endorphins might be interpreted as a decrease in dopaminergic activity; vice versa, the similarity of naloxone effect with that of dopamine agonists might be explained with an enhanced dopaminergic transmission.
The finding that naloxone shortens the ejaculation latency is of great interest, since it suggests that endogenous endorphins may have an inhibitory role in regulating the ejaculation mechanisms.
If the results obtained in rats apply to other species, then opioid agonists and antagonists might become potentially useful therapeutic agents for ejaculation and other sexual disturbances occurring in man.

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Levamisole inhibits mineral mobilisation, lactate production and lysosomal enzyme release from cultured bones

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Summary. Levamisole added to cultured calvarial bones inhibited spontaneous bone resorption, as indicated by reduced release of calcium and inorganic phosphate to the medium. In addition, levamisole reduced lactate production and release of lysosomal enzymes.

In a final effort to improve the periodontal condition of a patient suffering from both juvenile periodontitis and juvenile diabetes which had responded only poorly to conventional treatment, we used levamisole to try to correct the deficient leukocyte and cellular immune functions. The initial effect of the treatment was encouraging as the inflammatory symptoms were reduced, and during the following 1.5 years no progress of the alveolar bone destruction could be detected by radiological examination. Although the effect was most probably the result of a correction of the immune deficiencies, we considered the possibility that levamisole also had a more direct inhibitory effect on bone resorption with regard to its known inhibitory effect upon alkaline phosphatase[1-3]. We report here the results of a study upon the effect of levamisole (L-tetramisole) on bone resorption in vitro.

Material and methods. Paired halves of calvarial bones from 6–7-day-old mice (CsA type), were cultured separately at 37 °C on stainless grids in multi-well dishes (Linbro), containing 2.0 ml of a modification of CMRL 1066 medium (albumin added 0.1% w/v) and gassed with 5% CO_2 in air as described earlier[4]. In other series, each mouse was injected with 5 μCi ^{45}Ca at least 4 days prior to explantation and the calvarial halves were maintained in separate plastic Falcon dishes containing 5.5 ml of the medium, which was changed daily[4]. In each experiment, one of the calvarial halves was used as a control and the other cultured in the presence of levamisole (a gift from Janssen Division, AB Leo, Helsingborg, Sweden). The drug was dissolved directly in the medium. After culture the media were analyzed for their content of calcium (Ca^{2+})[5], inorganic phosphate (P_i)[6], β-glucuronidase[7], β-galactosidase[4,7,8], lactate dehydrogenase (LDH)[9], alanine aminotransferase (ALAT)[10], glucose[11] and lactate[12]. In addition the enzyme activities of the calvaria were analyzed as described[4]. All the enzyme assays included controls containing the test substance, and in no case did the substance affect the measurement of enzyme activity. All enzyme activities were calculated as units/half calvarium, where 1 unit refers to the decomposition of 1 μmole of substrate/min. The radioactivity in the media was analyzed in a liquid scintillation-counter[4] and the morphology of the bones was studied with conventional histological techniques.

Results. Levamisole, in doses higher than 10^{-4} M, was found significantly to reduce the spontaneous mobilisation of ^{45}Ca from prelabelled bones cultured for 24 h. The effect was virtually constant between 5×10^{-4} and 5×10^{-3} M. As shown in figure 1, the inhibitory effect of 10^{-3} M levamisole remained unchanged when the culture period was extended to 96 h. When the bones were cultured in the presence of the drug for 24 h and the culture continued in the absence of the drug for further 72 h, almost the same daily reduction of ^{45}Ca release was obtained as that seen when levamisole was present during the complete culture period. Levamisole did not influence the release of ^{45}Ca from heat-killed bones (data not shown).

The effect of 10^{-3} M levamisole on the release of LDH and ALAT from calvarial bones cultured for 24 h

	LDH (U/bone)		ALAT (U × 10^{-4}/bone)	
	Control (n = 11)	Levamisole	Control (n = 8)	Levamisole
Medium 0-24 h	32 ± 5	53 ± 16*	30 ± 2	41 ± 2**
Bone organ 24 h	297 ± 15	275 ± 11	37 ± 3	42 ± 3*
Medium + bone organ	328 ± 17	327 ± 13	67 ± 4	84 ± 3**

Values are means ± SEM. n = number of paired experiments. * $p<0.05$; ** $p<0.001$.

Further, in a parallel study a significant reduction of the mobilisation of non-radioactive Ca^{2+} and P_i was obtained when the calvaria were cultured in the presence of levamisole (figure 2). From figure 2 it also appears that, in the levamisole-containing culture, the activities of β-glucuronidase and β-galactosidase in the media were reduced. In contrast the media levels of LDH and ALAT were increased (table). The enzyme activities of the bones cultured in the presence of levamisole were slightly decreased as regards β-galactosidase (19%) and slightly increased as regards ALAT (14%), while no significant changes were found for β-glucuronidase and LDH. The glucose consumption remained unchanged but the lactate released to the medium was decreased in the presence of levamisole (figure 2).

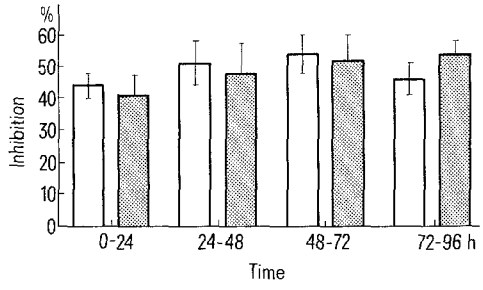

Fig. 1. The inhibitory effect of levamisole (10^{-3}M) on the spontaneous release of ^{45}Ca from calvarial bones cultured for 96 h. Unfilled columns indicate that the drug was present during the complete culture period. In this experiment the release of ^{45}Ca in the controls during the 4 consecutive days were 3827±221, 3248±314, 2091±243 and 1293±102 cpm/1.0 ml medium/bone respectively. Filled columns indicate that the drug was only present during the 1st 24 h. In this case the release of ^{45}Ca in the controls during the 4 days were 6492±496, 5315±562, 2262±358 and 1608±98 cpm/1.0 ml medium/bone respectively. The inhibitory effect is expressed as the percent reduction of ^{45}Ca (counts/min/1.0 ml medium/half calvarium) released by a treated bone as compaired to its paired control. Values are means±SEM for 5 paired experiments. $2p < 0.01$ for all observations.

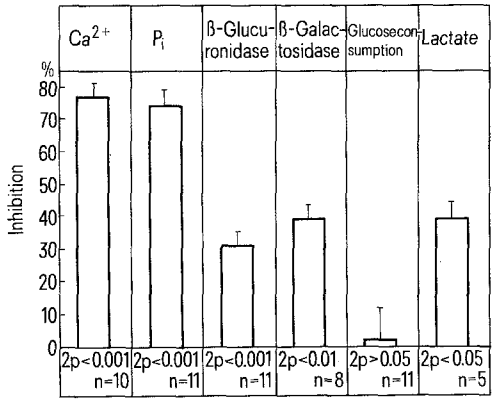

Fig. 2. The inhibitory effect of levamisole (10^{-3}M) on the spontaneous release of calcium (Ca^{2+}), inorganic phosphate (P_i), β-glucuronidase, β-galactosidase, lactate and glucose consumption by calvarial bones cultured for 24 h. The inhibitory effect is expressed as the percent reduction of Ca^{2+}, P_i, β-glucuronidase, β-galactosidase and lactate released and the glucose consumed by a treated bone as compaired to its paired control. The control released 29±3 µg Ca^{2+}/bone, 15±1 µg P_i/bone, 52±5 µU β-glucuronidase/bone (= 20% of total medium + bone), 115±18 µU β-galactosidase/bone, 294±33 µg lactate/bone and consumed 381±25 µg glucose/bone. Values are means±SEM. n= number of experiments.

Discussion. Our data indicate that levamisole in organ culture has an inhibitory effect upon bone resorption. The mechanism by which levamisole interferes with bone resorption is unknown.

The finding that the media levels of LDH and ALAT increased could be taken as evidence for a toxic effect of levamisole on the bone cells. However, the fact that the levels of the same enzymes in the bone explants were not reduced after culture, speaks against such a concept. In an earlier study, we found that the toxic substance NaN_3 caused a similar increase of ASAT in the media, but in this case the enzyme levels in the cultured bones were appreciably reduced[4]. The findings that the release of the lysosomal enzymes β-glucuronidase and β-galactosidase decreased and that the glucose consumption remained unchanged gives some support to the concept that the inhibitory effect of levamisole was not due to toxicity. The only morphological alteration which could be observed in the bones exposed to the drug was increased cellularity, which is not known to be a common consequence of drug toxicity. However, despite all these arguments a slight toxic effect cannot be ruled out completely. The observations by Reynolds and Dew[3] that 10^{-4} M levamisole inhibited the growth of chick bone explants raises the question of whether or not the action of the drug is due to toxicity or due to a specific pharmacological effect.

Another possibility may be that the drug influences the degradation of bone by inhibiting alkaline phosphatases[1-3]. It has recently been reported that a concentration of 10^{-4} M levamisole inhibits mouse bone alkaline phosphatase activity by 24%. At this concentration, however, we found no inhibition of bone resorption. Furthermore, in preliminary studies we have found that D-tetramisole, an isomer of levamisole, which has been shown to have almost no effect on mouse bone alkaline phosphatase[3], also inhibited the mobilisation of ^{45}Ca from cultured bones. Thus, the inhibitory effect of the tetramisoles on bone resorption would seem not to be related to the effect on alkaline phosphatase activity. A 3rd mechanism by which levamisole could exert its effects may be by interferring with release processes probably involved in bone resorption. This is indicated by the drug's capacity to reduce the levels of hydrolytic lysosomal enzymes in the media, as shown in the present study.

A 4th possibility suggested by our data is that levamisole reduced mineral mobilisation by diminishing lactate production. Whatever the mechanism may be, levamisole inhibits bone resorption in vitro. It has to be elucidated if this is also true in vivo.

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