between materials. The values however, will not be universally applicable for all experimental conditions for the reasons discussed above. Nevertheless a generalized qualitative classification distinguishing types of compaction behaviour can be made (Hersey & Rees 1971; York & Pilpel 1973) which has been found useful in several studies of pharmaceutical powders, simple mixtures and multicomponent formulations (Esezebo & Pilpel 1978; Kurup & Pilpel 1978; York 1978).

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The effect of neuroleptic drugs on serum and cerebrospinal fluid melatonin concentrations in psychiatric subjects

J. A. SMITH*, J. L. BARNES†, T. J. MEE, *Department of Pharmaceutical Chemistry, University of Bradford.* †*Ida Darwin Hospital, Fulbourn, Cambridge, U.K.*

The role of the pineal gland in normal physiology and in pathophysiological conditions is little understood. The active metabolite is thought to exert an inhibitory action on the pituitary-gonadal axis (Minneman & Wurtman 1975) and also to control cyclic variations in sleep and arousal (Quay 1974). We have recently established (Smith et al 1977b) a diurnal rhythm in normal human blood melatonin (MT) measured by radioimmunoassay (RIA) which is synchronous with human post mortem pineal synthetic enzymes where maximal and minimum values occurred around 0200 h and 1400 h respectively. The pineal gland has for two centuries been implicated in psychiatry, the evidence being recently reviewed by (Mullen & Silman 1977). In preliminary studies (Smith et al 1977a), we reported blood MT concentrations in psychiatric subjects. The results fell into two groups, those with high MT concentrations and little rhythm and those with very low MT concentrations, and hardly any rhythm. We suggested that the high level group correlated with those subjects being treated with chlorpromazine (CPZ). In an extension of these studies, we now confirm that chlorpromazine does indeed increase serum but not c.s.f. MT concentrations. Furthermore, the effect appears to be dose related. We also comment on the effect of other neuroleptic drugs.

Blood samples (10 ml) were collected at 4-hourly intervals for 24 h from normal and psychiatric subjects. Subjects were awakened from sleep in a dark room for night sampling. Serum (1 ml) and 2 M phosphate buffer (pH 10·1 1·5 ml) saturated with potassium chloride were extracted with 15 ml light petroleum (b.p. 40–60 °C), the aqueous phase was then extracted with chloroform (25 ml) (redistilled), and the organic phase evaporated under nitrogen at 37 °C. The residue was taken up in ethanol (1 ml) and transferred to the assay tube. The solvent was evaporated again under nitrogen at 37 °C and the MT measured by RIA as described by Arendt et al (1975). The antibody (raised in rabbits by the method of Arendt et al 1975) is specific for MT, exhibits no serious cross reaction with any of the major indoles, including 6-hydroxy-MT and *N*-acetyl-5-hydroxytryptamine, and is sensitive to 10 pg ml^{-1} of serum. The final antibody dilution is 150:1. Interassay and intra-assay coefficients of variation are 16 and 11% respectively. All glassware

* Correspondence.

was siliconized and recovery of authentic [^{3}H]MT (25 Ci mmol^{-1}, New England Nuclear) was 62 ± 8%.

Table 1 describes serum MT concentrations at 4 hourly intervals over 24 h in eight psychiatric subjects being treated with varying doses of CPZ (100–600 mg daily) and also in normal subjects. The average 1500 h daytime sample from psychiatric subjects was five times higher than the mean 1400 h normal MT concentration, whilst the 0300 h average concentration is only one and a half times more than the mean

Table 1. Variation with time of human serum melatonin concentration [pg ml^{-1} with (s.d.)].

Normal individuals average age 36 years (26–48) N = 7					
0200	0600	1000	1400	1800	2200 h
74 (26)	43 (15)	27 (11)	19 (7)	23 (9)	42 (21)
Psychiatric subjects being treated with CPZ (100–800 mg daily), average age 30 years (17–43) N = 8					
0300	0700	1100	1500	1900	2300 h
114 (33)	93 (34)	86 (20)	101 (29)	108 (36)	123 (38)
Psychiatric patients not being treated with drugs, average age 49 years (27–65) N = 5					
0200	0600	1000	1400	1800	2200 h
30 (0)	24 (6)	16 (5)	14 (4)	16 (5)	23 (11)

normal maximum at 0200 h. This suggests that CPZ has elevated the serum MT concentration producing high values with little rhythm. A similar effect has been observed by Ozaki et al (1976) in rats and confirmed by ourselves. Table 1 also shows serum MT concentrations in a group of psychiatric subjects not taking drugs. These values are much lower than those in subjects on CPZ and are lower than the values in normal subjects, but whether the drug-free serum MT concentrations were significantly lower than normal values is as yet unclear.

We have also measured serum MT concentrations in one newly presenting untreated psychiatric subject before and after CPZ treatment (300 mg daily). Before treatment. MT values at 2400 and 1500 h were 28 (s.d.3) and 27 (s.d.3) pg ml^{-1} respectively. Whilst the daytime concentration is normal, the night-time value is again abnormally low. After treatment with CPZ, however this subject's serum MT concentrations increased to 69 (s.d.7) and 39 (s.d.4) pg ml^{-1} at 2400 and 1500 h respectively. This confirms the elevation of MT serum values by CPZ in man. The possibility of cross reaction with the antibody by CPZ or its metabolites has been eliminated since our studies show that sera from subjects on large doses of CPZ (and other neuroleptics) spiked with MT produced a parallel line to our calibration curve, obtained from normal sera spiked with MT. This parallelism is evidence of non-cross reaction.

Investigations were made at 1100 h of simultaneous serum and c.s.f. MT concentrations in two groups of psychiatric subjects. In one group (3 subjects age 64–75 years) not receiving drug treatment, mean serum MT concentrations were significantly lower than the c.s.f. values (16, s.d.3 and 39, s.d.15 pg ml^{-1} respectively). This supports our earlier findings in leukaemic children (Smith et al 1976).

However in the other group (3 patients age 36–55 years) being treated with CPZ (200–600 mg daily), it is seen that mean serum MT concentrations are now significantly higher than the c.s.f. MT values (58, s.d. 21 and 33, s.d. 5 pg ml^{-1} respectively). These results show that CPZ has increased serum MT but left c.s.f. MT unaffected. Although we have shown in vitro that neuroleptics reduce MT production by inhibiting bovine hydroxyindole-*O*-methyltransferase (HIOMT), (Hartley et al 1972), we have found that these drugs have no effect in vivo on rat HIOMT (unpublished data). Our conclusion is therefore that CPZ reduces the rate of metabolism of MT in the liver rather than increasing the rate of synthesis in the pineal gland which would lead to an elevation of both serum and c.s.f. MT concentration. This is in agreement with proposals made by Ozaki et al (1976) for explaining similar increases in the serum MT of rats treated with CPZ.

When 1100 h MT serum values from psychiatric subjects are plotted against their total daily dose of CPZ, (Fig. 1) a positive correlation is seen such that increasing the total daily dose results in an increase in serum MT concentrations. One subject received a different dose on readmission after a relapse and Table 2 shows that at a total daily dose of CPZ of 450 mg, the 4 hourly serum MT values were consistently higher at each sampling time than at a 300 mg dose. This

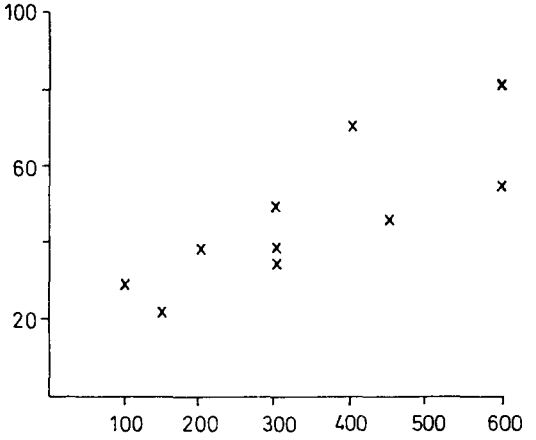

FIG. 1. Correlation of 11·00 h serum melatonin concentration (pg ml^{-1}) in psychiatric subjects (average age 45 years) with total daily dose of chlorpromazine (mg). Ordinate: serum melatonin concentration (pg ml^{-1}). Abscissae: total daily dose of CPZ. X represents triplicate assays of blood from one patient. Regression analysis gives a line with an intercept on y axis of 17·29 and a slope of 0·0865.

elevation of serum MT in subjects with abnormal mental states is therefore due to a dose-related effect of CPZ and not to an inherent difference from normal individuals. However, it is unclear whether the baseline serum MT in the psychiatric subjects is abnormally low or is normal before drug treatment. In the separate psychiatric subject studied, it appears that at least the night-time base value is very low. By apparently reducing the rate of MT metabolism in the liver, CPZ increases these MT concentrations.

Table 2. Serum melatonin concentration (pg ml^{-1}) in the same psychiatric patient at different total daily dosage of chlorpromazine. Age = 43 years.

Dose (mg)	0200	0600	1000	1400	1800	2200
300	80	64	36	50	48	94
450	119	83	47	81	94	102

Table 3. Serum melatonin concentration [pg ml^{-1} with (s.d.)] in psychiatric subjects.

(a) Treated with flupenthixol (40–100 mg every 2–4 weeks i.m.).
Average age 48 years (36–56) N = 4.

0300	0700	1100	1500	1800	2300 h
33 (9)	35 (10)	16 (4)	20 (17)	32 (27)	28 (10)

(b) Treated with fluphenazine (12·5–25 mg every 2–4 weeks i.m.
Average age 55 years (49–62) N = 2.

0200	0600	1000	1400	1800	2200 h
24 (8)	20 (2)	12 (2)	10 (6)	30 (13)	42 (25)

Neither flupenthixol nor fluphenazine elevated serum MT in psychiatric subjects the concentration of MT recorded being generally much lower than normal concentrations (Table 3). It is possible that in man those drugs reduce serum MT, but in the rat, at least, fluphenazine has no effect (unpublished data).

If the low values in drug-free patients can be shown conclusively to be associated with the disease, then further support might be given to the theory that in schizophrenia, HIOMT is defective (McIsaac et al 1961; Greiner 1970) or is out of phase with its substrate (Hartley & Smith 1973). It is tempting to suggest that CPZ exerts its antipsychotic activity by increasing serum MT concentration. However, with flupenthixol- and fluphenazine-treated subjects this effect is not seen and so the activity of these drugs cannot be explained in terms of elevated MT values. But in view of the implication of MT with sleep (Cramer et al 1974), high serum MT concentrations in CPZ-treated subjects might explain the initial sedative effect of CPZ before the onset of its antipsychotic action.

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