Lack of the effect of melatonin on the frog spermatogenic reaction

SIR,—Parenterally injected homogenised tissues from cow pineal glands diminish the spermatogenic response of the male frog to chorionic gonadotrophin injection (Juszkiewicz & Rakalska, 1963). Recently, Wurtman, Axelrod & Chu (1963) reported that some of the effects of the pineal gland on gonad function might be mediated by melatonin. Daily injections of microgram amounts of melatonin to rats decreased the incidence of oestrus and reduced ovarian weight. It was found that pinealectomy in rats was followed by an increase in the incidence of oestrus; this increase was inhibited by melatonin treatment (Chu, Wurtman & Axelrod, 1964).

However, there is some discrepancy in the reported effects of melatonin on the functioning of gonads. De la Lastra & Croxatto (1964) found that the whole human brain contains a substance producing depletion of the ovarian ascorbic acid, but they failed to obtain similar results in rats injected with melatonin. Moreover, Kappers (1962) reported that melatonin did not affect spermatogenesis in full grown rats.

It was of interest to find whether melatonin affected the spermatogenic response of the male edible frog, *Rana esculenta* L., to chorionic gonadotrophin injection. In the course of preliminary investigations, with the conditions we used we found that the most suitable dose was 20 I.U. of chorionic gonadotrophin (Gonadotrophine chorionique I.S.H., Paris) in 0·5 ml of saline solution; the best response was attained while examining the urine 3 and 6 hr after the injection of the hormone. Solutions of melatonin in ethanol were prepared freshly for injection with a subsequent 1:100 dilution with water. They were so adjusted that 0·5 ml contained 10, 100 or 500 μg of the substance. The preparations were injected into the dorsal lymph sac of the frogs. A saline solution of homogenised cow pineal glands (0·5 ml/100 mg of fresh tissue) was given once, 6 hr, and melatonin solutions twice, 12 hr and 30 min, before the injection of chorionic gonadotrophin. The experiment was made simultaneously on 242 male frogs in 6 groups.

TABLE 1. EFFECTS OF PARENTERALLY INJECTED MELATONIN AND HOMOGENIZED TISSUES FROM COW PINEAL GLANDS ON THE SPERMATOGENIC RESPONSE OF THE MALE FROG, *RANA ESCULENTA*, TO CHORIONIC GONADOTROPHIN INJECTION

Treatment		Spermatogenic response		
	No. of frogs	No. of frogs	Relative %	P*
Control group; chorionic gonadotrophin 20 I.U. ..	70	44	100	—
Melatonin 500 μg	35	1	4	0·001
Melatonin 10 μg; chorionic gonadotrophin 20 I.U. ..	35	21	95	0·9
Melatonin 100 μg; chorionic gonadotrophin 20 I.U. ..	34	19	89	0·5
Melatonin 500 μg; chorionic gonadotrophin 20 I.U. ..	34	22	103	0·8
Cow pineal 0·1 g; chorionic gonadotrophin 20 I.U. ..	34	5	23	0·001

* Compared with the control group by the χ^2 method with Yates' correction.

The results are in Table 1. The number of frogs which gave a positive spermatogenic response in a control group was taken as 100% (relative per cent). In relation to that figure the percentage of frogs reacting in the experimental groups was calculated.

We have been unable to find any action of injected melatonin on the frog spermatogenic reaction, whereas homogenised tissues from cow pineal glands

exhibited very significant inhibition, facts which support our previous experience (Juszkiewicz & Rakalska, 1963).

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References

Chu, E. W., Wurtman, R. J. & Axelrod, J. (1964). *Endocrinol.*, **75**, 238–242.
Juszkiewicz, T. & Rakalska, Z. (1963). *Nature, Lond.*, **200**, 1329–1330.
Kappers, J. A. (1962). *Gen. comp. Endocrin.*, **2**, 610–611.
de la Lastra, M. & Croxatto, H. (1964). *Nature, Lond.*, **204**, 583–584.
Snedecor, G. W. (1956). *Statistical Methods*, 5th ed., pp. 212–225. Ames, Iowa: Iowa State University Press.
Wurtman, R. J., Axelrod, J. & Chu, E. W. (1963). *Science*, **141**, 277–278.
Wurtman, R. J., Axelrod, J. & Potter, L. T. (1964). *J. Pharmacol.*, **143**, 314–318.

Mathematical treatment for oral sustained release drug formulations

SIR,—Recently a detailed mathematical treatment of drug release from oral sustained release dosage forms was presented by Rowland & Beckett (1964), who made a direct criticism of an equation presented by me to calculate the maintenance dose for sustained release (Nelson, 1957). These authors argued that the earlier presentation did not take into account the amount of drug that might be released from the maintenance portion of the dose during the time the initial dose was being absorbed. It should be pointed out that sustained release forms do exist from which only insignificant amounts of the maintenance portion of drug are released until the initial dose is absorbed (for example, Spansule and repeat action tablets). Therefore, the treatment presented by Rowland & Beckett (1964) applies only to the special case where release from the "free form" and "maintenance form" begins at the same time.

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References

Nelson, E. (1957). *J. Amer. pharm. Ass., Sci. Ed.*, **46**, 572–573.
Rowland, M. & Beckett, A. H. (1964). *J. Pharm. Pharmacol.*, **16**, *Suppl.* 156*T*–162*T*.