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Research Papers

Antifertility effect of orally formulated melatonin tablets in mice

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Summary

Melatonin, a putative pineal hormone, has been proved to be active orally. In the present investigation the efficacy of melatonin as an oral antifertility agent in mice was investigated. Melatonin tablets prepared by the solvent deposition technique (SDT) were found to be superior in their physical properties and the release rate of melatonin from the tablets was higher than those prepared by the conventional mixing method (CMM). The interaction of melatonin with polyvinylpyrrolidone (PVP) and lactose may be responsible for its high release rate. Melatonin tablets prepared by the SDT were used to evaluate its antifertility effect in mice. This effect was compared with that of its intramuscular injection. Oral administration of melatonin on the proestrus day resulted in 100% reduction in the mean number of corpora lutea and lutein cells. The ovulation blockage ratio was 100%. The mean duration of the oestrus phase was significantly decreased. Absence of corpora lutea was evident histologically. On the 4th day of pregnancy, oral administration of melatonin led to 100% interruption of pregnancy and 100% reduction in the mean number of implantation sites. Also, the average number of the blood capillaries and their transverse diameter were significantly decreased. Histological examination of the interrupted pregnant uteri showed completely degenerated embryo. The result of oral administration of melatonin mimicked the known effects of its intramuscular injection.

Introduction

There is now compelling evidence indicating that melatonin, a putative pineal hormone, has an antifertility effect. Melatonin has been proved to be effective in inhibiting implantation in mice (Vaughan et al., 1976; Mostafa, 1982). Epiphysan, a pineal extract, also proved to have an antifertility effect in mice (Hamed et al., 1984). Ron and Mead (1986) found that melatonin delayed implantation in the western spotted skunk.

Some studies on the action of pineal gland amines have indicated that melatonin also inhibited ovulation (Ying and Greep, 1973; Pomerantz and Reiter, 1974; Tamura and Kogo, 1989).

In these publications, melatonin was administered parenterally. However, Waldhauser et al. (1984) and Vakkuri et al. (1985) found that melatonin had a high bioavailability after oral dosing in human. This has led to the intensive investigation of alternative means of its prepara-

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tion including oral feeding (Kennaway and Seamark, 1980), silastic capsule (Lincoln and Ebling, 1985) and intravaginal implants (Nowak and Rodway, 1985).

It has been proven on the basis of data obtained in studies with different species that oral administration of melatonin can induce changes in reproductive activity similar to those after its injection. Oral administration of melatonin induced changes in prolactin and influenced the time of onset of the breeding season in sheep (Kennaway et al., 1982) and ewe (Poulton et al., 1986). Feeding melatonin affected the serum level of LH, FSH, prolactin, testosterone, triiodothyronine, thyroxin, cortisol and alkaline phosphatase in male white-tailed deer (Bubenik et al., 1986). Oral preparation of pineal melatonin caused hypnosis in man (Wright et al., 1986).

Melatonin is a poorly water-soluble hormone (the aqueous solubility is 0.96 g/l at 25 °C) and is used experimentally in low doses (100 μg–2 mg). The oral dosage form chosen for studying the efficacy of melatonin as oral antifertility agent in mice is moulded tablets. In the manufacture of tablet dosage forms of potent drugs, trituration by simple blending is commonly employed. The solvent deposition technique (SDT) may be a suitable method for ensuring rapid and reproducible dissolution rates of potent drugs (Monkhouse and Lach, 1972a,b; Ampolsuk et al., 1976). Johansen and Moller (1978) showed that a low drug to carrier ratio was necessary to give sufficiently increased dissolution rate of drug.

The aim of the present investigation was to study the antifertility effect of formulated melatonin tablets in mice. Comparison is made with parenterally administered melatonin.

Materials and Methods

Materials

Melatonin (Sigma) and all other chemicals were of analytical grade.

Methods

Preparation of injection

Melatonin was dissolved in absolute alcohol and diluted with Ringer's solution so that the ethanolic concentration was 3% and the final melatonin concentration was 1 mg/0.1 ml.

Preparation of moulded tablets

SDT: The calculated amounts of melatonin (1, 1.5 and 2 mg/tablet) and 3% PVP 44000 were dissolved in ethyl alcohol (85%). Lactose was mixed thoroughly with alcoholic solution. The paste formed was moulded into tablets and used within 1 week for the experimental studies. The properties of melatonin tablets prepared by this method were compared with that prepared by conventional mixing methods (CMM) (1.5 mg melatonin/tablet).

Evaluation of tablets

Tablets were evaluated for uniformity of weight, disintegration time (BP, 1988), hardness (Erweka hardness tester, TBT/S-Erweka Apparatebau, Heusentamm, Germany) and friability (Rock Friabilator, Erweka, TA3).

Uniformity of content (BP, 1988)

Ten tablets taken at random were dissolved in 100 ml distilled water. The absorbance of melatonin was measured spectrophotometrically (Shimadzu Spectrophotometer UV-150-O_2 Japan) at 277 nm. The concentration-absorbance curve followed the Beer-Lambert law over the range 1–16 μg/ml. No interference with the assay procedure was detected in the presence of the carriers used.

Dissolution rate

The USP XIX procedure was not applicable in the present study because of the low melatonin content (1.5 mg/tablet). The concentration of melatonin in 250 ml (least dissolution volume in USP procedure) was difficult to measure especially at the initial period of dissolution. The dissolution procedure adopted was as follows: One tablet was placed in a 100 ml beaker with 50 ml simulated gastric fluid and allowed to stand in a

constant-shaking water bath (Unitronic OR.P/selecta) adjusted to $37 \pm 0.1°C$ at a shaking rate of 30 rpm. At various time intervals, samples (1 ml) were withdrawn. Fresh aliquots of the dissolution medium were immediately added to compensate the samples withdrawn. The samples were assayed spectrophotometrically at 277 nm. Each experiment was performed in triplicate.

Ultraviolet spectroscopy (UV)

The UV spectrum of melatonin was compared with that in tablets (15 μg/ml) using the carriers as a blank (Unicam SP 1750, UV spectrophotometer).

Infrared spectroscopy (IR)

IR analyses of melatonin, excipients (PVP and lactose), and melatonin tablets prepared by SDT and CMM were performed using KBr discs (Unicam SP 1025 infrared spectrophotometer).

Antifertility effect of melatonin

Female albino mice, 3 months old and weighing 30–35 g were used in this study. The animals were divided into nine groups each of 10 animals.

Groups 1–4 were used to study the effect of oral melatonin on ovulation. Group 1 was treated with placebo tablets orally and maintained as a control group. Group 2 received intramuscular injection (i.m.) of 1 mg melatonin at 1, 2, 3 and 4 p.m. proestrus day as determined by vaginal smear test (Young et al., 1941). The other two groups (groups 3 and 4) were treated with 1 and 1.5 mg/tablet, respectively, in the same way as i.m. injection. The duration of the oestrus phase was determined by using vaginal smears. All animals were killed at 10 a.m. on the 4th day after treatment. The ovaries were examined for the number of corpora lutea and lutein cells. The ovulation blocking ratio was calculated (number of mice ovulated/total mice). Histological examination of the ovaries was carried out.

Groups 5–9 were used to study the effect of oral melatonin on implantation. Females were housed in groups of five, whereas fertile males were individually housed except at the time of mating. Females were examined daily and the finding of a copulation plug was utilized to determine the day of mating, which was designated as day 1 of pregnancy (Finn and McLarin, 1967). Group 5 was treated with placebo tablets orally and used as a control group. Group 6 received i.m. injection of 1 mg melatonin at 10 a.m. on the 2nd day of pregnancy. Groups 7–9 were treated with 1, 1.5 and 2 mg melatonin/tablet, respectively, in the same way as i.m. injection. All animals were injected with pontamine sky blue at 10 a.m. 15 min before killing on the 6th day of pregnancy for determination of implantation sites (Psychoyos, 1961). The uteri were examined for the number of implantation sites and the ovaries for the number of fresh corpora lutea. Histological examination of the uteri and ovaries of all animals (groups 5–9) was done to determine the number of blood capillaries (NBC) and to measure their transverse diameter (TDBC).

Differences in mean values between groups were compared by Student's *t*-test.

Results and Discussion

Evaluation of melatonin tablets

All the prepared tablets fulfilled the requirement of the BP (1988) for uniformity of weight and content. The data obtained on the properties of melatonin tablets formulated by SDT and CMM are listed in Table 1. Harder and less friable tablets were obtained by SDT. Moreover, melatonin was evenly distributed as indicated by low standard deviation in drug content.

Fig. 1 shows the dissolution pattern of melatonin from tablets prepared by SDT compared with those prepared with CMM. The percent of dissolved melatonin was found to be increased by about 2.9 and 1.4 fold from tablets prepared by SDT when compared with those prepared by CMM at 15 and 60 min, respectively. Evaluation of these data revealed that the dissolution pattern of melatonin from tablets prepared by both methods followed a first-order mechanism (Table 1).

No detectable changes in the UV spectrum of melatonin were observed as a result of its deposition on lactose and PVP.

TABLE 1

Properties of melatonin tablets preformulated by solvent deposition and conventional mixing methods (each tablet contained 1.5 mg melatonin)

Parameter	Conventional mixing	Solvent deposition
Weight (g) [a]	0.104 ± 0.006 (% deviation = 5.8)	0.101 ± 0.004 (% deviation = 3.9)
Hardness (kg) [b]	1.464 ± 0.131	1.500 ± 0.177
Friability (%) [c]	0.831 ± 0.043	0.690 ± 0.008
Content (% from claimed amount) [b]	97.800 ± 3.240	99.720 ± 0.012
Dissolution behaviour [d]		
Correlation coefficient	0.978	0.936
$t_{1/2}$ (min)	41.497 ± 7.461	28.857 ± 2.580
k $(min)^{-1}$	0.017 ± 0.003	0.024 ± 0.002

[a] Mean of 20 tablets.
[b] Mean of 10 tablets.
[c] Mean of five determinations of five tablets each.
[d] Mean of three tablets according to first-order mechanism.

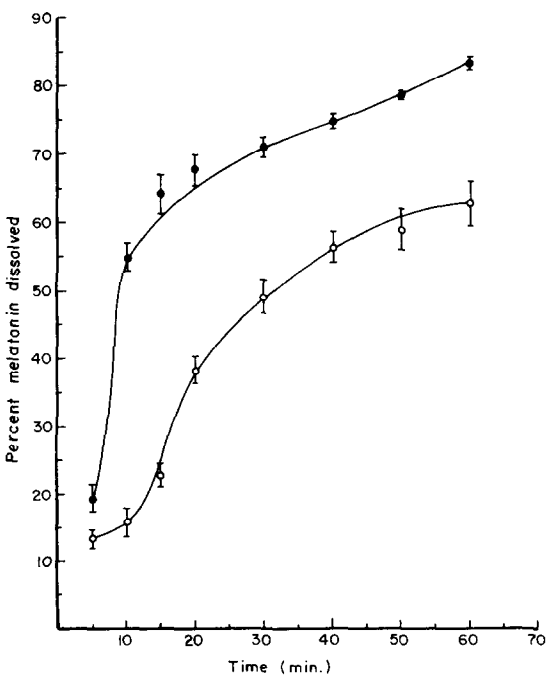

Fig. 1. Melatonin dissolution patterns: (○——○) tablets prepared by conventional mixing method; (●——●) tablets prepared by solvent deposition technique.

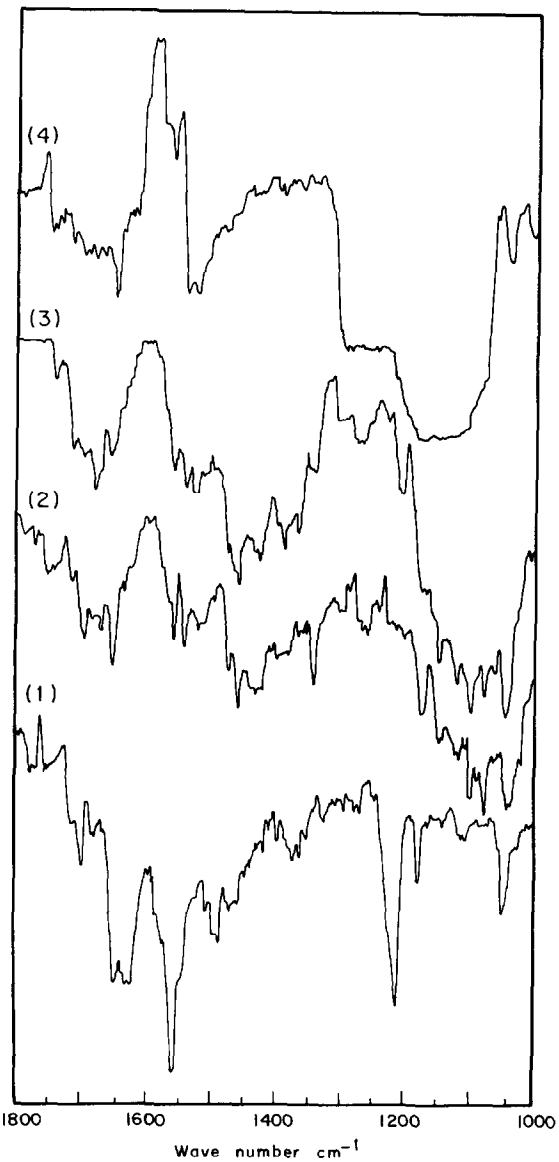

Fig. 2. Infrared absorption spectra of melatonin (1), tablet matrix (PVP/lactose) (2), tablets prepared by conventional mixing method (3), and tablets prepared by solvent deposition technique (4).

The IR spectra of melatonin, melatonin tablets prepared by both methods and inert matrix were compared (Fig. 2). The spectrum of melatonin tablets prepared by CMM is approximately the superposition of the patterns of both melatonin and tablet matrix. On the other hand, tablets prepared by SDT have a completely different

spectrum. For example, the absorption bands at 1000–1200 cm^{-1} in the melatonin spectrum appeared as one broad peak in the spectrum of tablets prepared by SDT.

In the melatonin spectrum the C = O absorption band of the primary amide group absorbed at 1690 cm^{-1} (amide 1 band) (Silverstein et al., 1974). In the spectrum of the solvent deposited sample, this band shifted to a lower frequency (1650 cm^{-1}). This occurred when this group was involved in hydrogen bond formation and this shift depends on the degree of hydrogen bonding (Silverstein et al., 1974). The observed spectral changes suggested a possible molecular interaction between the tablet matrix and melatonin. This interaction may be responsible for the increased dissolution rate of melatonin from tablets prepared by SDT.

Antifertility effect of melatonin

The oral effect of melatonin on the ovarian function of mice was studied using two different doses (1 and 1.5 mg/tablet). Administration of the highest dose (1.5 mg/tablet) at 1, 2, 3 and 4 p.m. proestrus day caused 100% decrease in the mean number of corpora lutea and lutein cells. The ovulation blockage ratio was 100%. Animals showed a significant decrease ($P < 0.001$) in the mean duration of the oestrus phase when compared with that of placebo treated animals (control group) (Table 2). Histological examination of these ovaries showed the complete absence of the corpora lutea. Many follicles were undergoing atresia. In these atretic follicles, there was complete fragmentation and dissolution of the granulosa and theca layer (Fig. 3a). Placebo treated ovaries revealed the presence of fresh corpus lutea which were characterized by hypertrophy and hyperplasia of the granulosa and theca cells (Fig. 3b).

The lower dose (1 mg/tablet) produced a similar effect except that the ovulation blocking ratio was 70% (Table 2).

The i.m. injection of melatonin (1 mg/injection) caused similar effects to oral administration (1.5 mg/tablet) (Table 2).

The effect of orally administered melatonin on pregnant mice was also studied using three different single doses (1, 1.5 and 2 mg/tablet). Administration of the highest dose at 10 a.m. on the 2nd day of pregnancy produced interrupted pregnancy in 100% of treated mice. Interrupted pregnant animals showed 100% reduction in the mean number of implantation sites. A significant decrease ($P < 0.001$) in the mean number of blood

TABLE 2

Effect of melatonin on duration of oestrus phases, number of corpora lutea and lutein cells in mice

Treatment	Duration of oestrus phase (h)		Ovulation blockage ratio	Corpora lutea		Lutein cells	
	Mean	Percent decrease		Mean	Percent decrease	Mean	Percent decrease
Placebo	15.38 ±1.11 [a]	–	0.0	8.00 ±0.56	–	56.20 ±3.17	–
i.m. injection (1 mg/injection)	7.63 [c] ±1.03	50.39	100	0.0	100	0.0	100
Oral treatment (mg/tablet)							
1	11.00 [b] ±1.22	28.48	70	2.90 [c] ±0.35	63.75	42.80 [b] ±2.80	23.84
1.5	8.25 [c] ±0.99	64.36	100	0.0	100		100

[a] Standard error.
[b] Significant at 1% level of probability when compared with controls.
[c] Significant at 0.1% level of probability when compared with controls.

capillaries (NBC) and their transverse diameter (TDBC) was observed when these values were compared with that of the control group (Table 3). Histological examination of the interrupted pregnant uteri showed that the uterine lumen was open and irregular. No decidual cell transformation was observed in the stroma (Fig. 4a). Some specimens showed degenerated embryos in the middle of the uterine lumen (Fig. 4b and c). Fig. 4d shows the uterus of placebo treated pregnant animals (con-

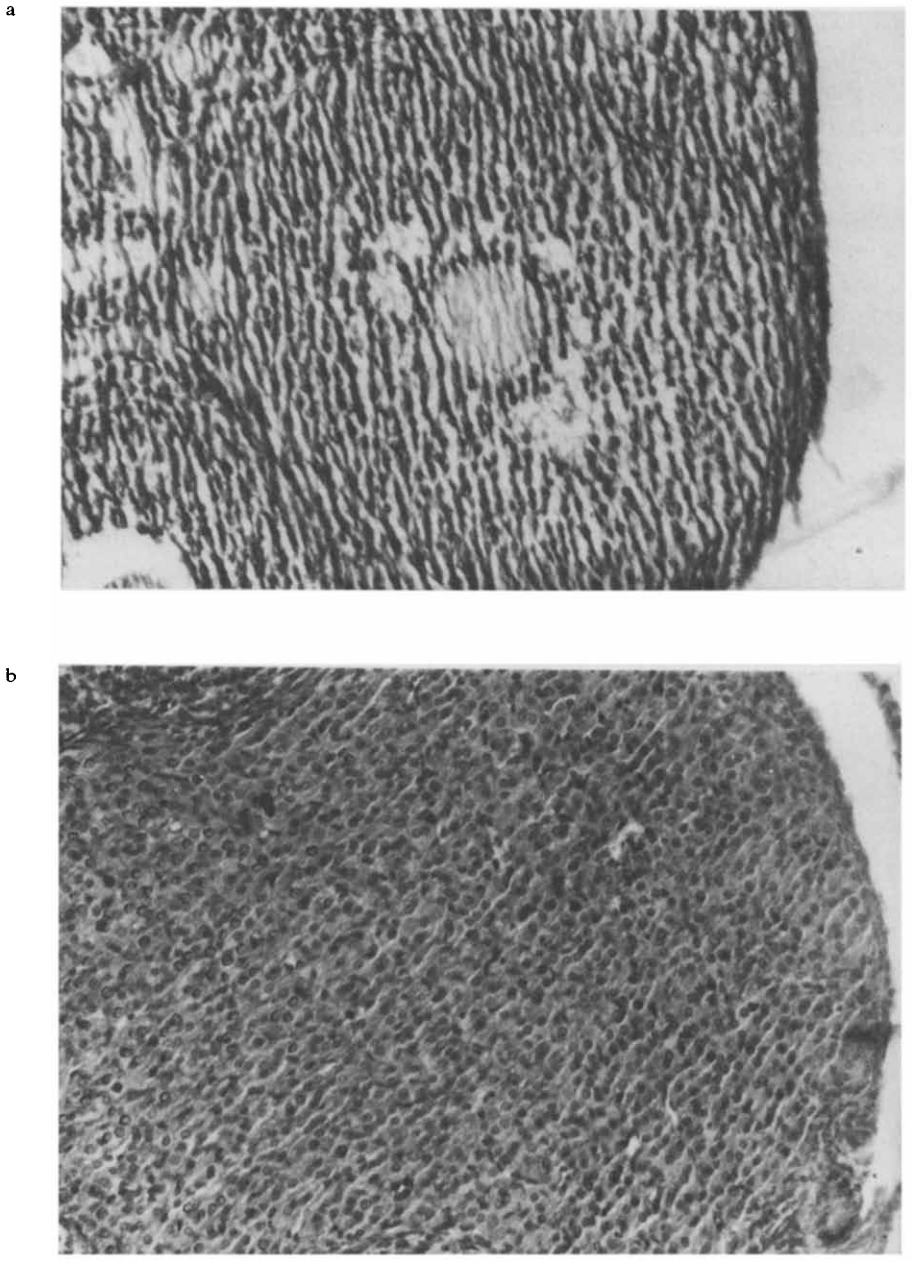

Fig. 3. Photomicrographs of ovary × 200. (a) Oral melatonin treated tissue revealed the presence of atretic follicles. Note that the granulosa layer is completely fragmented. (b) Placebo treated tissue showing the presence of fresh corpus luteum which is characterized by hypertrophy and hyperplasia of the granulosa and theca cells.

trol group). It reveals the presence of the embryo at the egg-cylinder stage and the stroma shows a decidual cell reaction.

The other two doses (1 and 1.5 mg/tablet) produced similar effects except for the incidence of interrupted pregnancy which was 60 and 80%, respectively (Table 3).

The i.m. injection of melatonin (1 mg) caused a similar effect to the oral dosage of 2 mg/tablet (Table 3).

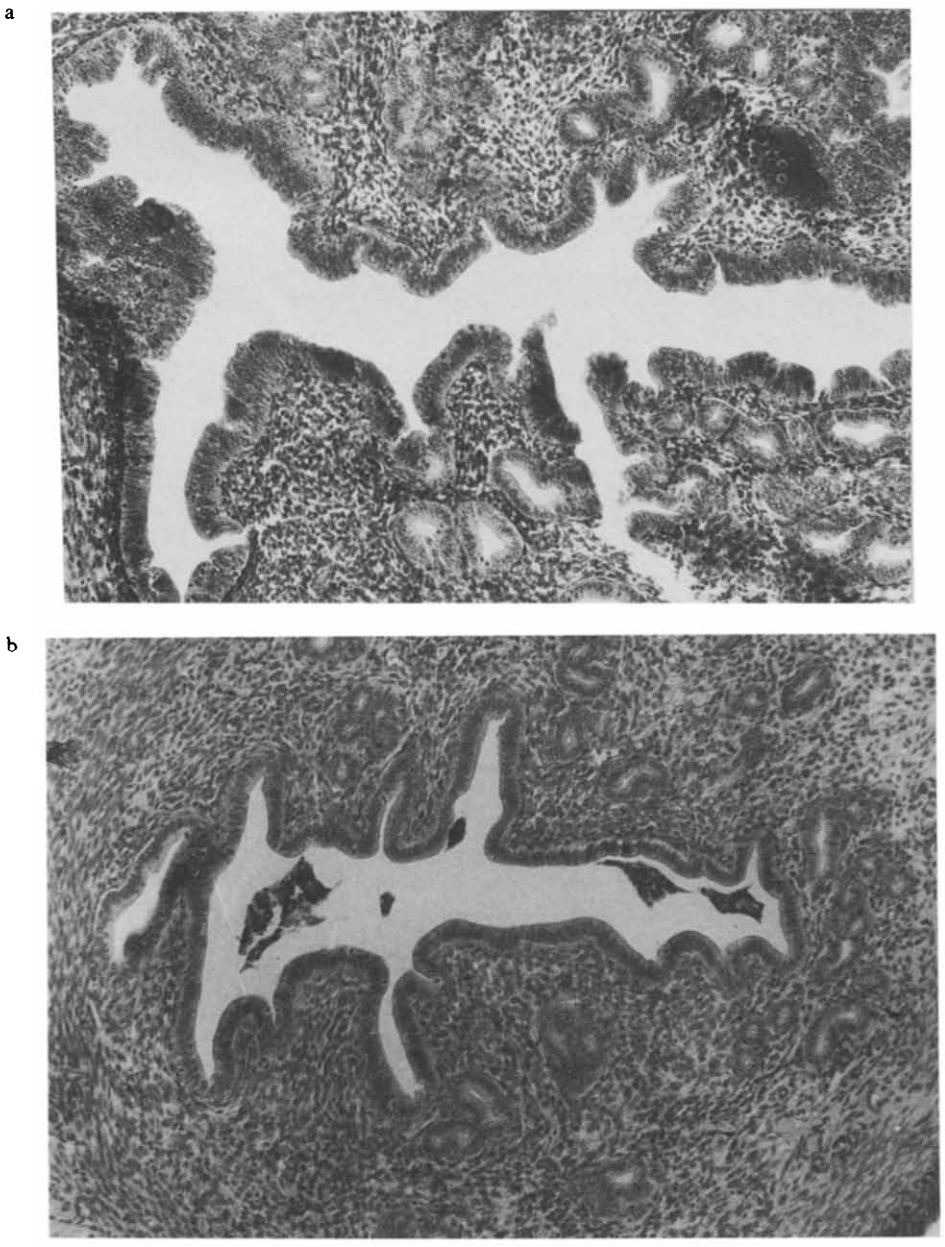

Fig. 4. Photomicrographs of uterus × 200. (a) Oral melatonin treated tissue showing the presence of opened and irregular uterine lumen. No decidual cell transformation is observed in the stroma. (b) Completely degenerated embryo. (c) Degenerated embryo at the egg-cylinder appearance. (d) Placebo treated tissue revealing the presence of normal embryo in the center of the uterine lumen.

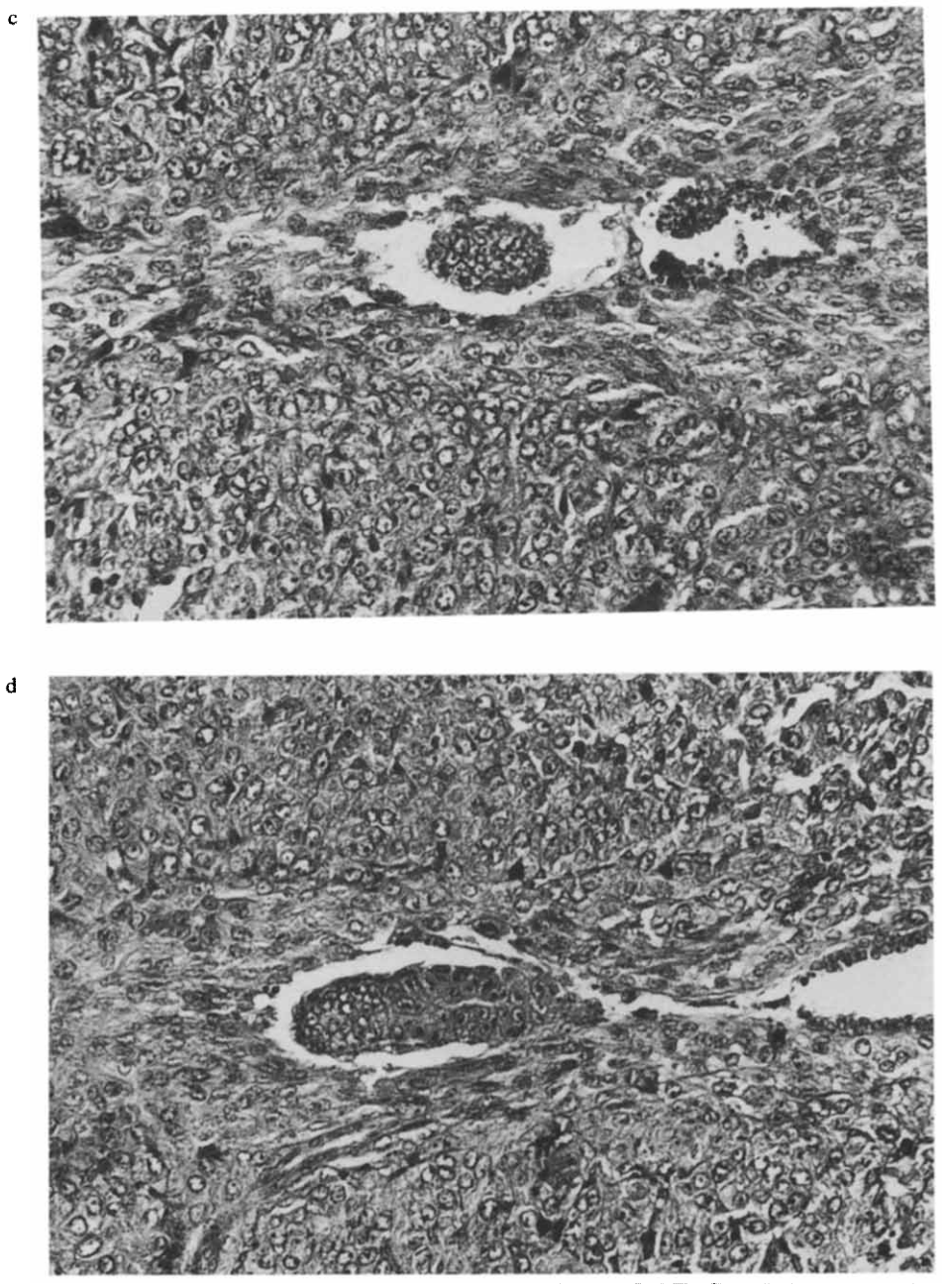

Fig. 4 (c,d).

These results suggest that oral melatonin has an antigonadotrophic effect and may have a direct effect on the vascularity of the uterus. The results of the present study demonstrate that the effects of oral administration of melatonin on the reproductive activity of mice are similar to those after its i.m. injection.

The oral administration of melatonin has proved a practical and reliable method of raising serum melatonin levels in sheep and goats (Ken-

TABLE 3

Effect of melatonin on implantation in pregnant mice

Treatment	Effective percentage	Implantation site		Corpora lutea		Mean NBC	Mean TDBC (μm)
		Mean No	Decrease (%)	Mean No	Decrease (%)		
Control	0.0	7.58 ±0.64 *	–	7.80 ±0.51	–	18.85 ±1.51	53.70 ±3.31
i.m. injection (1 mg/injection)	100	0.0	100	4.43 [c] ±0.31	43.21	9.01 [c] ±0.63	27.11 [c] ±1.55
Oral (mg/tablet)							
1	60	0.0	100	5.80 [b] ±0.41	25.64	16.17 ±0.40	47.99 ±3.14
1.5	80	0.0	100	4.95 [c] ±0.41	36.54	13.33 [a] ±1.49	38.14 [b] ±2.50
2	100	0.0	100	4.30 [c] ±0.34	44.87	10.11 [c] ±0.63	30.67 [c] ±2.39

* Standard error.

[a] Significant at 5% level of probability when compared with controls.

[b] Significant at 1% level of probability when compared with controls.

[c] Significant at 0.1% level of probability when compared with controls.

NBC, mean number of blood capillaries; TDBC, mean transverse diameter of blood capillaries.

naway and Seamark, 1980) and humans (Wetterberg et al., 1978).

The effect of oral administration of melatonin as an antifertility drug is very encouraging. This may lead in the future to its use as a postcoital contraceptive agent.

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