ratio to β-adrenergic stimulation is clearly less than that produced by TSH, it is apparently faster, reaching its maximum effect within 2 min, as opposed to about 10 min for TSH[2]. Adrenaline was reported to produce a half maximal response in cyclic AMP at a concentration of 3×10^{-7} M in calf thyroid slices[4], although iodide uptake could be stimulated by as little as 5×10^{-7} adrenaline in isolated bovine thyroid cells[5]. Our observation of an increase in kinase activity with as little as 10^{-8} M *iso*proterenol suggests that lower concentrations of this pure β stimulator are required than are needed for an effect of the combined α and β stimulator, adrenaline.

Comparison of the action of *iso*proterenol and TSH reveals that *iso*proterenol acts more rapidly but does not raise cyclic AMP or stimulate kinase activity to the same degree as TSH. These findings, as well as the lack of effect of propranolol on TSH suggest that the two agents act on different receptor sites. The inhibition of the kinase response to *iso*proterenol by *l*- but not *d*-propranolol, as well as the lack of effect of phentolamine, indicates that the *iso*proterenol effect is mediated by β receptors.

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Influence of the pineal on wound healing

REMOVAL of the pineal gland affects endocrine functions in both birds and mammals, and has been shown to cause increased growth and spread of malignant tumours in the rodent. There are several hypotheses as to the mechanism by which the pineal affects these processes[1–4], but no role for the gland or its hormonal product melatonin has been suggested in normal processes. We have now found that pinealectomy can slow wound healing and that this effect can be reversed by treatment with melatonin.

Twenty 4-week-old inbred C57BL mice were divided into four groups of five. The mice in group 1 were pinealectomised; group 2 was sham-operated; group 3 received melatonin (1 mg kg^{-1} body weight) intraperitoneally daily, beginning 24 h after pinealectomy and continuing for 10 d; and group 4 served as untreated controls. All animals were kept in transparent plastic cages in a 10×12 foot room at 74–78 °F (23–26 °C) with 40% relative humidity. A 60-W bulb provided light during the day, and was turned off at night. The animals were fed Rockland hamster diet and water *ad libitum*.

Pinealectomy and sham operation were performed as described previously[2], and 10 d later a 2-mm semicircular wound was made in the right ear of each animal, including the controls. After 72 h, colchicine (2 mg kg^{-1} body weight) was injected intraperitoneally. All animals were killed 48 h later, always at 1030, to control for diurnal variations in pineal hormone interaction. Daily weighing during the experiment showed no weight loss in any of the animals.

Table 1 Original data transformed to square root

	Control	Sham	Melatonin	Pineal
Observations (*n*)	250.00	250.00	250.00	250.00
Mean	2.09	2.15	2.25	1.58
Median	2.00	2.24	2.27	1.73
Variance	0.60	0.46	0.57	0.53

For the sake of uniformity, each wound was divided into three areas. Samples from the margins of each area were excised, fixed in neutral formalin, dehydrated and embedded in paraffin. All samples were treated simultaneously to reduce the chance of artefact to a minimum. Serial sections (7 μm) were cut, then stained with haematoxylin and eosin. Each ear yielded 200 sections and all were mounted individually. We selected 50 slides from each area of each wound randomly, and counted the cells in each high-power field (×40); the average number in each field was constant from animal to animal. Obvious spindles and hyperchromatic nuclei with brush borders were considered to represent nuclei in mitosis and were included in the count.

We took as response (y) the number of mitoses per high-power field in each slide. Hence, the response was a discrete variable $0 \leqslant y \leqslant 20$ with 50 slides from each of five animals. We had 250 mitotic values for each group. The data showed that the responses did not follow normal distribution and the variances were heterogeneous. Therefore, the square root transformation was used in the final analysis. Table 1 summarises the transformed data. The ANOVA (Table 2) clearly indicates that at least two group means are significantly different at the 1% level of significance. To distinguish the pinealectomised groups from the others, a multiple comparison test was done. The result indicated that control, sham-operated, and melatonin-treated animals form one group, with means not significantly different, whereas the pinealectomised group differed significantly from the others at the 5% level.

Table 2 ANOVA using square root transformation

Sources of variation	Degrees of freedom	Sum of square	Mean square	*F*
Between groups	3	68.1248	22.7083	42.3
Between animals within groups	16	11.4246	0.7140	1.33
Residual	980	526.0679	0.5368	
Total	999	605.6173		

The small number of mitoses in the healing wounds of pinealectomised mice is possibly the result of a direct hormonal effect on wound healing. This observation is in contrast to the previous reports of gonadal hypertrophy and increased growth of malignant tumours after the removal of the pineal[2–4]. The gonadal hypertrophy has been postulated to be the result of hyperplasia or of hypertrophy of individual cells of the target organ. In the case of transplantable malignant tumours, hormonal influence has been proposed as one of the causes of increased growth and spread.

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