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Examination of Blood Clobazam Levels and Several Pupillary Measures in Humans

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Abstract □ The State–Trait Anxiety Inventory was administered to 15 subjects before initiation of the experiment. Three subgroups of five subjects were defined by computing the unweighted sum of the state and trait anxiety scores. A 40-mg dose of clobazam, a 1,5-benzodiazepine, was administered to each subject and repeated with two additional dosage forms following a 2-week washout period. Blood samples were withdrawn, and blood levels were determined by fluorometric analysis. Additionally, pupillary measures of critical flicker fusion, constriction, and dilation in response to a cognitive task were obtained at 0, 2, 4, and 6 hr. A repeated measures analysis of variance revealed that blood levels were, as expected, statistically different over time and dosage form. The pupillary constriction mirrored the blood levels in statistical patterns. The pupillary measure of cognition related to the anxiety state after the performance effects of the cognitive task were statistically removed. The results suggest that clobazam has less immediate human effect than does diazepam.

Keyphrases □ Clobazam—analysis, fluorometry, blood levels, effect on pupillary constriction, and anxiety level □ Pupillary constriction—clobazam effect, blood level, and anxiety level □ Tranquilizers, minor—clobazam, fluorometric analysis, blood levels, effect on pupillary constriction, and anxiety level

Clobazam, a 1,5-benzodiazepine, is effective in the treatment of anxiety neurosis (1, 2). The immediate and long-term effects of the benzodiazepines on human performance ability likewise have been demonstrated. Generally, the benzodiazepines decrease human ability to perform complex tasks (3–5). Additionally, the benzodiazepine drug class affects physiological measures such as auditory reaction times and complex visual reaction times (6–8).

Pupillometrics is defined as the aspect of psychology that deals with the assessment of pupillary alterations elicited by any stimulus other than light (9). Pupil dilation in response to complex cognitive tasks can be observed with proper instrumentation and experimental controls (10). A sensitive means of generating pupillary cognition curves by verbally presenting randomized digits to subjects was described (11, 12). Furthermore, diazepam consumption was shown to alter the pupil cognition curves and recall ability (12).

The objective of this research was to determine if clobazam consumption related to pupillometry involving human cognition and two noncognitive pupillary measures. Critical flicker fusion and miotic effect of the drug were selected as noncognitive measures.

EXPERIMENTAL

Fifteen male volunteers were recruited and subjected to the State–Trait Anxiety Inventory (13). A high anxiety subgroup, consisting of five members, was defined operationally by a combined state–trait anxiety score of >72.0. A five-membered, low anxiety group was defined by a combined score of <64.0. The remaining five subjects were assigned to the middle anxiety group.

A randomized Latin-square design was employed to assign the subjects to a three-level dosage form treatment group. Each subject received separate 40-mg doses by tablet, solution, and capsule dosage forms in randomized and matched sequences. Blood samples were obtained for up to 144 hr following ingestion (14). The blood levels were determined by a fluorometric assay, which did not distinguish between the drug and its active major metabolite, the *N*-desmethyl compound (15).

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Table I—Analysis of Variance of Blood Clobazam Levels (Nanograms per Milliliter)

Source	Degrees of Freedom	*F*	P
Anxiety	2	N.S.[a]	
Dosage form	2	5.0818	0.014
Dosage form–anxiety	4	2.8937	0.044
Time	3	461.5114	0.001
Time–anxiety	6	N.S.	
Dosage form–time	6	2.6644	0.022
Dosage form–time–anxiety	12	N.S.	

[a] Not significant.

Table II—Analysis of Variance of Pupillary Diameter (Millimeters)

Source	Degrees of Freedom	*F*	P
Anxiety	2	N.S.[a]	
Dosage form	2	7.0835	0.004
Dosage form–anxiety	4	N.S.	
Time	3	9.3013	0.001
Time–anxiety	6	N.S.	
Dosage form–time	6	N.S.	
Dosage form–time–anxiety	12	N.S.	

[a] Not significant.

Pupillary measures were obtained for all subjects and all doses immediately preceding ingestion of the 40-mg dose. Additional pupillary observations were obtained immediately preceding the 2-, 4-, and 6-hr blood samplings.

The total pupillary recordings required approximately 7 min for each subject–dosage form–time set. The subjects entered an experiment room illuminated at 5 foot-candles, and critical flicker fusion measures were obtained. Ascending and descending measures were recorded individually for each eye. The flicker fusion apparatus was calibrated to balanced maximum brightness, a neutral density wedge setting of 3, and 15% light[1].

The cognitive task methodology outlined previously (11, 12) was employed after determination of the critical flicker fusion rates. Thus, the subject's pupils were accommodated to the experimental room illumination level before the cognition recordings commenced. The subjects were required to remember the exact order of seven randomized digits between zero and nine. The digits were presented in monotone fashion at 1-sec intervals. The pupillary dilation pattern was simultaneously recorded[2] and continued for several seconds following the presentation of the seventh digit, which permitted the subjects to resort the numbers and finalize their thought processes. Each trial covered 14 sec, and two trials were executed at each subject–dosage form–time interval and averaged to reduce variability. Finally, a measure of satisfactory completion of the cognitive task was obtained by recording the number of digits correctly recalled at each trial.

All pupillary and blood level data were reduced to machine-readable form. To isolate the pupillary cognition curve from the constriction effect of the drug, the initially recorded diameter at the 1st sec of the cognitive task was subtracted from that at each remaining second of the cognitive curve. In this manner, a constriction factor was created. A program was written that computed the areas under the "cleansed" cognition curves, employing the trapezoidal rule (16).

Thus, the final data base contained 15 subjects categorized into one of three anxiety levels. Each subject's file contained fluorometric blood level recordings, pupillary constriction measures, pupillary cognitive area under the curve measures, a recording of the correct number of responses, and critical flicker fusion measures. All measures were repeated at 0, 2, 4, and 6 hr and further repeated using three dosage forms of tablet, solution, and capsule. The data were analyzed by means of an analysis of variance and covariance (17). The anxiety level was input as the grouping factor.

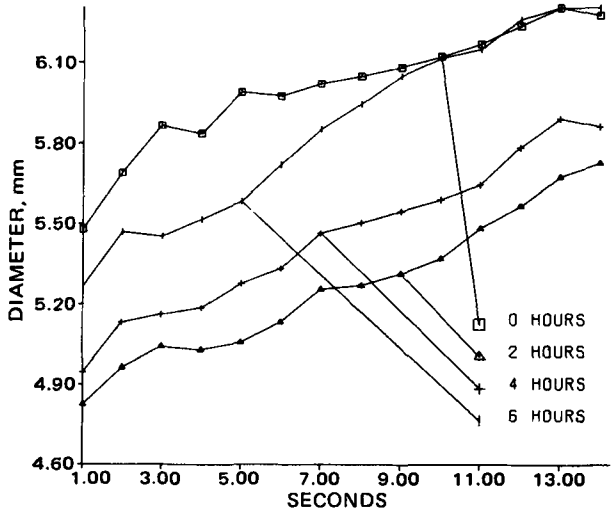

Figure 1—*Average pupil cognition curves for 15 male subjects following consumption of 4 × 10-mg clobazam tablets.*

[1] Control model 12025 and (viewing chamber) model 12024, Lafayette Instrument Co., West Lafayette, Ind.

[2] Polymetric model V11651R, U. S. Testing Co., Hoboken, N.J.

RESULTS

The ability of the random digit task to stimulate pupil dilation was substantiated. Figures 1–3 display the average pupil dilation pattern for the three dosage forms and four time levels. It was immediately apparent that the pupil-constricting effect of clobazam was substantial.

The blood levels were highly significant for the time and dosage form factors, as expected (Table I). The anxiety level did not relate to the blood level, but the dosage form–anxiety interaction proved significant. In a similar pattern, the pupil-constricting effect of the drug proved highly significant for the time and dosage form factors. Table II displays the analysis of variance of the constriction measures, and Table III compares the mean blood levels and pupillary constriction at each time level, summing over the three dosage forms. The pupil was constricted to the greatest degree at the 2-hr point, at which time the greatest average blood level was recorded. The pupil diameter returned to the baseline at the 6-hr point, but the blood level of the parent compound and the metabolites exceeded 350 ng/ml. The factors of critical flicker fusion and number of correct responses proved insignificant for the time factor, which was not anticipated.

An initial analysis of variance of the cognition curves over time and dosage forms proved significant for the anxiety and dosage form factors. However, the variance terms were not homogeneous, and previous research indicated that the ability of subjects to recall randomized digits correctly affects the cognition curves (11, 12). Therefore, the number of digits correctly recalled was input in the analysis as covariates, and the analysis of variance was repeated. In such a manner, the effect of recalling all, or less than all, of the randomized digits was removed from the pupillary cognition curves.

The results of the combined analysis of variance and covariance are presented in Table IV. Only the anxiety factor remained statistically significant in terms of the area under the cognition curve. The covariates removed the statistical significance from the dosage form factor. The

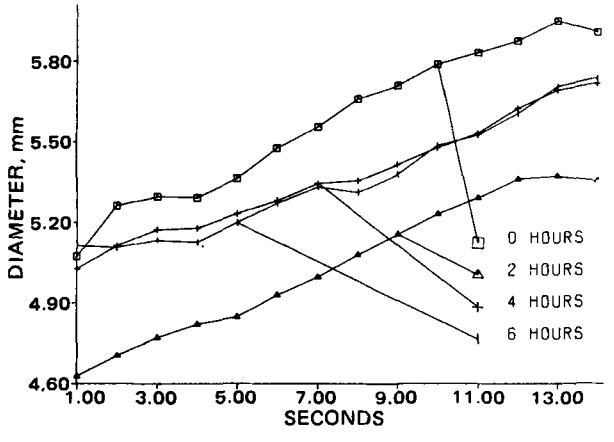

Figure 2—*Average pupil cognition curves for 15 male subjects following consumption of 4 × 10-mg clobazam capsules.*

Table III—Comparison between Blood Levels and Pupil Diameter at Four Time Levels for All Dosage and Anxiety Levels

Hours	Pupil Diameter, mm	Blood Level, ng/ml
0	5.3567	3.2444
2	4.8289	436.4221
4	5.0711	363.9110
6	5.3622	352.3110

mean areas under the cognition curves, corrected for accuracy of recall, were 5.9631, 4.1535, and 7.1379 mm-sec for the low, medium, and high anxiety groups (n = 5), respectively.

CONCLUSIONS

The major conclusion of this study was that the drug and its metabolite reduced the diameter of the pupil when blood levels peaked. However, pupil diameters did not remain constricted at the 6-hr point when blood levels exceeded 350 ng/ml. These results are similar to reports that many psychomotor functions such as coordination tests, tracing tests, sorting tests, and letter cancellation tests returned to baseline values 5 hr after subjects consumed 20 mg of diazepam (18). However, the average blood diazepam level exceeded 300 ng/ml at the 5-hr point. Furthermore, at 105 min, blood diazepam levels exceeded 800 ng/ml and most psychomotor functions were impaired (18). Thus, the benzodiazepines clobazam and diazepam appear to produce a detectable human response at higher blood levels.

Anxiety state proved to be significantly related to the area under the cognition curve after correction was made for recall ability. The low and high anxiety subgroups generated greater areas under the curve than did the middle anxiety group at all times and with all dosage forms. Apparently, anxiety is partly related to the nonresponsive phenomenon, and the nonsensitive subjects were assigned to one of the three groups. The anxiety results manifest the high degree of intersubject pupillary variation in response to the cognitive task.

Some evidence suggests considerable variance among the benzodiazepines in their ability to alter human performance. In fact, clobazam demonstrated little effect on adaptive tracking and reaction times when compared to diazepam and chlordiazepoxide (3). However, the results of this experiment demonstrated a measurable alteration in human pupillary response at higher blood levels of clobazam and its metabolites.

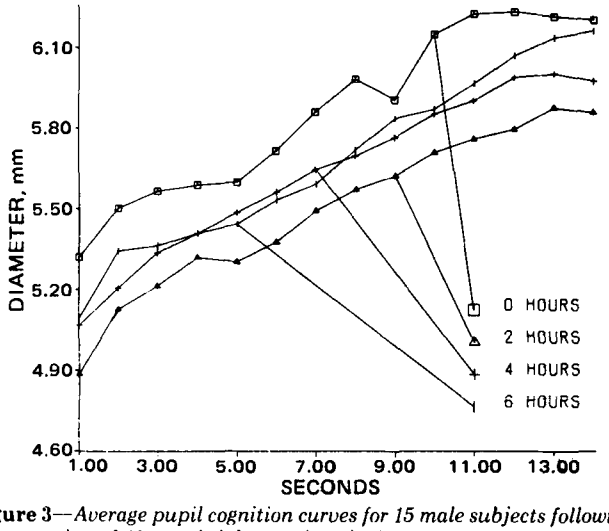

Figure 3—*Average pupil cognition curves for 15 male subjects following consumption of 40 mg of clobazam in solution.*

Table IV—Analysis of Variance and Covariance of Area under Cognition Curve (Millimeters per Second)

Source	Degrees of Freedom	F	P
Anxiety	2	4.9656	0.040
Recall covariate time 0 hr	1	8.2676	0.021
Recall covariate time 2 hr	1	N.S.[a]	
Recall covariate time 4 hr	1	5.5813	0.046
Recall covariate time 6 hr	1	N.S.	
Dosage form	2	N.S.	
Dosage form–anxiety	4	N.S.	
Recall covariate time 0 hr	1	4.0503	0.058
Recall covariate time 2 hr	1	N.S.	
Recall covariate time 4 hr	1	N.S.	
Recall covariate time 6 hr	1	N.S.	
Time	3	N.S.	
Time–anxiety	6	N.S.	
Dosage form–time	6	N.S.	
Dosage form–time–anxiety	12	N.S.	

[a] Not significant.

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