid itself of viruses and other intracellular parasites. Conceivably, then, the enhancement of interferon synthesis and the augmentation of T-lymphocyte activity would suggest a dual role for vitamin C in its putative protective participation against viral disease.

The effect of levamisole on phosphodiesterase activity

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Summary. Phosphodiesterase activity of mouse liver homogenates was estimated in presence and absence of levamisole. The enzyme activity was 1394 and 1399 nmoles/mg protein/30 min respectively. Our data show that levamisole does not affect the phosphodiesterase activity.

Levamisole, a widely used anthelminthic drug, was found to restore host defense mechanisms by stimulating phagocytes and lymphocytes when they are defective, and it has a potential therapeutic value as an immuno-modulating agent in domestic animals and in man[1]. In man, encouraging results have been reported under a wide range of conditions, many of which are to unknown etiology, but all with suspected defects in host defense mechanisms[2].

The mode of action of levamisole is not known and many hypotheses have been formulated. The variety of cells and the multitude of functions that are affected make it likely that levamisole influences a basic mechanism common to

1 Editorial: Lancet *1*, 151 (1975).
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all cells. It is interesting to note that theophylline depressed immunological phagocytosis, where as levamisole stimulated[3]. Theophylline inhibits phosphodiesterase and may thus increase intracellular cyclic adenosine monophosphate (cAMP). Hadden et al.[4] also demonstrated that levamisole increased cellular levels of cyclic guanosine monophosphate and decreased levels of cyclic AMP in mouse lymphocytes, and correlated this with increased responsiveness in vitro to phytohaemagglutinin. It therefore seems possible that levamisole could act by modifying nucleotide levels through activation of phosphodiesterase[3]. We therefore studied the effect of levamisole on phosphodiesterase activity.

We used 10 female mice as source of liver phosphodiesterase. The liver of each mouse was homogenized in 0.25 M sucrose containing 1 mM EDTA, centrifuged and the supernatant was used as enzyme source. Phosphodiesterase activity of the homogenates was estimated by using a procedure previously described which is based on measurement of the disappearance of tritiated cyclic (^{3}H) AMP[5]. The fractionation of the tritiated adenine nucleotides was obtained by chromatography on alumina column. Duplicate samples with and without levamisole were assayed for phosphodiesterase activity. Protein concentration of the liver homogenates was determined colometrically by the procedure of Lowry et al.[6]. Levamisole solutions were prepared each day before use in 50 mM Tris-HCl buffer pH 7.6.

Phosphodiesterase activity of mouse liver

Number of experiment	Phosphodiesterase activity (nmoles cAMP hydrolyzed/mg/30 min)	
	without levamisole	with levamisole
1	1395	1365
2	1465	1475
3	1425	1450
4	1355	1375
5	1389	1370
6	1405	1395
7	1375	1410
8	1365	1380
9	1386	1340
10	1435	1380
Mean ± S.D.	1399.50 33.85	1394 40.87

As shown in the table, the levels of phosphodiesterase activity of mouse liver did not change significantly in the present of various concentrations of levamisole. The mean levels of phosphodiesterase activity of mouse liver was 1399.50 nmoles of cyclic nucleotide hydrolyzed per mg protein per 30 min, at 37 °C. In the presence of 10^{-2} to 10^{-7} M levamisole the enzyme activity was 1394 nmoles cAMP/mg protein/30 min.

Pre-incubation of homogenates, at 37 °C for 30 min, with different concentrations of levamisole before assaying, gave no alterations on enzyme activity. However, there was a considerable increase in the level of phosphodiesterase activity when we added imidazole.

It is clear from our data that levamisole does not affect the phosphodiesterase activity in vitro. It seems worth investigating whether levamisole may stimulate intracellular phosphodiesterase activity in vivo.

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Neuroendocrine effects of a non-steroidal compound of testicular origin[1]

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Summary. The effects of the compound (+)-1,4-diphenylbutane-2,3-diol (DPB, synthetized in the testes) on gonadotropin secretion have been studied in castrated male rats. DPB, when injected subcutaneously, does not modify serum levels of LH and FSH. On the contrary, the local implantation of DPB in the median eminence of the hypothalamus results in a significant elevation of serum FSH. It is suggested that DPB may play a physiological role in the control of FSH release.

The mechanisms which control gonadotropin secretion in male animals are still controversial. Testosterone is generally believed to suppress the secretion of LH after having been converted in the anterior pituitary and in the brain into 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) and 5α-androstan-3α, 17β-diol[4–7]. Androgens also exert an inhibitory effect on FSH release[5–8]. This effect is probably linked to the 'aromatization' to estrogens, a process which occurs both in the periphery and in the central nervous system (mainly in the hypothalamus and in the limbic structures)[9,10]. The possibility that a proteic factor (Inhibin) originating in the testis might specifically inhibit FSH secretion has recently been revived[11–15].

The non-steroidal compound (+)-1,4-diphenylbutane-2,3-diol (DPB) has been isolated from bull and rat testicular tissue[16] and from dog spermatic vein blood[17,18]. The amounts of this compound have been shown to be higher in the testes of sexually mature animals than in those of prepuberal ones[16]. The present study was aimed at investigating whether DPB might exert any activity on gonadotropin secretion.

Materials and methods. 3 types of experiments were performed. Experiment 1: Adult male rats of the Sprague Dawley strain (initial weight 200 g ± 12) were castrated and DPB (dissolved in sesame oil) was administered subcutaneously in 2 daily doses of 50 μg/rat for 8 days beginning the day of castration. Treatments were performed at