CLOZAPINE: THE CORRELATION BETWEEN CLINICAL IMPROVEMENT AND LABORATORY PARAMETERS.

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1. AIM

The aim of this study was to ascertain whether certain laboratory parameters could be utilized to give an indication of the clinical efficacy of clozapine in the treatment of acute schizophrenia. The following laboratory parameters were investigated:

(i) the serum levels of clozapine alone and clozapine together with its metabolites were determined in order to ascertain whether any correlation exists between these serum levels and clinical improvement.

(ii) the serum levels of prolactin were determined in order to ascertain whether clozapine, like other neuroleptics, causes a rise in prolactin serum levels and also to ascertain whether any correlation exists between changes in prolactin serum levels and clinical improvement.

(iii) the 5-hydroxytryptamine-induced platelet aggregation in order to ascertain whether clozapine therapy enhanced this aggregation and whether this enhanced aggregation correlates with clinical improvement.

(iv) the plasma cholinesterase and red blood cell acetylcholinesterase activity prior to and at the end of,

the treatment period in order to ascertain whether these parameters could be used as diagnostic aids in the diagnosis of schizophrenia and to monitor possible changes induced by clozapine treatment.

Throughout the duration of the study, side effects, pulse rate, and blood pressure were also monitored in order to ascertain whether any correlation exists between these factors and serum levels of clozapine or clozapine and its metabolites and also to ascertain the effect of clozapine on these factors.

2. INTRODUCTION

2.1 The pharmacology of clozapine

2.1.1 Chemical structure

Clozapine is a piperazine derivative of dibenzodiazepine. It is a tricyclic compound with an asymetric 7-member central ring. Its chemical structure is illustrated in figure 1.

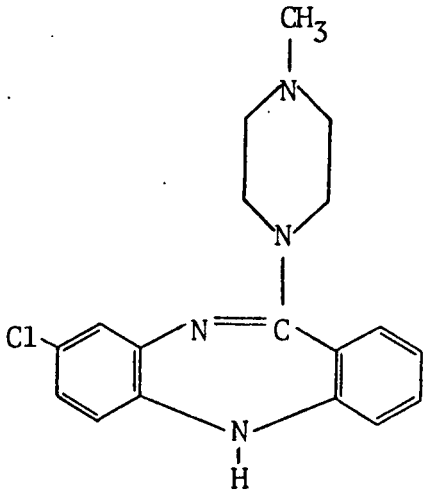


Figure 1: 8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine.

2.1.2 Pharmacodynamics

Clozapine has an antipsychotic action but is practically devoid of extrapyramidal motor side effects[2]. Therefore it cannot be classified as a "classic" neuroleptic drug since the definition of a "classic" neuroleptic drug incorporates both an antipsychotic effect and extrapyramidal motor side effects[1].

Pharmacologic studies in animals have shown that clozapine resembles most other neuroleptics in many respects but its noradrenolytic, anticholinergic, antihistaminic, antianaphylactic, and motility and arousal reaction inhibiting effects are stronger than most neuroleptics[2].

However, clozapine differs from most classic neuroleptics in that it is not cataleptogenic in animals[3,4,5]. It is not an apomorphine antagonist and only weakly antagonizes the effect of amphetamine[5]. Clozapine has a pronounced central anticholinergic action[5], a property not shared by any of the other neuroleptic drugs. This anti-acetylcholine property could explain why clozapine fails to produce catalepsy and to antagonize apomorphine and amphetamine stereotypes in animals[6].

Like all other neuroleptic drugs clozapine increases the turnover of dopamine in the brain as

determined by measuring the concentration of homovanillic acid (HVA), the principal metabolite of dopamine. However, unlike other neuroleptics, at all the doses tested, it caused a greater percentage rise of HVA in the limbic system than in the corpus striatum[6].

Clozapine also produced hypersalivation in rats - an effect which hitherto remains unexplained[5]. This effect has not been observed with other neuroleptics.

2.1.3 Pharmacokinetics

Clozapine is extensively metabolized in man and the metabolites are excreted in the urine principally in the unconjugated form[28].
Clozapine (see Figure 1), its N-desmethyl derivative (Figure 2) and an unknown compound, probably a phenolic derivative of the N-desmethyl compound, were detected in urine in roughly equal amounts whereas the amount of the N-oxide (Figure 3) was about twice as much.

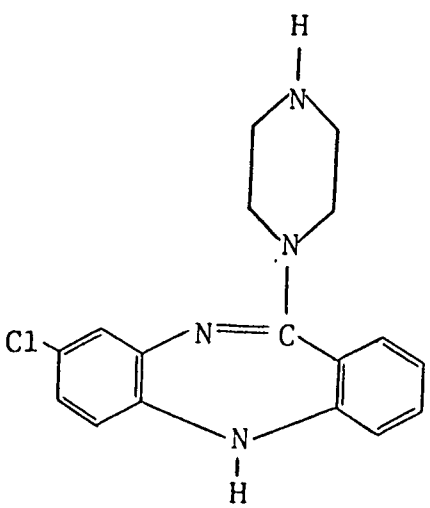


Figure 2: N-desmethyl-clozapine.

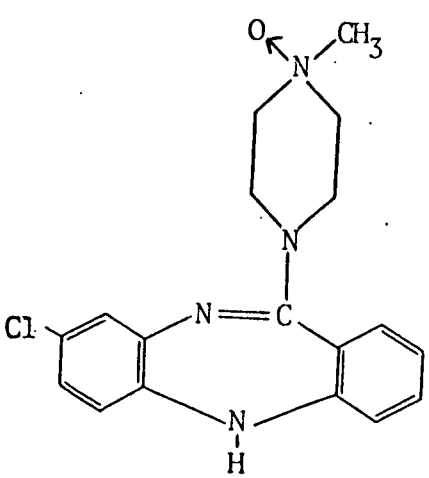


Figure 3: Clozapine N-oxide

The N-oxide is therefore the principal metabolite of clozapine in human urine. Since N-oxidation of tertiary amines and subsequent reduction back to the corresponding base have been established as common metabolic routes and since clozapine and its N-oxide are so readily converted to each other in vivo, Meier[32] concludes that both these compounds should be determined in any pharmacokinetic study.

The elimination half-life varies between 10 and 16 hours[29,30].

2.1.4 Clinical Efficacy

Since 1962 when it was first tested by Gross and Langner in Vienna[8], clozapine has been administered to more than 3 000 acute and chronically ill hospitalized and out patients for a few days to as long as 5 years in uncontrolled and controlled, open and double blind trials[2].

Clozapine has been used successfully in the treatment of a variety of mental illnesses, for example,

(i) the treatment of hypomanic, manic and other acute psychoses of diverse etiologies[9,10,11].

(ii) behavioral disorders in non-psychotic individuals[12] and psychopathic prisoners[13]

and

(iii) endogenous depression[14].

However, by far the most important indication is the treatment of acute and chronic schizophrenia and clozapine has been used extensively for the treatment of this disease throughout the world. Numerous clinical trials have been conducted testifying to the efficacy of the drug in the treatment of this condition[9-12,15-27].

2.1.5 Dosage

For the treatment of schizophrenia, the recommended dosage is 200 - 400 mg daily[31].

2.1.6 Side effects

Sedation, hypersalivation, hypotension, and tachycardia are the principal side effects of clozapine occurring in up to 50% of patients. These appear soon after therapy is initiated and, with the exception of hypersalivation and tachycardia, tend to abate within 10 - 20 days. Hypotension and orthostatic collapse frequently necessitate temporary dosage reduction[2].

Clozapine sometimes causes hyperthermia. The onset is usually between the 10th and 15th treatment day. It is usually mild and lasts for 5 - 7 days and rarely necessitates interruption of treatment[2].

In sharp contrast to classical neuroleptics, clozapine seldomly causes any extrapyramidal reaction[2]. Less frequently reported side effects are weakness and fatigue, dry mouth, headache, nausea, constipation, decreased sexual interest, impotence, hyperhidrosis, pruritis, and vertigo[2].

Leucopenia occurs rarely during treatment with clozapine[2] while agranulocytosis has occurred in isolated instances. The estimated frequency for cases outside Finland in which clozapine is regarded as a possible or probable causative factor is 0,3 per 1 000[33]. In Finland 18 cases of severe blood disorder, 9 of them fatal, were reported in June and July 1975 in conjunction with clozapine treatment, 6 months after the introduction of the drug in February 1975. It is estimated that between 1500 and 2 000 patients were treated with clozapine[34]. The reason for this high incidence of blood disorders is unknown.

2.2 Correlation between serum levels of psychotropic drugs and clinical efficacy

A number of studies with nortriptyline[50,51,52] have shown that a curvelinear relationship exists between plasma levels of the drug and clinical efficacy in the treatment of depression. Plasma levels of nor-

triptyline below 50 ng/ml or above 150 ng/ml result in a poor therapeutic response. These studies comprised a very homogenous group of patients suffering only from moderate to severe endogenous depression, and the findings could not be confirmed by other studies[53,54]. However, the diagnostic criteria, analytical techniques, design, and the drugs used in the latter studies vary greatly from those in the former. It would thus appear that in patients suffering from moderate to severe endogenous depression a therapeutic range for nortriptyline plasma levels of 50 - 150 ng/ml is to be recommended.

Concerning chlorpromazine, a relationship between the plasma concentration and clinical response has been found during the first two weeks of drug treatment[55]. Two more recent clinical investigations[56,57] have provided some evidence of a direct relationship between clinical response and the concentration of 7-hydroxy-chlorpromazine while patients who responded poorly had relatively high plasma levels of chlorpromazine sulphoxide.

Very little research concerning the correlation between plasma levels and clinical efficacy has been done on other neuroleptic drugs. Berling et al could find no relationship between plasma levels and clinical effects of thioridazine and thiothixene[58]. Sensitive radio-immunoassay methods exist for the determination of plasma levels of both pimozide and clozapine but no

investigations have been reported to date showing any correlation between clinical efficacy and plasma levels.

2.3 The effect of neuroleptics on serum prolactin levels

Meltzer et al have demonstrated that serum prolactin levels are within normal limits for unmedicated severely disturbed schizophrenic patients[35]. However, it is well established that phenothiazines and other neuroleptic drugs increase serum prolactin levels in man and laboratory animals[36-43]. Meltzer, Daniels, and Fang[44] have also demonstrated markedly increased serum prolactin levels in rats treated with different dosages of clozapine. To date no studies reporting on the effect of clozapine on serum prolactin levels in humans have appeared in the literature.
Kolakowska and Wiles have also demonstrated a correlation between mean prolactin levels and mean chlorpromazine levels in psychiatric patients[40].

2.4 The effect of chlorpromazine on 5-hydroxy-tryptamine-induced platelet aggregation

Boullin et al have demonstrated an inhibition of 5-hydroxy-tryptamine-induced platelet aggregation by

chlorpromazine and 7 of its major metabolites *in vitro*[45] but an enhancement of the aggregation in psychiatric patients receiving chlorpromazine therapy[46]. Furthermore, they concluded that the enhanced aggregation response to 5-HT seen after chlorpromazine treatment is due to a change in the properties of the platelets rather than a factor in the plasma[47]. It was hoped that further studies would correlate this enhancement of 5-HT-induced platelet aggregation with the therapeutic effect of chlorpromazine in psychoses in order to by-pass all the problems involved in inter- and intra-patient variation in chlorpromazine metabolism[46]. This hope has however not materialized to date.

2.5 Cholinesterase and acetylcholinesterase activity in schizophrenics

Domino et al have demonstrated that cholinesterase and acetylcholinesterase activity in acute schizophrenics fall within the normal range while the cholinesterase activity in chronic schizophrenics was significantly reduced[48,49]. The reason for their study is related to their working hypothesis that an abnormal blood fraction can be etiologic or diagnostic of schizophrenia[48].

3. MATERIALS AND METHODS

3.1 Patient selection

Fifteen hospitalized Black patients of both sexes suffering from acute schizophrenia and meeting the following requirements, were included in the study:

1. Age: between 18 and 60 years;
2. Symptom groups: (i) disturbances of affect
   (ii) thought disorders
   (iii) disturbances of behaviour.

All patients had not been treated with any psychotropic drugs during the 10 days preceeding the study.

Patients suffering from severe somatic or neurologic disease, cardiovascular diseases, brain trauma, or a chronic brain syndrome were excluded from the study. Appropriate haematologic and biochemical tests were performed including the following:

(i) a full blood count,
(ii) urea, uric acid, and creatinine,
(iii) Al-transaminase, As-transaminase, bilirubin, alkaline phosphatase, total protein, and albumin.

In the event of severe disturbances of one or more systems, the patient was excluded from the study.

Before the start of the study, an explanation was given to each patient or his guardian concerning the purpose, nature, and risk of the study. Written consent was

obtained from each patient and also from the legal guardian (the Medical Superintendent of the mental hospital concerned).

3.2 Medication and dosage

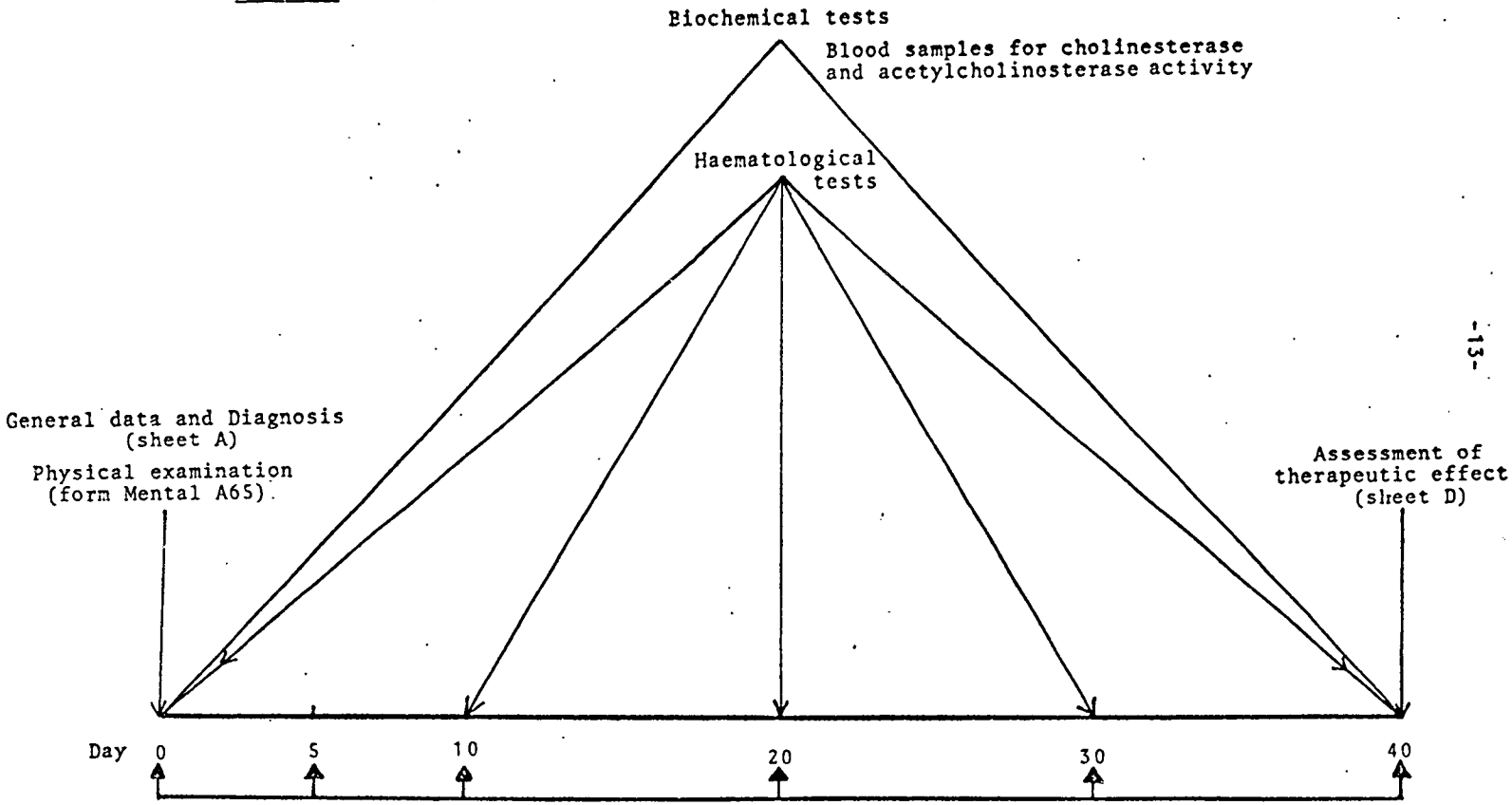
Tablets containing 25 mg or 100 mg of clozapine and manufactured by Sandoz (Pty.) Ltd. under the trade name of Leponex were used. The tablets were administered orally three times daily at 07h00, 12h00 and 16h00.

During the first 6 days of treatment the dosage was gradually increased as indicated in table 2 until a dosage of approximately 4 mg/kg/day was reached. Alterations in dosage were only made if the clinical effect on 4 mg/kg/day was inadequate or if intolerable side effects occurred.

Day	Dosage per administration (mg) 07h00	12h00	16h00	Total (mg/day)
1	-	-	50	50
2	50	-	50	100
3	50	50	50	150
4	50	50	100	200
5	100	50	100	250
6	100	100	100	300

Table 2: Dosage schedule.

Figure 4: Study design.



B.P.R.S. (sheet B_2), assessment of symptoms (sheet B_1), somatic symptoms (sheet C), side effects, blood pressure, and pulse rate (sheet C).

Blood samples for determination of clozapine and metabolites, prolactin, 5-HT-induced platelet aggregation.

3.3 Study design

This was an open study of clozapine in tablet form administered to hospitalized acute schizophrenic patients.

Patients were evaluated over a period of 42 days. There were 3 periods in the course of the study:

(i) A pre-treatment period of at least two days during which basal assessments were performed.

(ii) A build-up period (1 week).

(iii) A maintenance period (5 weeks).

The full design is illustrated graphically in figure 4.

Patients were assessed at least two days prior to treatment, and on days 5, 10, 20, 30, and 40 after introducing clozapine. To enhance the sensitivity and reliability of the assessments, an attempt was made to ensure that the same psychiatrist assessed the same patient at each assessment.

3.3.1 Data concerning each patient, the diagnosis, the progress of the disease, and the case history was entered on sheet A prior to the commencement of the administration of clozapine. A standard physical examination was also performed and recorded on the Mental A 65 form.

3.3.2 The therapeutic efficacy was determined as follows:

SANDOZ BASLE

Neuroleptic Drug:

Study No.

PATIENT No. ☐

Initials .....

A

NL

Lochen 1

Case history No .....

(1) ☐ (1) * ♂ (1) ☐ * ♀ (2)

Date of birth (2) ☐ year

Investigator: Country:

Clinic: Place:

Concomitant illness, if any: ..... WHO No. (3) ☐

Outpatient (4) ☐ * Inpatient (4) ☐ *

Body-wt. (kg) (5) ☐

Height (cm) (6) ☐

INDICATION FOR TRIAL DRUG *

schizophrenia (U.K. classification)

(7) ☐ simple

(8) ☐ hebephrenic

(9) ☐ paranoid-hallucinat.

(10) ☐ catatonic

(11) ☐ others, which .....

(12) ☐ mania

(13) ☐ alcoholism (14) ☐ agitation secondary to other illnesses, which .....

(15) WHO - Classifikation ☐

Characterisation of illness *

(16) ☐ acute (1st breakdown) (22) ☐ insidious onset

(17) ☐ relapse after symptom-free (or almost) interval

(18) ☐ exacerbation in chronic course

(19) ☐ chronic-productive

(20) ☐ chronic residual

(21) ☐ others, which .....

Severity of illness *
(before beginning trial)

severe (23) 3 ☐ ++

moderate (23) 2 ☐ +

mild (23) 1 ☐ (+)

resistent to therapy till now (24) ☐ *

Course of illness

1st onset of illness (25) ☐ years ago

No. of previous episodes (26) ☐ no.

Average duration of epis. (27) ☐ months ø

min. (28) ☐ months

max. (29) ☐ months

Onset of present episode (30) ☐ days before treatment with trial drug

Duration of present hospitalisation (31) ☐

Pretreatment of present episode

Preparation:

Interruption of treatment (32) ☐ days ago

Date (33) day ☐ month ☐ year ☐

Comments:

Stamp, signature: Date:

* Cross when applicable

July, 15 th 1971 Dr.KF/DBo
transl. Dr. v. Or.

II. Edition K. Fischer-Cornelssen

DIAGNOSE
DIAGNOSIS

NAAM
NAME

Geregistreerde
Registered No.

Geslag
Sex

Opgeneem
Admitted

Distrik
District

Geboortedatum
Date of birth

Artikel van Wet
Section of Act

Epilepties
Epileptic

Geboorteplek
Birthplace

Datum van order
Date of order

Neigings tot selfmoord
Suicidal

In Republiek van S.A.
In Republic of S.A.

Vorige aanval
Previous attack

Gevaarlik
Dangerous

Ras of stam
Race or tribe

Ouderdom by eerste aanval
Age first attack

Huwelikstaat
Marital state

Tevore behandel
Previously treated

Oorsake
Causes

Kinders
Children

Duur van huidige aanval
Duration present attack

Woonplek
Domicile

Beroep
Occupation

Geloof
Religion

Adres van naasbestaande
Address of nearest relative

LIGGAAMLIK
PHYSICAL

Voedsel
Nourishment

Gewig
Weight

Lengte
Height

Temperatuur
Temperature

Tuberkulose reaksie
Tubercular reaction—Von Pirquet, etc.

Wanstaltigheid
Deformities

Littekens, kneusels
Scars, injuries

Vel
Skin

Kliere
Glands

Skedel
Genium

Gesig
Face

Ore
Ears

Verhemelte
Palate

Hart- bloedvatstelsel
Cardiovascular system

Asemhalingstelsel
Respiratory system

Spysverteringstelsel
Alimentary system

Tande
Teeth

Keel
Throat

Tong
Tongue

Urogenitaalstelsel
Urogenital system

Urien
Urine – S.G.

Reaksie
Reaction

Eiwit
Albumen

Suiker
Sugar

Besinksels
Deposits

Penis

Testes

Maandstonde
Menstruation

Senuweestelsel
Nervous system

Gesig
Sight

Nistagmus
Nystagmus

Gehoor
Hearing

Stabismus

Ptose
Ptosis

Bewegings
Movements

Oogpupille
Pupils

Omtrek
Outline

Grootte
Size

Reaksie:
Reaction:

Lig
Light

Skaduwee
Shade

Konsensueel
Consensual

Aanpassing
Accommodation

Horingvliesreflekse
Corneal reflex

Keelreflekse
Pharyngeal reflex

Fundi, oculi

Ander harsingsenuwees
Other cranial nerves

Spraak
Speech

Skrif
Writing

Beweeglikheid
Mobility

Kramps
Spasms

Atrofie
Atrophy

Bewings
Tremors

Reflekse: driekoppier, supinator, knie, enkel, voetsool, hangspier, buikspier
Reflexes: triceps, supinator, patellar, achilles, plantar, cremaster, abdominal

Gang
Gait

Houding
Station

Ataksie
Ataxia

Gevoeligheid, aanraking, plekbepaling, pyn, parestesie, temperatuur, spiergevoeligheid
Sensibility, touch, localization, pain, paraesthesia, thermal, muscular vibration

Harsingvog en ruggraatvog
C.S. fluid

Bloed
Blood

SANDOZ BASLE

SANDOZ BPRS

Neuroleptic Drug:

Study No.

Rater:

PATIENT No.

Initials .....

B2 NL

Examination No.

Day of treatment (41)

Date (40) Day . Month . Year 19 . .

Dosage at the day of examination, mg

	1st dose	2nd dose	3rd dose	4th dose	Daily total
	(42)	(43)	(44)	(45)	(46)

1. Somatic concern: Degree of concern over present bodily health. Rate the degree to which physical health is perceived as a problem by the patient, whether complaints have a realistic basis or not. (47)

2. Anxiety: Worry, fear, or over-concern for present or future. Rate solely on the basis of verbal report of patient's own subjective experience. Do not infer anxiety from physical signs or from neurotic defense mechanisms. (48)

3. Emotional withdrawal: Deficiency in relating to the interviewer and to the interviewer situation. Rate only the degree to which the patient gives the impression of failing to be in emotional contact with other people in the interview situation. (49)

4. Conceptual disorganization: Degree to which the thought processes are confused, disconnected or disorganized. Rate on the basis of integration of the verbal products of the patient; do not rate on the basis of patient's subjective impression of his own level of functioning. (50)

5. Guilt feelings: Over-concern or remorse for past behavior. Rate on the basis of the patient's subjective experiences of guilt as evidenced by verbal report with appropriate affect; do not infer guilt feelings from depression, anxiety or neurotic defenses. (51)

6. Tension: Physical and motor manifestations of tension "nervousness", and heightened activation level. Tension should be rated solely on the basis of physical signs and motor behavior and not on the basis of subjective experiences of tension reported by the patient. (52)

7. Mannerisms and posturing: Unusual and unnatural motor behavior, the type of motor behavior which causes certain mental patients to stand out in a crowd of normal people. Rate only abnormality of movements; do not rate simple heightened motor activity here. (53)

8. Grandiosity: Exaggerated self-opinion, conviction of unusual ability or powers. Rate only on the basis of patients's statements about himself or self-in-relation-to-others, not on the basis of his demeanor in the interview situation. (54)

SCORE

1 not present
2 very mild
3 mild
4 moderate
5 moderately severe
6 severe
7 extremely severe

Stamp, signature:

SANDOZ BASLE

SANDOZ BPRS

Neuroleptic Drug:

Study No.

Rater:

PATIENT No.

Initials .....

B2 NL

Examination No.

Day of treatment (41)

Date (40) Day . Month . Year 19 . .

9. Depressive mood: Despondency in mood, sadness. Rate only degree of despondency; do not rate on the basis of inferences concerning depression based upon general retardation and somatic complaints. (55)

10. Hostility: Animosity, contempt, belligerence, disdain for other people outside the interview situation. Rate solely on the basis of verbal report of feelings and actions of the patient toward others; do not infer hostility from neurotic defenses, anxiety nor somatic complaints. (Rate attitude toward interviewer under "uncooperativeness".) (56)

11. Suspiciousness: Belief (delusional or otherwise) that others have now, or have had in the past, malicious or discriminatory intent toward the patient. On the basis of verbal report, rate only those suspicions which are currently held whether they concern past or present circumstances. (57)

12. Hallucinatory behavior: Perceptions without normal external stimulus correspondence. Rate only those experiences which are reported to have occurred within the last week and which are described as distinctly different from the thought and imagery processes of normal people. (58)

13. Motor retardation: Reduction in energy level evidenced in slowed movements. Rate on the basis of observed behavior of the patient only; do not rate on the basis of patient's subjective impression of own energy level. (59)

14. Uncooperativeness: Evidence of resistance, unfriendliness, resentment and lack of readiness to cooperate with the interviewer. Rate only on the basis of the patient's attitude and responses to the interviewer and the interview situation; do not rate on basis of reported resentment or uncooperativeness outside the interview situation. (60)

15. Unusual thought content: Unusual, odd, strange, or bizarre thought content. Rate here the degree of unusualness, not the degree of disorganization of thought processes. (61)

16. Blunted affect: Reduced emotional tone, apparent lack of normal feeling or involvement. (62)

17. Excitement: Heightened emotional tone, agitation, increased reactivity. (63)

18. Disorientation: Confusion or lack of proper association for person, place or time. (64)

SCORE

1 not present
2 very mild
3 mild
4 moderate
5 moderately severe
6 severe
7 extremely severe

Stamp, signature:

3.3.2.1 The Brief Psychiatric Rating Scale (B.P.R.S.) (sheet B_2). The B.P.R.S. has proved to be a valid indication of the clinical improvement in patients suffering from schizophrenia[62]. Furthermore, the B.P.R.S. has been validated as an indicator of clinical improvement in Black patients suffering from schizophrenia[63,64]. Each of the 18 items in the B.P.R.S. were standardized by posing specific questions carefully chosen to adapt to the cultural background of Black patients and translated into South Sotho. The scale is scored as indicated on sheet B_2. The maximum score is 126 and the minimum score 18.

SANDOZ BASLE

Neuroleptic Drug

Study No.

Rater:

PATIENT Nr.

Initials .....

B1 NL

Examination No.

-18-

Day of treatment (41)

Date (40) Day . Month . Year 19 . .

Dosage at the day of examination, mg

	1st dose	2nd dose	3rd dose	4th dose	Daily total
	(42)	(43)	(44)	(45)	(46)

SYMPTOMS

MOOD	Score
tension, irritation (200)	
anxious mood (201)	
depressed mood (202)	
dysphoria (203)	
indifference (204)	
euphoria (205)	

AFFECT	
blunted or blocked (206)	
inadequate (207)	
incontinent (208)	
labile (209)	
ambivalent (210)	

PSYCHOMOTOR BEHAVIOUR	
retardation, inhibition (211)	
motor stiffness (212)	
mannerism (213)	
stereotyped (214)	
agitation, excess. uncoordin. motor activity (215)	

ORIENTATION, CONSCIOUSNESS	
disorientation (216)	
confusion (217)	
delirium (218)	

* cross where applicable

SLEEP	Score
disturbed onset of sleep (219)	
interrupted sleep (220)	
early morning wakening (221)	

THOUGHT PROCESSES	
retardation (222)	
blocking (223)	
disconnection (224)	
flight of ideas (225)	
paralogia (226)	

THOUGHT CONTENTS	
lack of insight into illness (227)	
increased self-estimation (228)	
delusions of grandeur (229)	
phobias (230)	
obsessions (231)	
delusional ideas and experiences (232)	

OTHER SYMPTOMS

yes (246) [1] * no (246) [2] *

if yes, which:

..... (247)

..... (248)

..... (249)

..... (250)

SENSORY DISTURBANCES	Score
hallucinations (233)	

PERSONALITY	
depersonalisation (234)	
autism (235)	

SOCIAL BEHAVIOUR	
mutism (236)	
lack of personal contact (237)	
negativism (238)	
lack of inhibition (239)	
aggressive tendency (240)	
aggressive action (241)	
autoaggressivity (242)	
social maladaptation (243)	
reduced working capacity (244)	

APPETITE	
lack of appetite (245)	

SCORE

[3] severe
[2] moderate
[1] mild
[0] absent
[?] not evaluable

Stamp, signature:

SYMPTOM-SCORE CHECK-LIST

1.7.71 Dr.KF/DBo

3.3.2.2 The Symptom Score Check List - a rating scale designed by Dr. K. Fischer - Cornelssen (F.C. Rating Scale (sheet B_1). This check list assesses 46 important psychopathological symptoms and is scored as indicated on sheet B_1. The maximum score is 138 and the minimum score 0.

EVALUATION OF THE THERAPEUTIC EFFECT

to D

Symbol SCORE	(119) Effect of drug (irrespective of tolerance)	(120) Tolerance	(121) Fitness for discharge	(122) Working capacity (eventual)	(123) Global evaluation Overall therap. usefullness to patient
very good 3 ++	Practically symptomfree, "total remission", unimportant residual symptoms	Practically no side-effects	Patient discharged to care of family doctor	Patient resumes full activity in old or new employment	very good
good 2 +	Main symptoms substantially improved. No important impairment by illness. Social remission	Mild to moderate side-effects causing little inconvenience.	Patient can be discharged providing regular medical care is ensured.	Working capacity somewhat reduced.	medium to good
fair 1 (+)	Main symptoms partially or not substantially influenced. Patient still disturbed.	Side-effects tolerable but disturbing	Patient cannot be discharged or only under constant close psychiatric and social care	Working capacity very limited, but occupation possible	moderate
failure Ø ø	No or questionable improvement; detericration.	Side-effects strong. One or cluster of side-effects necessitates interruption of therapy.	No discharge possible	No working capacity, occupation restricted	little or none

In chronic residual schizophrenics and long-standing inpatient this evaluation has to be modified.

EVALUATION OF THE THERAPEUTIC EFFECT (score, see overleaf A)

Drug effect	Tolerance	Fitness for discharge	Working capacity	Overall therap. usefulness
(119)	(120)	(121)	(122)	(123)

19.7.1971
Dr. KF/DBo

3.3.2.3 The evaluation of the therapeutic effect (sheet D). The investigator's subjective overall impression as to the therapeutic benefit includes, and is based on, four separate factors and is scored as indicated on sheet D.

SANDOZ BASLE

Neuroleptic Drug:

Study No.

PATIENT No. [ ]

Initials .....

C NL

Examination No. [ ]

Day of treatment (41) [ ]

-22-

Date (40) Day [.] Month [.] Year [19 . .]

SOMATIC SYMPTOMS* or SIDE-EFFECTS*

SCORE

[0] absent [1] mild [2] moderate [3]* severe / therapy interruption

CENTRAL NERVOUS SYSTEM

drowsiness/sleepiness	(70)	
disturbed sleep	(71)	
restlessness	(73)	
agitation	(74)	
confusion, delirium	(75)	

G. I. DISTURBANCES

nausea, vomiting	(76)	
constipation	(77)	
diarrhoea	(78)	

VEGETATIVE SYMPTOMS

inhibition of micturition	(79)	
dryness of the mouth	(80)	
hypersalivation	(81)	
sweating	(82)	
disturbances of visual accommodation	(83)	

CIRCULATORY SYMPTOMS

dizziness	(84)	
collapse (score 2:syncope)	(85)	

HEADACHE

headache tension - "-	(86)	

NEUROLOGICAL DISTURBANCES

hypokinesia	(87)	
hyperkinesia	(88)	
dyskinesia	(89)	
rigor	(90)	
tremor	(91)	
akathisia tasikinesia	(92)	

OTHERS

yes (93) [1] *

no (93) [2] *

if yes, which

.....(94) [ ]

.....(95) [ ]

.....(96) [ ]

BLOOD PRESSURE mmHg

lying systolic	(100)	
lying diastolic	(101)	
standing systolic	(102)	
standing diastolic	(103)	

PULSE RATE / min.

lying	(104)	
standing	(105)	

COMMENTS

yes (97) [1] *

no (97) [2] *

if yes, use separate sheet!

LABORATORY TESTS

normal (98) [1] *

pathological (98) [2] *

NO SIDE EFFECTS

(99) [1] *

ADDITIONAL DRUGS

yes (106) [1] * no (106) [2] *

Preparation	Daily dose	treatment day No. from	to	Date from	to

Stamp, signature:

* cross where applicable

3.3.3 Somatic symptoms of the psychosis were assessed according to sheet C. Unspecified somatic symptoms were entered under "other". Somatic symptoms were scored as indicated on sheet C.

3.3.4 Subjective side effects were also entered on sheet C and marked accordingly with a ■ on days 5, 10, 20, 30, and 40. No direct or suggestive questions were posed- only spontaneous complaints were registered. The side effects were scored according to the scale on sheet C. If no side effects were present the appropriate box on sheet C was marked with an X.

3.3.5 Blood pressure and pulse rate - at least 10 minutes after lying down, and 3 minutes after standing - was recorded on sheet C prior to the administration of clozapine and on days 5, 10, 20, 30, and 40.

3.3.6 Biochemical tests including urea, uric acid, creatinine, total protein and albumin, bilirubin, Al-transaminase and As-transaminase, and alkaline phosphatase were performed prior to the administration of clozapine and on day 40.

3.3.7 Haematological tests including a total and differential count were performed prior to the administration of clozapine and on days 10, 20, 30, and 40.

SANDOZ BASLE	Drug:	Study No.	PATIENT No. / Initials: .....

F

-24-

TEMPERATURE AND DAILY DOSAGE

180) treatment day	0	1	2	3	4	5
181) date	day month year					
182) temperature	(1)	(2)	(3)	(4)	(5)	(6)
183) daily dose mg	(1)	(2)	(3)	(4)	(5)	(6)
180) treatment day	6	7	8	9	10	11
181) date						
182) temperature	(7)	(8)	(9)	(10)	(11)	(12)
183) daily dose mg	(7)	(8)	(9)	(10)	(11)	(12)
180) treatment day	12	13	14	15	16	17
181) date						
182) temperature	(13)	(14)	(15)	(16)	(17)	(18)
183) daily dose mg	(13)	(14)	(15)	(16)	(17)	(18)
180) treatment day	18	19	20	21	22	23
181) date						
182) temperature	(19)	(20)	(21)	(22)	(23)	(24)
183) daily dose mg	(19)	(20)	(21)	(22)	(23)	(24)
180) treatment day	24	25	26	27	28	29
181) date						
182) temperature	(25)	(26)	(27)	(28)	(29)	(30)
183) daily dose mg	(25)	(26)	(27)	(28)	(29)	(30)
180) treatment day	30	31	32	33	34	35
181) date						
182) temperature	(31)	(32)	(33)	(34)	(35)	(36)
183) daily dose mg	(31)	(32)	(33)	(34)	(35)	(36)
180) treatment day	36	37	38	39	40	41
181) date						
182) temperature	(37)	(38)	(39)	(40)	(41)	(42)
183) daily dose mg	(37)	(38)	(39)	(40)	(41)	(42)

Comments:

Stamp, signature:

3.3.8 Environmental and body temperature (recorded under the arm) was recorded daily and registered on sheet F with the dialy dose.

3.3.9 Blood samples were taken prior to the administration of clozapine for the determination of the following:

3.3.9.1 prolactin serum levels

3.3.9.2 5-hydroxytryptamine-induced platelet aggregation

3.3.9.3 plasma cholinesterase and red blood cell acetylcholinesterase activity.

Further blood samples were taken on days 5, 10, 20, 30, and 40 for the determination of the serum levels of clozapine and its metabolites and also for the determination of prolactin serum levels and the effect of the treatment with clozapine on 5-HT-induced platelet aggregation. On day 40 cholinesterase and acetylcholinesterase activity was also determined.

All blood samples were taken 3 to 4 hours after the 07h00 dosage of clozapine.

3.4 Determination of serum levels of clozapine and its metabolites.

Blood samples obtained were centrifuged on the same day and the serum divided into four equal portions and frozen. Determinations of the serum levels of clozapine and its metabolites for all the patients were carried out simultaneously after all fifteen

patients had completed the study.

Serum levels of clozapine and of clozapine plus its metabolites were determined by means of a radio-immunoassay developed by Sandoz (Pty.) Ltd. utilizing a specific antibody for the determination of clozapine only and a non-specific antibody for clozapine plus its metabolites.

The following protocol for all determinations was followed:

(i) Incubation steps, 20 minutes at $4^{o}C$,

(ii) Separation of free from bound clozapine by adsorption on charcoal,

(iii) Centrifugation and sample liquid scintillation counting of supernatant solution.

Reagents provided and preparation:

1. ^{3}H-clozapine to which 9,0 ml saline buffer was added at time of use.
2. Clozapine-standard stock solution. The bottle contains 4,8 µg clozapine in 1,0 ml ethanol. From this solution, 0,50 ml is withdrawn and diluted with 5,0 ml saline buffer.
3. Antiserum - specific for the determination of clozapine only
   - non-specific for the determination of clozapine plus its metabolites.

   This bottle contains an amount of lyophilized

antiserum sufficient for 100 tubes. The dry antiserum was dissolved in 10,0 ml distilled water at time of use.

4. Saline buffer which was adjusted to a final volume of 200 ml with distilled water.
5. Charcoal which was suspended in 50 ml saline buffer and agitated continually on a magnetic stirrer.

Appropriate dilutions for preparing clozapine-standards were prepared as follows: the clozapine-standard stock solution contains 48 ng per 0,1 ml. To generate a standard curve, 0,50 ml is diluted with 0,50 ml saline buffer (≙24 ng/0,1 ml); to 0,50 ml of this solution 0,50 ml saline buffer is again added (≙12 ng/0,1 ml), and so on to a final dilution of 1,5 ng/0,1 ml.

Radioimmunoassay system:

Reagents and respective samples were added to the tubes (11 X 70 mm) in the following order:

1.

Tube No.	Saline buffer (ml)	Sample Identification
1 - 2	1,4	total radioactivity
3 - 4	0,9	charcoal binding
5 - 6	0,8	zero binding control
7 - 18	0,7	standard dilutions
19 - 100	0,8	unknown samples

2. 1 - 18 0,1 ml clozapine-free serum was added.
   19 - 20 0,1 ml known concentration of clozapine was added which served as a control (≙40 ng/ 0,1 ml).
   21 - 100 0,1 ml replicates of unknown samples were added.

3. 7 - 18 0,1 ml clozapine-standard dilutions (1,5 - 48 ng) were added.

4. 5 - 100* 0,1 ml antiserum was added. Each tube was agitated on a vortex mixer and on completion of all tubes, an incubation period of 10 minutes at 4°C was allowed.

5. 1 - 100 0,1 ml ^{3}H-clozapine was added. Each tube is agitated and on completion of all tubes, an incubation period of 10 minutes at 4°C was again allowed.

6. 3 - 100 0,5 ml charcoal suspension was added, the tubes mixed well and allowed to stand for 5 minutes at room temperature.

7. 3 - 100 were centrifuged for 10 minutes at approximately 1500 X g.

8. 1 - 100 aliquots of 0,1 ml of supernatent solution were counted for a period of 10 minutes each in a liquid scintillation spectrometer (Packard Tri-Carb Model 3385) by adding 10

ml Aquagel to each vial.

*48 tubes per determination were used.

A punch-tape was then made using the counts obtained per aliquot. The concentration of clozapine or clozapine plus metabolites was then determined using a radioimmunoassay program compiled by members of the Department of Pharmacology, University of the Orange Free State, Bloemfontein, a Hewlett-Packard 2748B tape reader and a Hewlett-Packard Model 10 calculator.

The binding ability of this assay system is between 30 to 50%. Charcoal binding amounts to approximately 5%.

3.5 Determination of serum levels of prolactin

Serum obtained from blood samples taken prior to the administration of medication and on days 5, 10, 20, 30, and 40 was frozen and determined after all fifteen patients had completed the study. Prolactin levels were determined by means of a radio-immunoassay using reagents obtained from CIS. The reference standard for this assay was MRC 71/222. The normal range accepted by our Chemical Pathology laboratory is 100 - 500 International Units for females and 100 - 350 International Units for males.

3.6 5-hydroxytryptamine-induced platelet aggregation

Blood was obtained by clean venopuncture using a plastic syringe prior to the commencement of the administration of medication and on days 5, 10, 20, 30, and 40. The blood was immediately placed in 5 ml plastic tubes (11 X 70 mm) containing 0,5 ml sodium citrate and mixed well. 5-HT-induced platelet aggregation was carried out within 4 hours after the sample had been taken.

Blood was centrifuged at 1000 revs./minute for 10 minutes in order to obtain platelet-rich plasma (P.R.P.) and at 5000 revs./minute to obtain platelet-poor plasma (P.P.P.). Platelets in the P.R.P. sample were then counted using a coulter counter and adjusted to give a final platelet concentration of ~ 300 000/µl. A Payton Dual Channel Aggregation Module and two Vitatron recorders were used for all determinations. For each patient sample 0,45 ml of the P.R.P. with a platelet concentration of ~ 300 000/µl was incubated in the aggregation module at 37°C and the recorder adjusted to register 100% transmission while 0,5 ml of the P.P.P. was used to adjust the recorder to register 0% transmission. The P.R.P. was then stirred at 900 revs./minute at 37°C and 20 µM 5-HT (5-hydroxytryptamine creatinine sulphate - Sigma Chemical Co., St. Louis, Mo, U.S.A.) in 50 µl added. Responses were quantitated as changes in optical density and expressed as a percentage.

3.7 Determination of cholinesterase and acetylcholinesterase activity

Blood was taken prior to the administration of clozapine and on day 40 and placed in a 5 ml glass tube containing sodium heparin. Cholinesterase and acetylcholinesterase activity was determined according to a modification of the method of Ellman et al[59,60]. The normal range accepted by our department of Pharmacology for plasma choliensterase activity is 2000 - 3500 mU/ml serum and for red blood cell acetylcholinesterase activity is 2000 - 3500 mU/µmol Hb.

Throughout this study, wherever possible, results were compared using the Student's Paired t test. The criterion for statistical significance was $2p < 0,05$.

4. RESULTS AND DISCUSSION

4.1 Patient data

Fifteen hospitalized Black patients meeting the criteria discussed in 3.1 were included in the study. Detail concerning the patients' age, sex, type of schizophrenia, the duration of the present illness, whether a first break-down or a relapse, and the severity of the illness is described in table 3.

No.	Initials	Age (years)	Sex		Type of schizophrenia					Duration of present illness (days)			Severity	
			Male	Female	Simple	Hebephrenic	Paranoid-Hallucinatory	Catatonic	Undifferenciated		First Breakdown	Relapse	Severe	Moderate
1.	MJM	38	X				X			12	X		X	
2.	LD	45		X			X			12	X			X
3.	BM	23		X				X		8	X		X	
4.	AP	23		X	X					8	X		X	
5.	SL	28	X					X		6	X		X	
6.	AM	28		X		X				15	X		X	
7.	MM	31	X				X			10	X		X	
8.	JN	27		X			X			14	X		X	
9.	MM	35		X					X	14	X		X	
10.	MM	45		X			X			5		X	X	
11.	SD	23		X		X				4	X		X	
12.	CR	21		X				X		4	X		X	
13.	AM	18		X			X			20	X		X	
14.	AM	22	X				X			6	X		X	
15.	PNM	35	X					X		12		X	X	
Total:		18 - 45 (mean 29)	4	10	1	1	7	4	1	4 - 20 (mean 10)	13	2	14	1

Table 3: Patient data

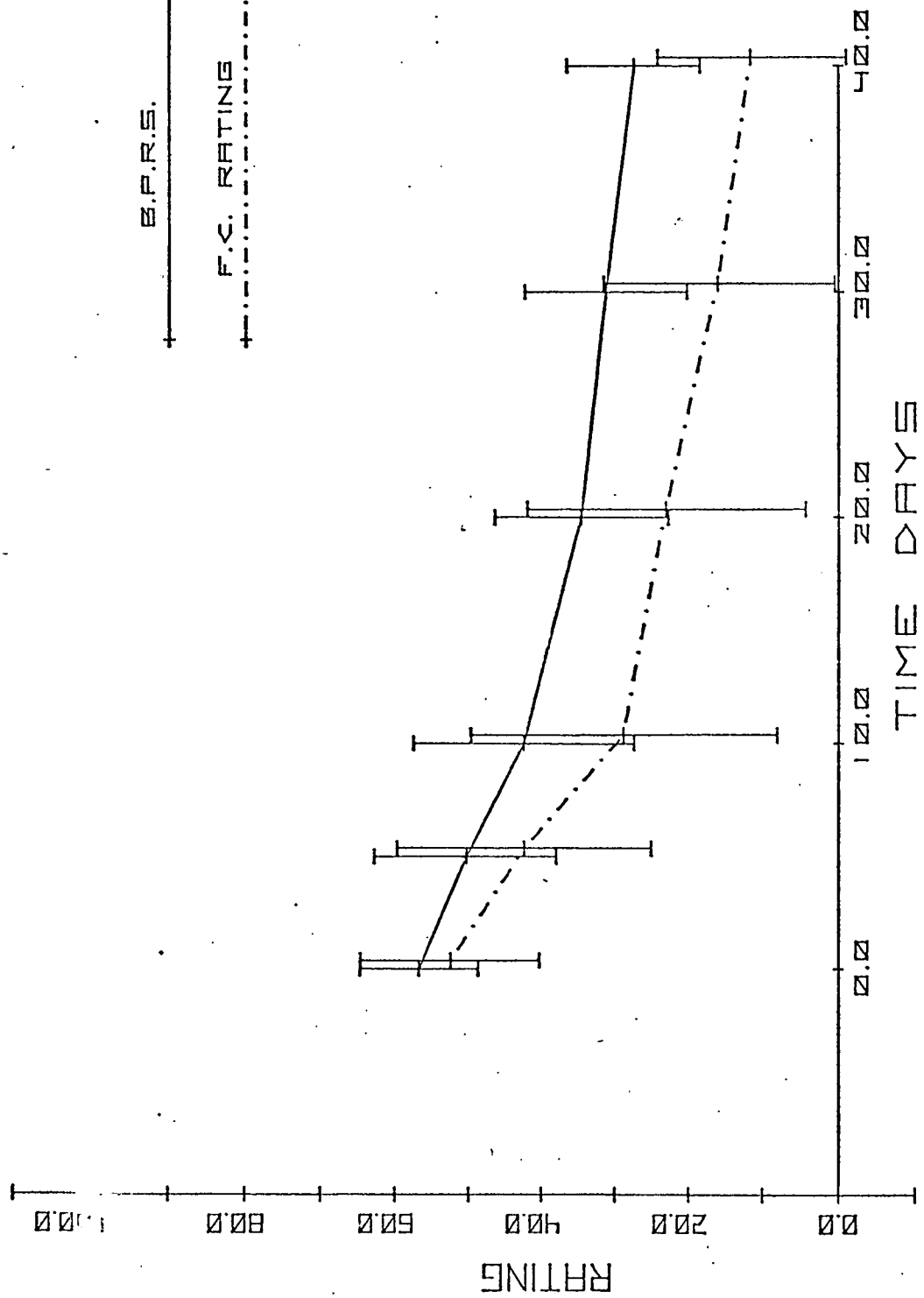


Figure 5: Mean B.P.R.S. and F.C. ratings on days 0, 5, 10, 20, 30, and 40.

Patient No.11 was terminated on day 20 and Patient No.15 on day 30 because of clinical improvement requiring no further psychotropic therapy.

4.2 Therapeutic efficacy

The therapeutic efficacy of clozapine, as determined by the B.P.R.S. and the F.C. rating scales, is illustrated in Figure 5. The mean values ± standard deviation and level of statistical significance for each rating when compared with the previous rating is described in table 4.

	Day 0	2p	Day 5	2p	Day 10	2p
B.P.R.S.	57 ± 8	<0,02*	50 ± 12	<0,005*	42 ± 15	<0,001*
F.C. rating	52 ± 12	<0,005*	42 ± 17	<0,001*	29 ± 21	<0,02*

	Day 20	2p	Day 30	2p	Day 40
B.P.R.S.	36 ± 12	<0,02*	31 ± 11	>0,1N.S.	27 ± 9
F.C. rating	23 ± 19	<0,005*	16 ± 16	>0,1N.S.	12 ± 13

Table 4: Mean values of B.P.R.S. and F.C. ratings ± standard deviation and 2p values as determined by the paired t test comparing ratings on day 0 with day 5, day 5 with day 10, day 10 with day 20, day 20 with day 30, and day 30 with day 40.

*indicates statistical significance at the 95% confidence level.
N.S. indicates not significant at the 95% confidence level.

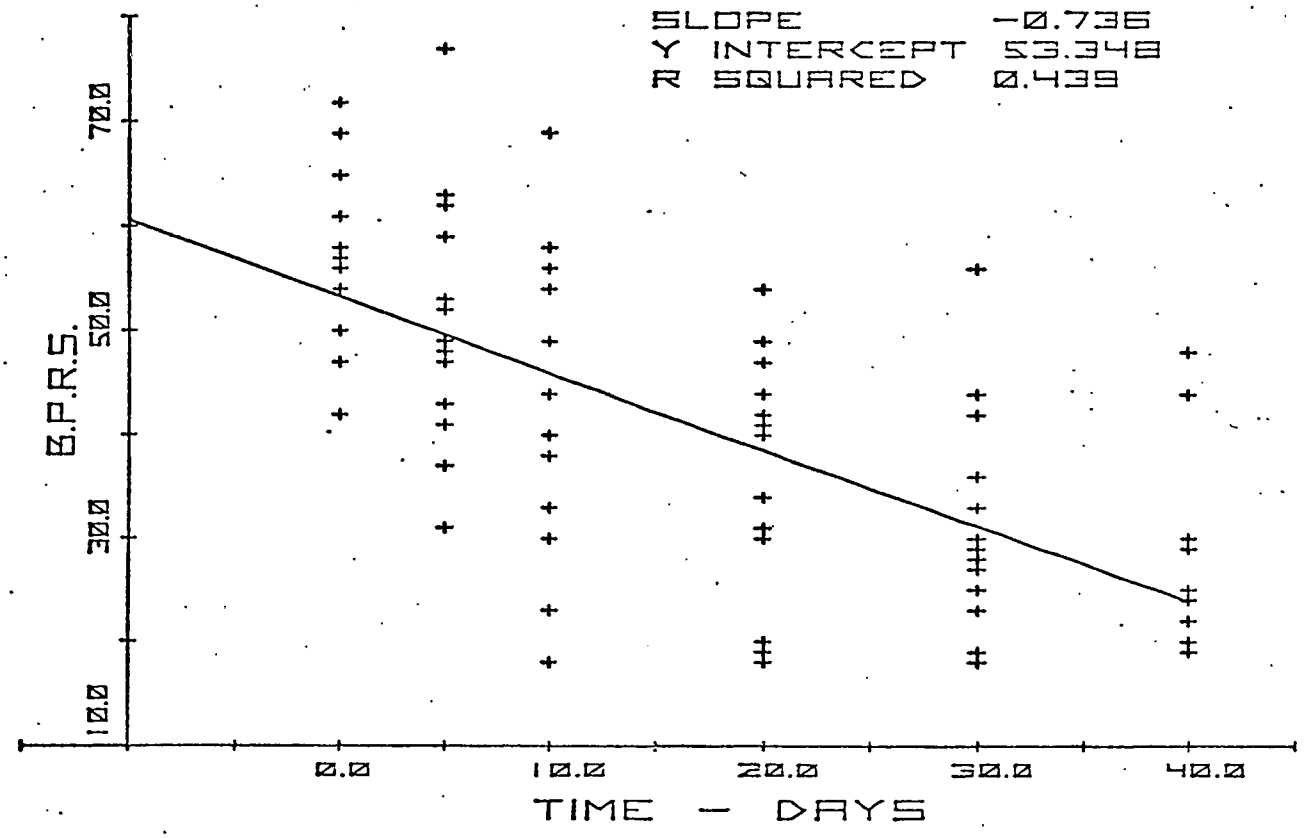


Figure 6: Linear regression curve of B.P.R.S. scores on days 0, 5, 10, 20, 30, and 40. Regression coefficient: r = 0,6625 (p < 0,001).

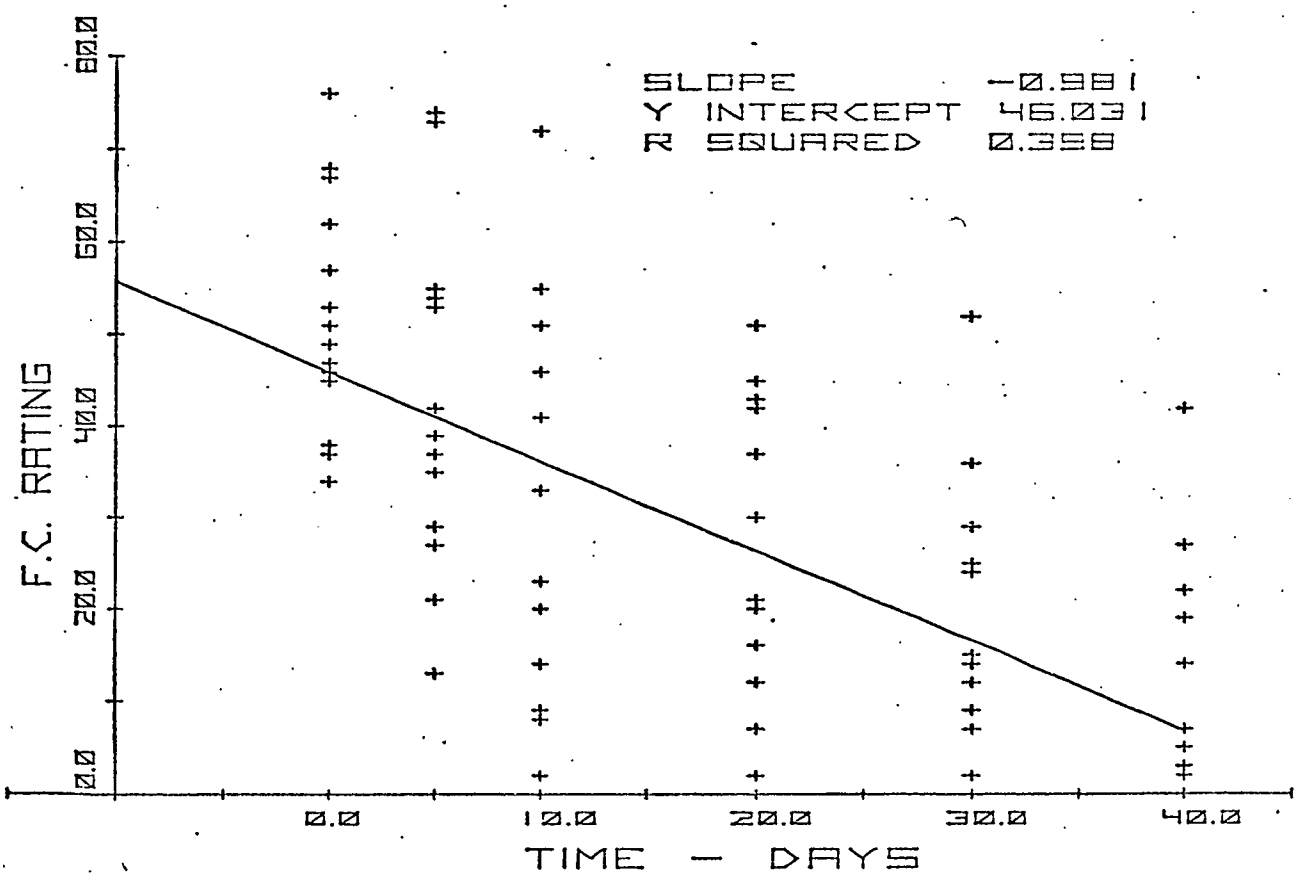


Figure 7: Linear regression curve of F.C.-rating on days 0, 5, 10, 20, 30, and 40. Regression coefficient: r = 0,6308 (p < 0,001).

Figures 6 and 7, illustrating linear regression curves of the B.P.R.S. and F.C. Ratings on the six assessment days, illustrate the fact that clinical improvement correlated with time during the course of the trial. The regression coefficients of both curves are significant at the 99% confidence limit ($p < 0,001$). When the mean scores for both the B.P.R.S. and F.C. rating on day 0 were compared with each of the subsequent mean scores on days 5, 10, 20, 30, and 40 it was found that each mean rating differed significantly from the mean baseline scored ($2p < 0,05$ for all comparisons).

It would thus appear that clozapine caused a significant clinical improvement in these 15 patients (as determined by the mean B.P.R.S. and F.C. rating scores) and that maximal clinical improvement took place upto day 30. Hereafter clinical improvement was only slight and did not reach a level of statistical significance.

An overall evaluation of the therapeutic effect regarding the efficacy of the drug, tolerance, fitness for discharge, working capacity, and global utility indicating the overall therapeutic usefullness to the patient can be seen in table 5.

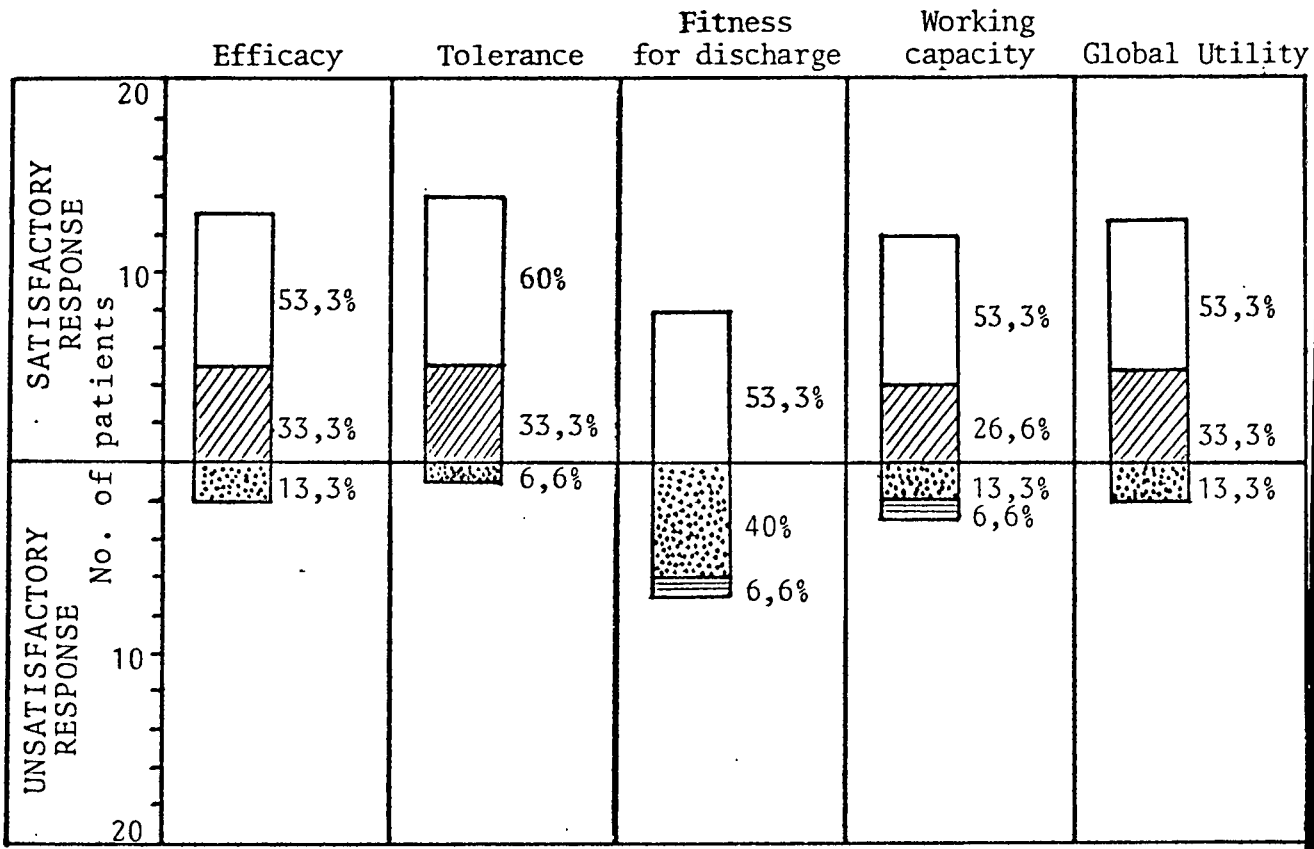


Table 5: Overall evaluation of therapeutic effect.

= 3 = very good

= 2 = good

= 1 = moderate

= 0 = no change

From the results illustrated in table 5, the following deductions can be made:

(i) clozapine caused some improvement in all 15 patients treated. For the majority (86,6%) this improvement was marked while only 2 patients (13,3%) showed only a moderate improvement.

(ii) clozapine was well tolerated by all the patients and no patients had to be removed from the study as a result of intolerability.

(iii) more than half of the patients (53%) were fit for unconditional discharge at the termination of the study. The duration of the study, namely 40 days, was probably too short to effect a radical cure with sufficient improvement for more patients to be discharged unconditionally at that stage. With continued treatment for a longer period a larger percentage of patients would probably qualify for unconditional discharge. One patient was totally unfit for discharge and was regarded as a therapeutic failure, while the rest, namely 40%, could be discharged only under close psychiatric monitoring and social care.

(iv) the working capacity of the majority of the patients (80%) was satisfactory while the working capacity of 2 patients was very limited and one

patient was totally unfit to resume any occupation. This was the same patient mentioned above who was regarded as a therapeutic failure.

4.3 Side effects

The incidence of side effects can be seen in table 6. Patients with an increase in symptom intensity by comparison with pre-treatment scores were included. All other symptoms complained of spontaneously by the patient and not present at the pretreatment evaluation were regarded as side effects.

Side Effect	Day 5			Day 10			Day 20			Day 30			Day 40			Total[1]	Percentage
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
. Drowsiness/sleepiness	5	-	5	2	2	3	8	2	1	5	-	1	1	1	1	12	80
. Disturbed sleep	-	-	-	1	-	-	-	-	-	2	-	1	-	-	-	3	20
. Nausea, vomiting	3	-	1	5	2	1	2	1	-	4	-	1	1	-	-	10	66,6
. Constipation	1	-	-	1	1	-	1	-	1	-	-	-	-	-	-	3	20
. Diarrhoea	-	-	-	1	-	-	1	-	-	1	-	1	-	-	-	4	26,6
. Inhibition of micturition	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1	6,6
. Hypersalivation	2	-	5	3	2	1	2	2	-	1	2	-	3	4	-	11	73,3
. Sweating	-	1	-	-	-	-	1	-	-	-	-	-	1	-	-	3	20
. Disturbance of visual accommodation	2	3	-	2	1	-	-	1	-	-	-	-	-	-	-	6	40
. Dizziness	2	-	3	3	1	4	2	-	3	3	-	1	2	1	-	10	66,6
. Collapse	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	2	13,3
. Headache	2	-	1	5	-	1	1	-	1	1	-	-	2	-	-	8	53,3
. Dyspepsia	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	2	13,3
. Epigastric pain	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	6,6
Total[2]	17	5	17	24	9	10	18	6	6	17	2	6	11	6	1		

Table 6: Side effects.

Total[1]: Total number of patients exhibiting the specific side effect during the course of treatment.

Total[2]: Total number of patients exhibiting mild, moderate or severe side effects on each treatment day.

1 = mild 2 = moderate 3 = severe.

As can be seen from table 6, the most common side effect was daytime sedation which occurred in 12 patients (80%) at some stage during the course of treatment. This effect was most pronounced during the early stages of treatment although it persisted throughout the entire treatment period in a few patients. However, this can be regarded as a positive feature in soothing and sedating the patient in the initial stages of treatment.

Nausea and vomiting occurred in 10 patients (66,6%) during the course of treatment with a maximum incidence on day 10. This side effect caused considerable patient inconvenience and its high incidence in this study cannot readily be accounted for. While investigating 100 Black patients suffering from acute schizophrenia, Wessels[61] observed that on admission, before treatment was instituted, the most common complaints were related to the abdomen. The second most common complaint was headache. In the present study the high incidence of gastro-intestinal related side effects and headache can possibly be related to the fact that these two somatic symptoms may not be solely drug-related. Dizziness was complained of by 10 patients (66,6%) during the course of treatment also with a maximum incidence on day 10 after which it rapidly subsided. It is doubtful whether this side effect can be related to a lowering in blood pressure by clozapine as no significant changes

in blood pressure was found during treatment (see section 4.4).

Hypersalivation occurred in 11 patients(73,3%) during the treatment period. There did not appear to be any peak incidence of this side effect on any specific evaluation day and it occurred with equal frequency throughout the course of the study. Hypersalivation may be a parkinson-like effect of clozapine as anticholinergic antiparkinson drugs have been found to relieve it[2]. However, no satisfactory explanation for this side effect has been offered to date.

Headache, usually only mild and not troublesome, occurred in 8 patients (53,3%) during the course of treatment. The same possibility that was proposed for the high incidence of nausea and vomiting is valid in the case of headache.

Disturbance of visual accommodation occurred in 6 patients (40%) during the early stages of the study probably as a result of the anticholinergic effects of clozapine. This side effect, however, rapidly subsided and was not complained of by any patients on day 30 or 40.

Constipation and diarrhoea occurred in 3 and 4 patients respectively (20%, 26,6%) during the course of treatment. Both were of a mild nature and were not unduly troublesome to the patients. Disturbed sleep and

sweating each occurred in 3 patients (20%) while 2 patients (13,3%) had complaints of dyspepsia. One patient (6,6%) complained of problems with micturition probably due to the anticholinergic effect of clozapine on two occasions and one patient complained of one episode of moderate epigastric pain.

Two patients (13,3%) collapsed during the course of treatment - one on day 5 and the other on day 39. Both incidents were found to be the result of a brief fall in orthostatic blood pressure.

As can be seen from table 6, by studying total^2, the highest incidence of severe side effects occurred on day 5 (total^2 = 17). The incidence of severe side effects however rapidly decreased upto day 40 with the occurrence of only 1 severe side effect. It would thus appear that these patients developed a tolerance to the troublesome pharmacodynamic effects of clozapine during the treatment period while no such tolerance developed to the beneficial pharmacodynamic effects. This can be concluded from the fact that clinical improvement continued throughout the treatment period (see figure 5).

A possible explanation for this phenomenon is that as the mental status of the patient improves and the patient realizes that the medication is beneficial, the troublesome side effects are better tolerated and although possibly still present, do not cause the patient as much discomfort as originally.

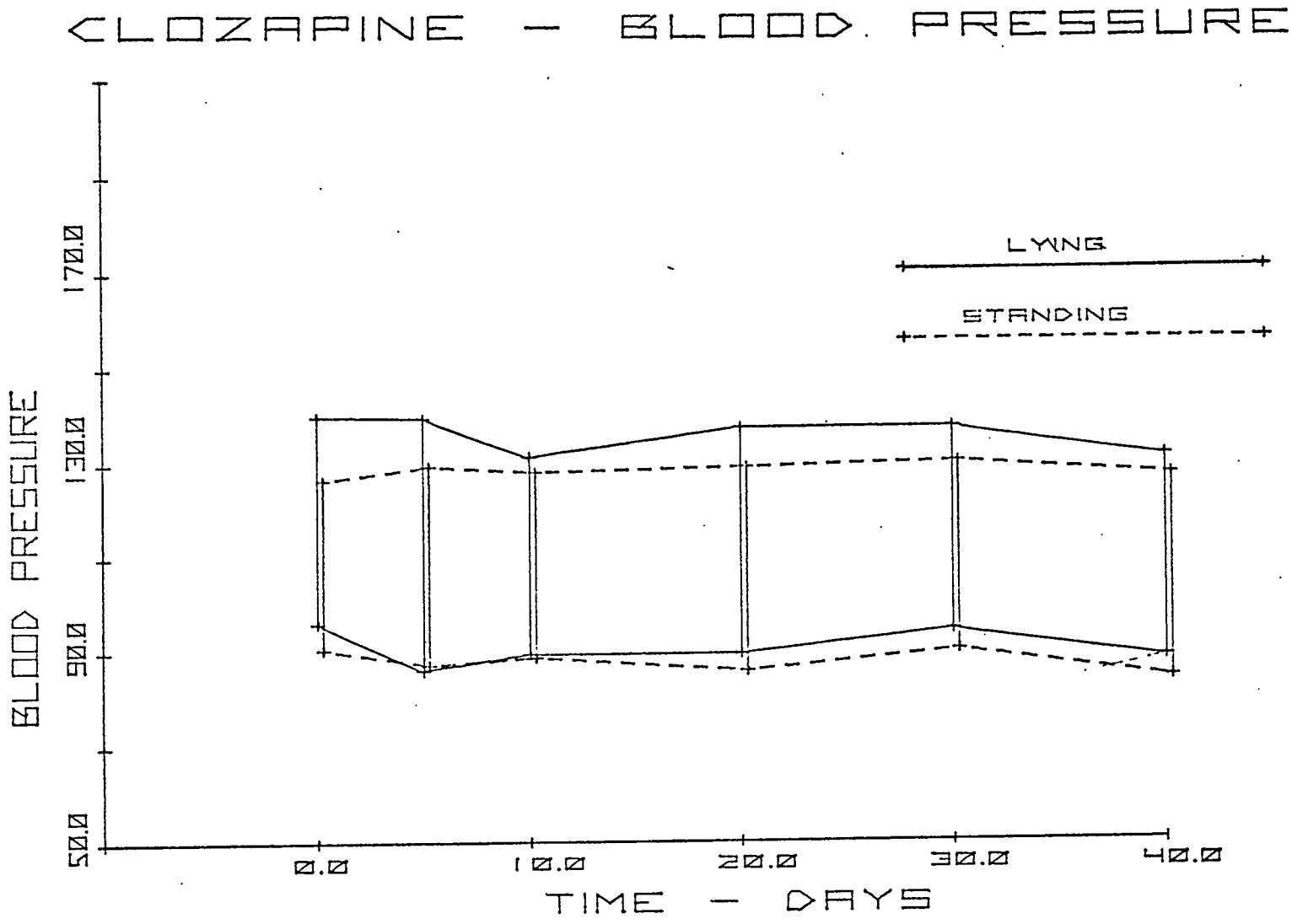


Figure 8: Graphical representation of blood pressure recordings during the course of the study with clozapine.

It should be noted that clozapine did not induce any extrapyramidal side effects in any of the 15 patients studied. This failure to cause extrapyramidal side effects is one of the outstanding features of the drug[2].

4.4 Blood pressure

The mean (± standard deviation) systolic and diastolic blood pressure recordings both lying and standing are tabulated in table 7.

		Pre-treatment	Day 5	Day 10	Day 20	Day 30	Day 40
LYING	Systolic	140 ± 19	139 ± 10	131 ± 10	137 ± 18	137 ± 15	131 ± 12
	Dyastolic	96 ± 16	86 ± 9	89 ± 15	89 ± 10	94 ± 10	88 ± 6
STANDING	Systolic	126 ± 17	129 ± 18	128 ± 14	129 ± 21	130 ± 22	127 ± 13
	Dyastolic	90 ± 16	87 ± 11	86 ± 12	86 ± 15	90 ± 15	84 ± 16

Table 7: Mean (± S.D.) systolic and dyastolic blood pressure recordings in mm Hg pre-treatment and on the subsequent evaluation days during the course of treatment with clozapine.

Statistical analysis using the paired t test to compare pre-treatment recordings with subsequent recordings on the 5 evaluation days during treatment with

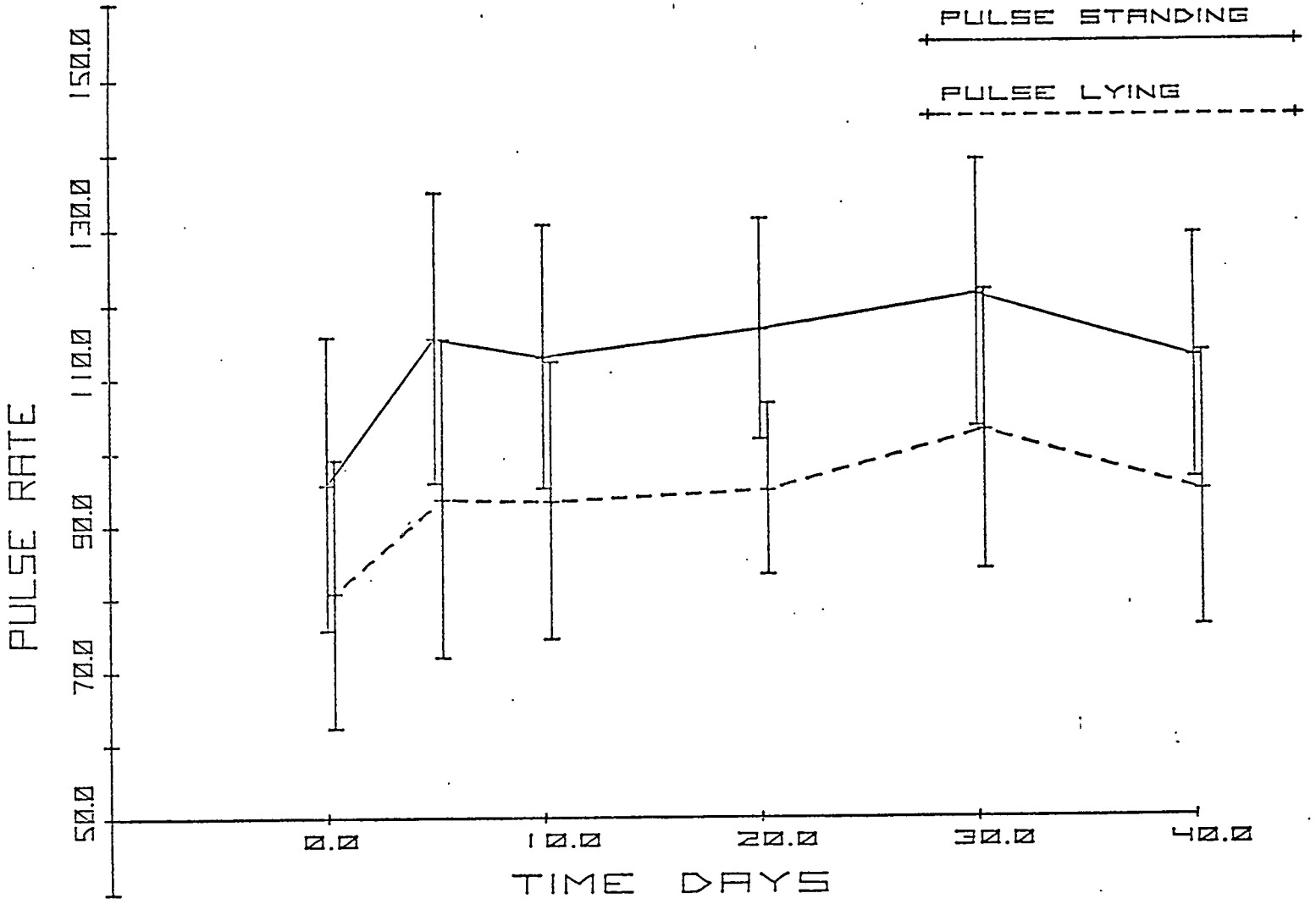


Figure 9: Graphical representation of mean (± S.D.) pulse rate recordings during the course of the study with clozapine.

clozapine failed to reveal any significant differences.

A graphical representation of the blood pressure recordings during the course of the study can be seen in figure 8.

It can thus be concluded that in these patients in this study, clozapine had no significant effect on blood pressure.

4.5 Pulse rate

The mean (± standard deviation) pulse rate recordings, lying and standing, are tabulated in table 8.

Pulse Rate	Pre-treatment	Day 5	Day 10	Day 20	Day 30	Day 40
Lying	81 ± 18	94 ± 22	93 ± 19	95 ± 12	103 ± 19	95 ± 19
Standing	96 ± 20	116 ± 20	113 ± 18	117 ± 15	121 ± 18	113 ± 16

Table 8: Mean (± S.D.) pulse rate recordings lying and standing pre-treatment and on subsequent evaluation days during treatment with clozapine.

The mean (± S.D.) pulse rate recordings are illustrated graphically in figure 9.

Statistical analysis using the paired t test comparing pre-treatment pulse rate recordings with recordings on

each subsequent evaluation day during the course of treatment with clozapine revealed a statistically significant difference at the 95% confidence level for both lying and standing pulse rate recordings.

It can thus be concluded that in these patients clozapine caused a significant and sustained rise in pulse rate in both the lying and standing positions.

Comment: It occurred on two separate occasions during the course of the study that clozapine was not administered to two patients during day 1 to 5 of the study while the investigator was under the impression that it had been administered. When the pulse rates of these two patients were recorded on day 5 and no increase in pulse rates were noted, the investigator immediately checked the prescription charts of the patients and found that he had forgotten to prescribe the clozapine to be administered during day 1 to 5. In these two cases the investigator's suspicion that the patients were not receiving their medication was based solely on the fact that no rise in pulse rate above pre-treatment recordings was noted. Clozapine so consistently caused a rise in pulse rate above pre-treatment recordings that the investigator is of the opinion that the pulse rate can be used as a convenient aid to ascertain whether the patient is taking the medication regularly. Thus the pulse can be used as an aid to check patient compliance when the patient is being treated with clozapine.

4.6 Biochemical and haematological tests

No biochemical or haematological abnormalities were detected in any patients during the course, or on completion, of the study.

4.7 Body temperature

No significant changes of body temperature were found throughout the course of the study.

4.8 Serum concentrations of clozapine, clozapine plus metabolites, and metabolites.

The mean serum concentrations (± S.D.) of clozapine, clozapine plus metabolites, and metabolites on each of the evaluation days during the course of the study is tabulated in table 9.

Serum concentration of	Day 5	Day 10	Day 20	Day 30	Day 40
clozapine	459 ± 266	467 ± 190	569 ± 400	515 ± 142	493 ± 219
clozapine plus metabolites	658 ± 321	699 ± 339	751 ± 427	817 ± 226	750 ± 260
metabolites	200 ± 132	277 ± 216	165 ± 137	302 ± 206	257 ± 192

Table 9: Mean serum concentration (± S.D.) in ng/ml of clozapine, clozapine plus metabolites, and metabolites only on each evaluation day during the course of the study.

The actual serum concentrations of clozapine and the dosage for each patient is tabulated in table 10, for clozapine plus metabolites in table 11, and for metabolites only in table 12.

Patient No.	Dosage mg/kg	Day 5	Day 10	Day 20	Day 30	Day 40
1	4,35	620	576,2	807	569,7	644,4
2	3,95	603,9	778,2	1626,3	763,9	1016,2
3	3,81	405,8	311,35	821	416,5	551,8
4	4,07	467,5	496,2	520,55	605	217,35
5	3,85	918,3	884,5	836,25	727	560,4
6	3,79	878,4	307,8	515,4	337,65	383,3
7	4,17	289,65	320,2	338	395,3	370,85
8	4,04	547,1	453,9	170,9	522,8	226,95
9	3,66	614,8	664,5	227,4	520,45	380,55
10	3,79	21,25	235,75*1	95,2	337,4	265,25
11*2	4,17	-	136,42	103,49	-	-
12	3,79	128,4	307,5 *3	860,3	644	586,05
13	3,85	160,45	638,9	703,2	360,65	588,05
14	3,85	291	493,15	576,8*4	600,75	618,6
15*5	3,78	487,10	691,05	339,35	411,65	-

Table 10: Serum concentration (ng/ml) of clozapine and dosage (mg/kg) for each patient on each evaluation day during the course of the study with clozapine.

*1 Dosage increased to 4,55 mg/kg on day 10.

*2 Patient No. 11 terminated on day 20 and *5 patient No. 15 terminated on day 30 because of clinical improvement requiring no further psychotropic therapy.

*3 Dosage increased to 4,55 mg/kg on day 10.

*4 Dosage increased to 4,62 mg/kg on day 20.

Patient No.	Dosage mg/kg	Day 5	Day 10	Day 20	Day 30	Day 40
1	4,35	910,45	785	952,4	763,4	672,9
2	3,95	1004,4	1117,85	1961,85	987,4	1187,7
3	3,81	751,15	498,35	1029,7	601,45	765,95
4	4,07	680,6	613,05	654,6	635,1	233,3
5	3,85	970,8	918,4	908,05	885,55	773,25
6	3,79	967,7	558,35	558	636,9	704,35
7	4,17	554,7	538,95	534,7	633,8	580,45
8	4,04	813,75	767,1	480,45	769,9	586,85
9	3,66	929,55	1133,15	631,95	1131,7	888,1
10	3,79	149,86	382,56*1	168,16	863,9	678,7
11*2	4,17	-	175,98	123,22	-	-
12	3,79	133,22	1042,65*3	873,05	1349,45	1256,2
13	3,85	204,91	1310,1	814,25	893,9	673,6
14	3,85	357,29	962,85	714,8*4	762,3	743,6
15*5	3,78	780,2	702,15	852,6	524,9	-

Table 11: Serum concentration (ng/ml) of clozapine plus metabolites and dosage (mg/kg) for each patient on each evaluation day during the course of the study with clozapine.

*1 Dosage increased to 4,55 mg/kg on day 10.

*2 Patient No. 11 terminated on day 20 and

*5 Patient No. 15 terminated on day 30 because of clinical improvement requiring no further psychotropic therapy.

*3 Dosage increased to 4,55 mg/kg on day 10.

*4 Dosage increased to 4,62 mg/kg on day 20.

Patient No.	Dosage mg/kg	Day 5	Day 10	Day 20	Day 30	Day 40
1	4,35	290,45	208,85	145,25	193,7	28,49
2	3,95	400,5	339,65	335,55	223,5	171,5
3	3,81	355,35	187	208,7	184,95	214,15
4	4,07	213,1	116,85	134,05	30,1	15,95
5	3,85	52,5	33	71,8	158,5	212,85
6	3,79	89,3	250,55	42,6	299,25	321,05
7	4,17	265,05	218,75	196,7	238,5	209,6
8	4,04	284,65	313,2	309,55	247,1	359,9
9	3,66	314,75	468,66	404,55	611,25	507,65
10	3,79	128,61	146,81*1	72,96	526,5	413,45
11*2	4,17	-	39,56	19,73	-	-
12	3,79	4,8	735,15*3	12,75	705,45	670,15
13	3,85	44,46	617,2	111,05*4	533,25	85,55
14	3,85	66,29	469,7	138	161,55	125
15*5	3,78	293,1	11,1	513,25	113,25	-

Table 12: Serum concentration (ng/ml) of the metabolites of clozapine and dosage (mg/kg) for each patient on each evaluation day during the course of the study with clozapine.

*1 Dosage increased to 4,55 mg/kg on day 10.

*2 Patient No. 11 terminated on day 20 and *5 patient No. 15 terminated on day 30 because of clinical improvement requiring no further psychotropic therapy.

*3 Dosage increased to 4,55 mg/kg on day 10.

*4 Dosage increased to 4,62 mg/kg on day 20.

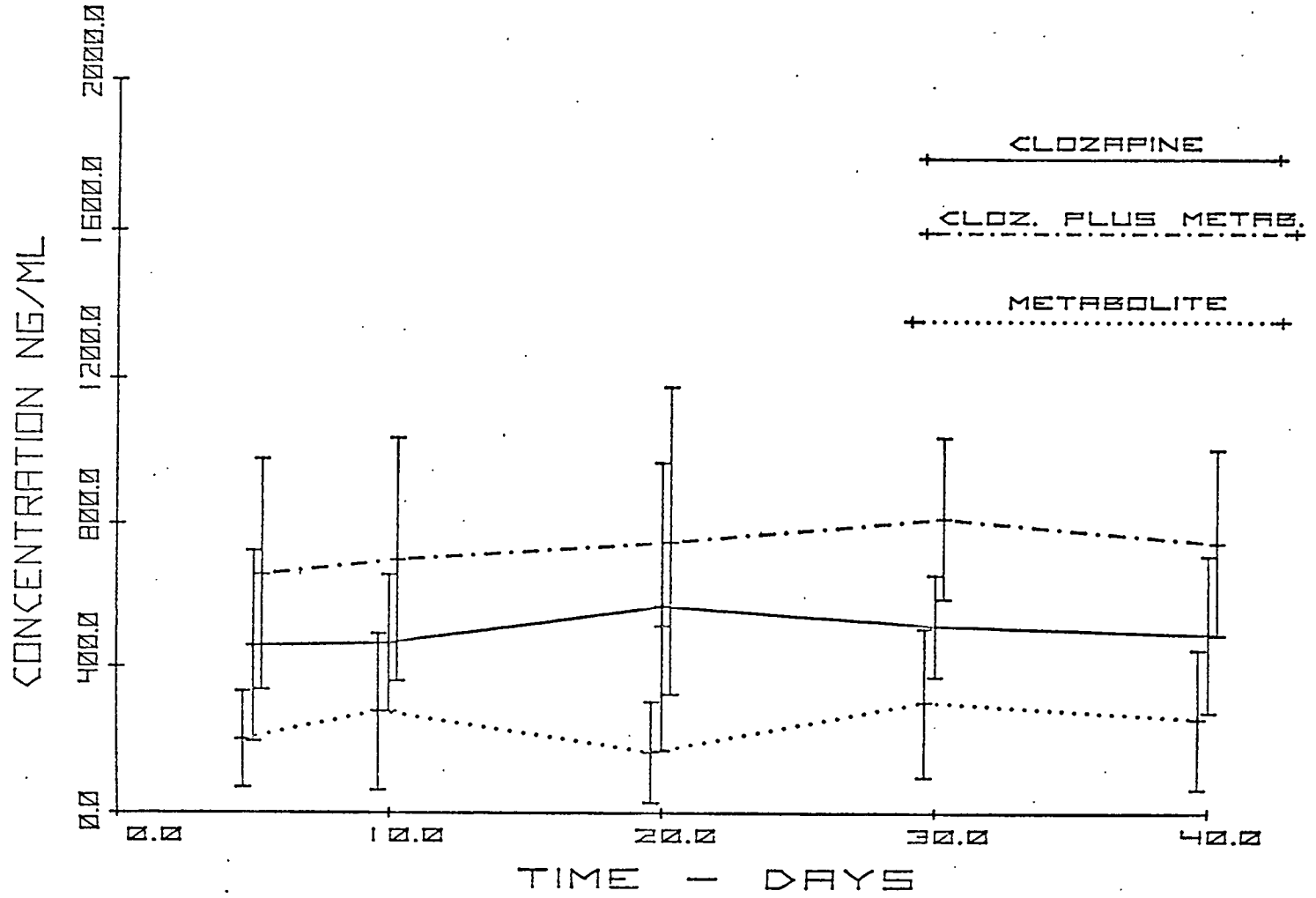


Figure 10: Graphical illustration of the mean (± S.D.) serum concentrations of clozapine, clozapine plus metabolites, and metabolites only on each evaluation day during the course of the study with clozapine.

Figure 10 graphically illustrates the mean (± S.D.) serum concentrations of clozapine, clozapine plus metabolites, and metabolites only on each evaluation day during the course of the study.

From tables 10, 11 and 12 it can be seen that the dosage of clozapine ranged from 3,66 mg/kg to 4,62 mg/kg. On this dosage range the serum levels of clozapine ranged between 21,25 ng/ml to 1626,3 ng/ml, the serum levels of clozapine plus metabolites between 149,86 ng/ml to 1961,85 ng/ml, and the serum levels of the metabolite of clozapine only, between 11,1 ng/ml to 735,15 ng/ml.

By studying table 9 and figure 10 the following conclusions can be reached:

(i) on treatment day 5 steady state serum levels of clozapine and of clozapine plus its metabolites had been reached. Using the paired t test, no statistically significant differences between the serum levels of clozapine, clozapine plus its metabolites or its metabolites only could be demonstrated at the 95% confidence level, when those on day 5 were compared with those on day 40.

(ii) no auto-induction of the metabolism of clozapine appears to occur during the treatment period of 40 days.

(iii) no accumulation of clozapine or its metabolites

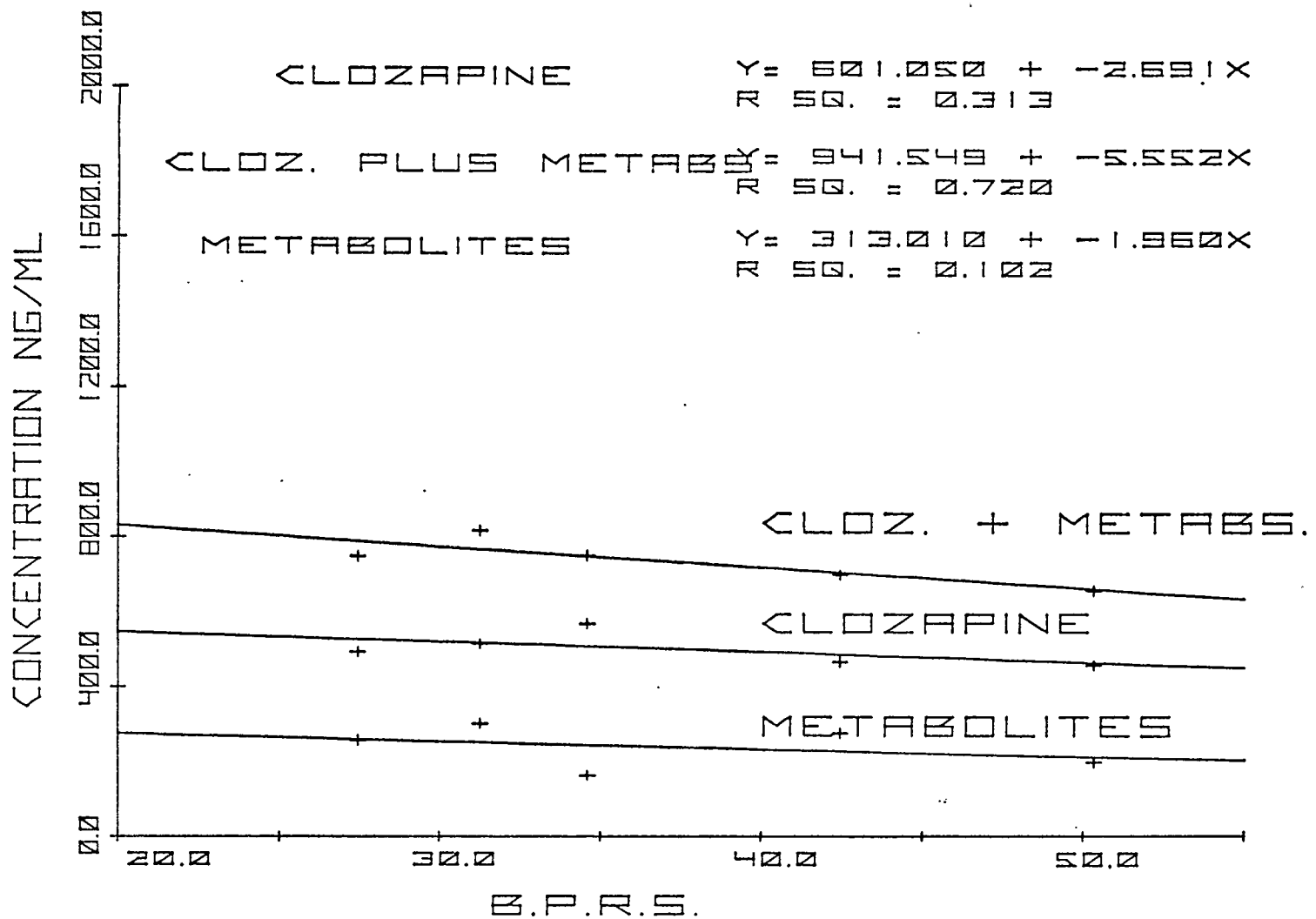


Figure 11: Linear regression curves of mean serum concentrations of clozapine, clozapine plus metabolites and metabolites only, against mean B.P.R.S. scores for each evaluation day.

appears to occur during the treatment period of 40 days.

In an attempt to ascertain whether any correlation existed between clinical improvement and serum levels of clozapine, clozapine plus its metabolites, or the metabolites of clozapine only, a linear regression curve was drawn plotting the mean serum concentrations of each against the mean B.P.R.S. scores for each evaluation day. The three curves are represented in figure 11. It appears that no correlation exists between clinical improvement and the serum concentrations of clozapine, clozapine plus its metabolites, or the metabolites only. None of the regression curves reached statistical significance at the 95% confidence level.

In a further attempt to correlate serum concentrations with clinical improvement, separate linear regression curves were drawn for each evaluation day plotting actual serum concentrations of clozapine plus metabolites agains B.P.R.S. scores. The five curves are illustrated in figure 12.* Once again none of the regression curves reached statistical significance at the 95% confidence level emphasizing the fact that no correlation exists between clinical improvement and serum levels of clozapine plus its metabolites.

It seems justifiable to conclude that a certain period of exposure to clozapine and/or its metabolites is necessary to effect clinical improvement. This assertion can be

*see annex 1, page 81.

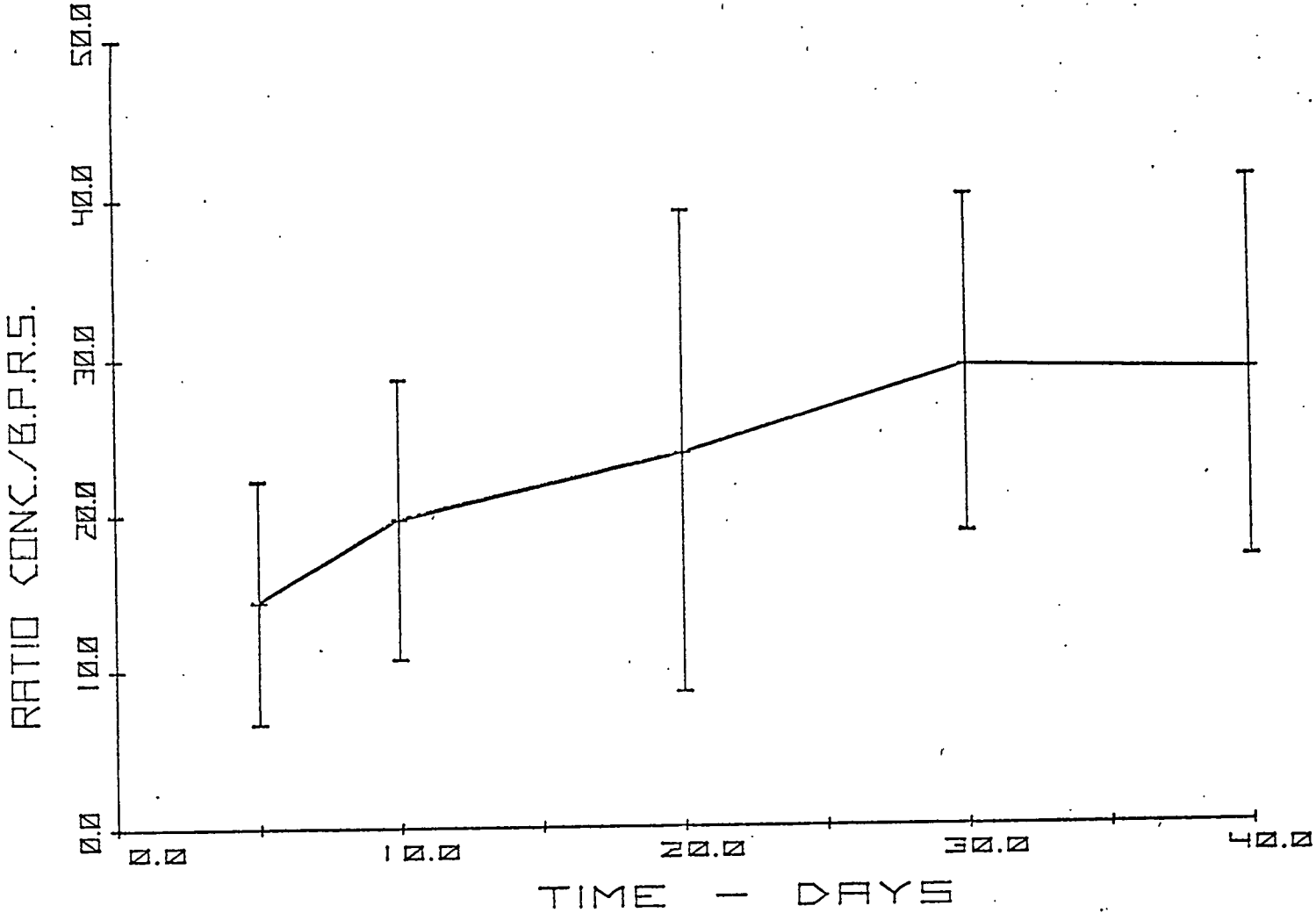


Figure 13: Graphical representation of the ratio of the mean serum concentration of clozapine plus its metabolites and mean B.P.R.S. scores for each evaluation day during the study with clozapine.

illustrated graphically by plotting the ratio of the mean serum concentrations of clozapine plus its metabolites and the mean B.P.R.S. scores (± S.D.) for each evaluation day during the course of the study. This graphical representation can be seen in figure 13. Because the mean serum concentrations of clozapine plus its metabolites remains fairly constant throughout the duration of the study while the B.P.R.S. scores are decreased on each evaluation day, the ratio increases during the course of the study.

Because treatment with clozapine caused such a consistent rise in pulse rate it was decided to ascertain whether a correlation existed between mean serum concentrations of clozapine plus its metabolites and mean pulse rate recordings on each evaluation day. Linear regression curves plotting the mean concentration of clozapine plus its metabolites against pulse rate recordings for each evaluation day are illustrated in figure 14 (lying pulse rate recordings) and figure 15 (standing pulse rate recordings). The linear regression curve illustrated in figure 14 has an r value of 0,921 and is statistically significant at the 95% confidence level. However, the linear regression curve illustrated in figure 15 has an r value of 0,715 and fails to reach statistical significance at the 95% confidence level. It can therefore be concluded that the lying pulse rate of patients being treated with clozapine offers a reasonable indication of the serum level of clozapine plus its metabolites.

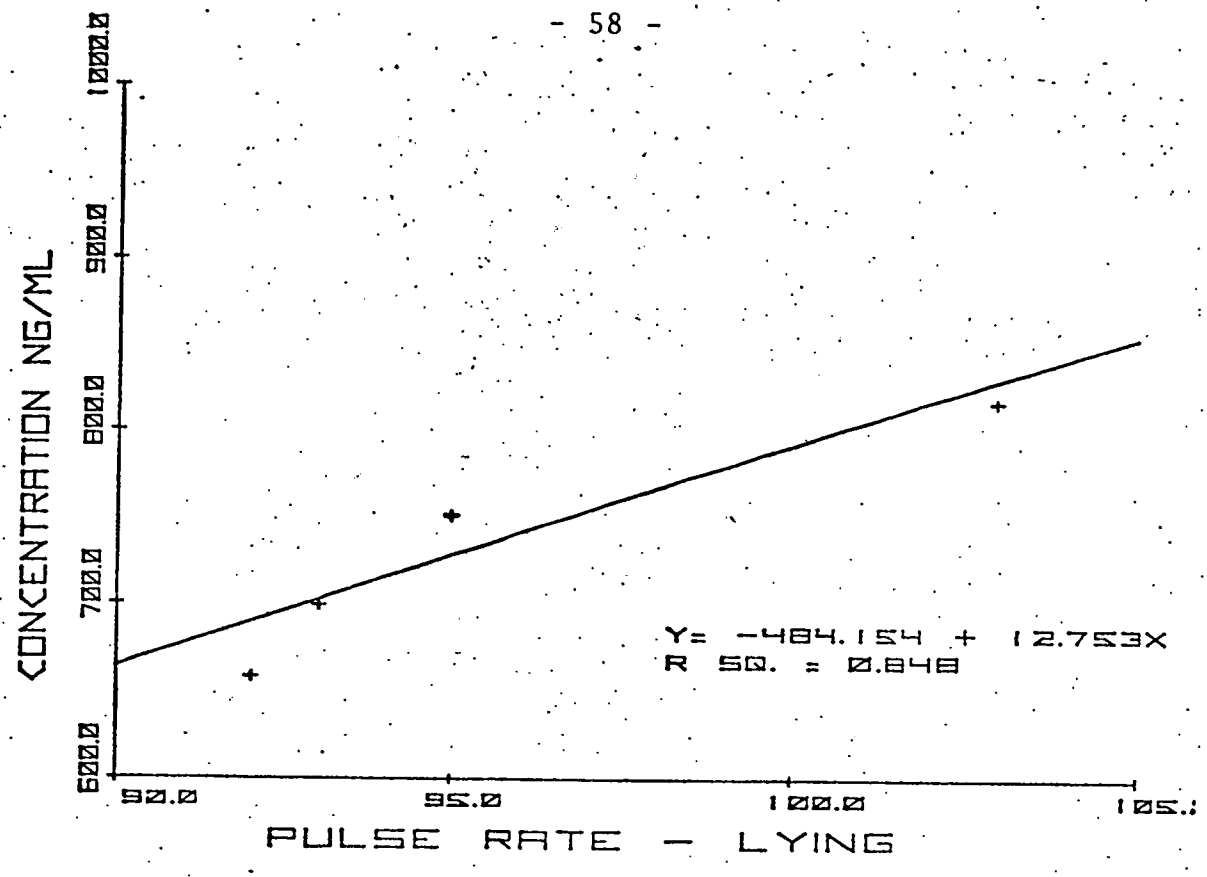


Figure 14: Linear regression curve of mean serum concentrations of clozapine plus metabolites and mean lying pulse rate recordings. r = 0,921 (2p < 0,05).

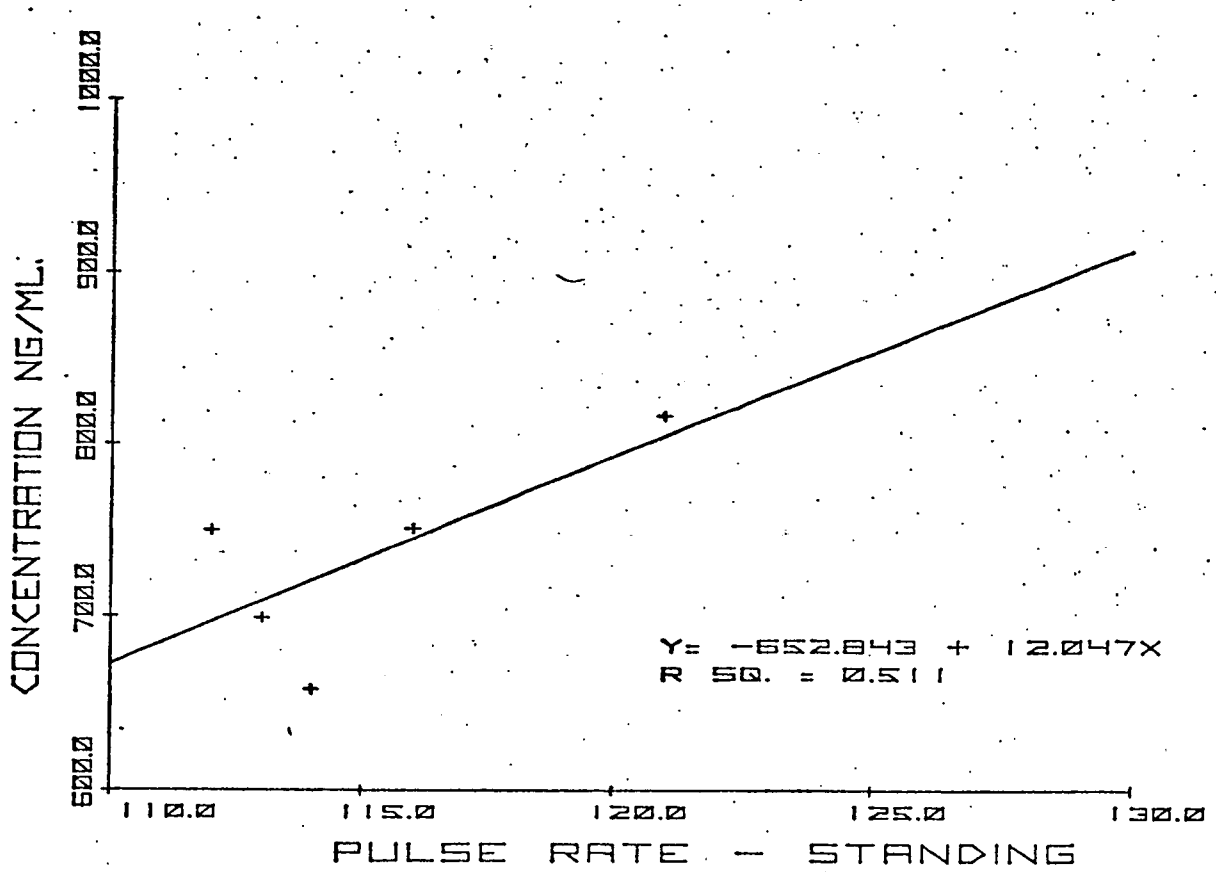


Figure 15: Linear regression curve of mean serum concentrations of clozapine plus metabolites and mean standing pulse rate recordings. r = 0,715 (2p > 0,1).

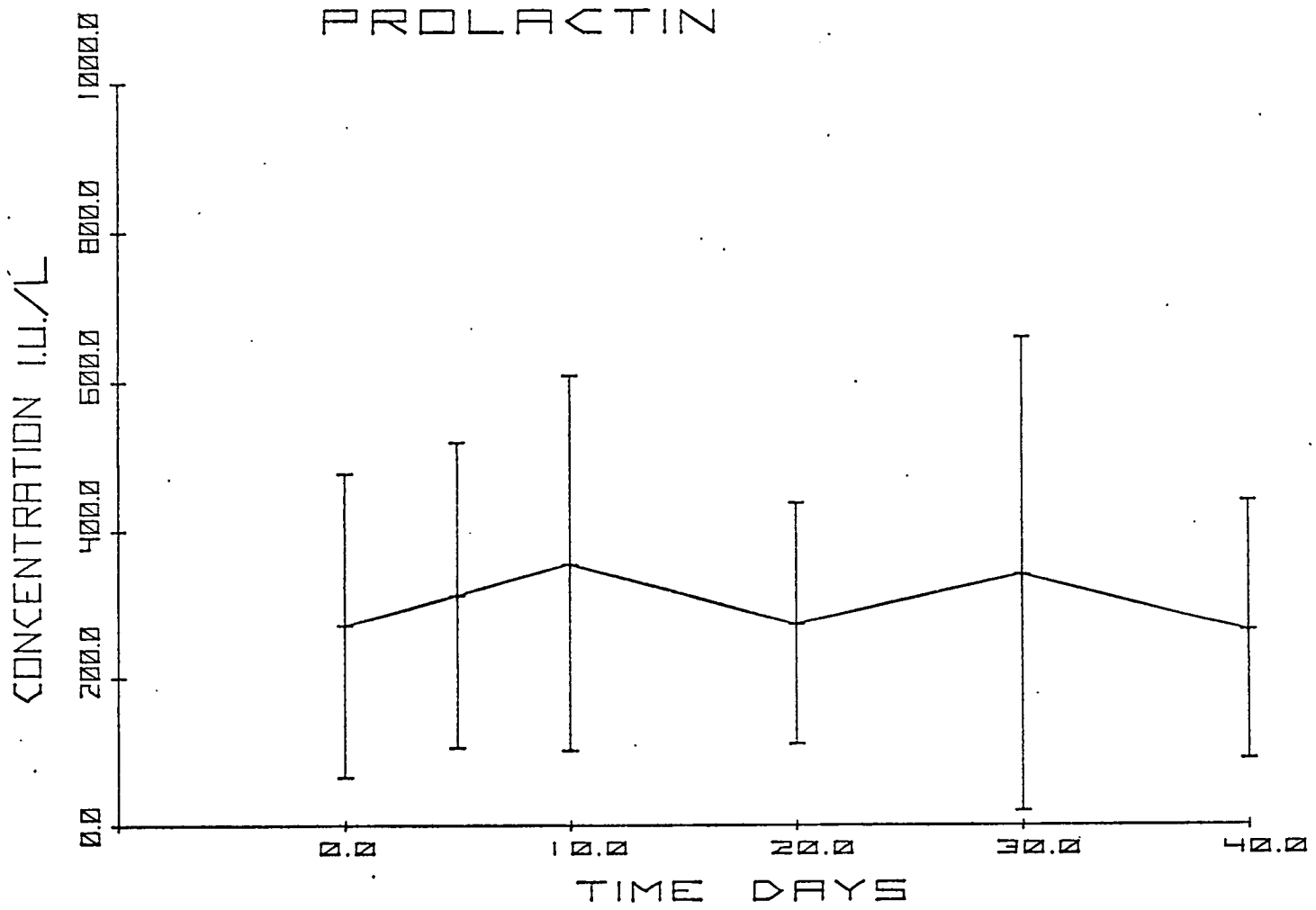


Figure 16: Mean serum prolactin levels (± S.D.) prior to treatment and on each subsequent evaluation day after institution of treatment with clozapine.

4.9 Prolactin serum levels

Prolactin serum levels obtained for each patient prior to the commencement of treatment with clozapine and on each subsequent evaluation day after the institution of treatment with clozapine is tabulated in table 13.

Patient No.	Pre-treatment	Day 5	Day 10	Day 20	Day 30	Day 40
1	580	188	228	200	130	154
2	<100	242	190	390	190	242
3	216	388	454	476	332	216
4	200	258	214	200	440	188
5	348	636	980	676	1360	720
6	142	290	810	178	290	190
7	370	258	440	290	370	370
8	130	178	154	132	166	198
9	116	214	190	132	142	<100
10	178	178	214	218	310	328
11	240	242	228	228	-	-
12	116	<100	<100	<100	118	132
13	870	920	224	236	132	118
14	242	264	556	496	556	480
15	362	328	344	158	236	-
Mean (± S.D.)	273 ± 208	312 ± 208	355 ± 254	274 ± 164	341 ± 321	265 ± 176

Table 13: Prolactin serum levels for each patient prior to treatment and on each subsequent evaluation day.
Concentrations expressed in International Units / 1.

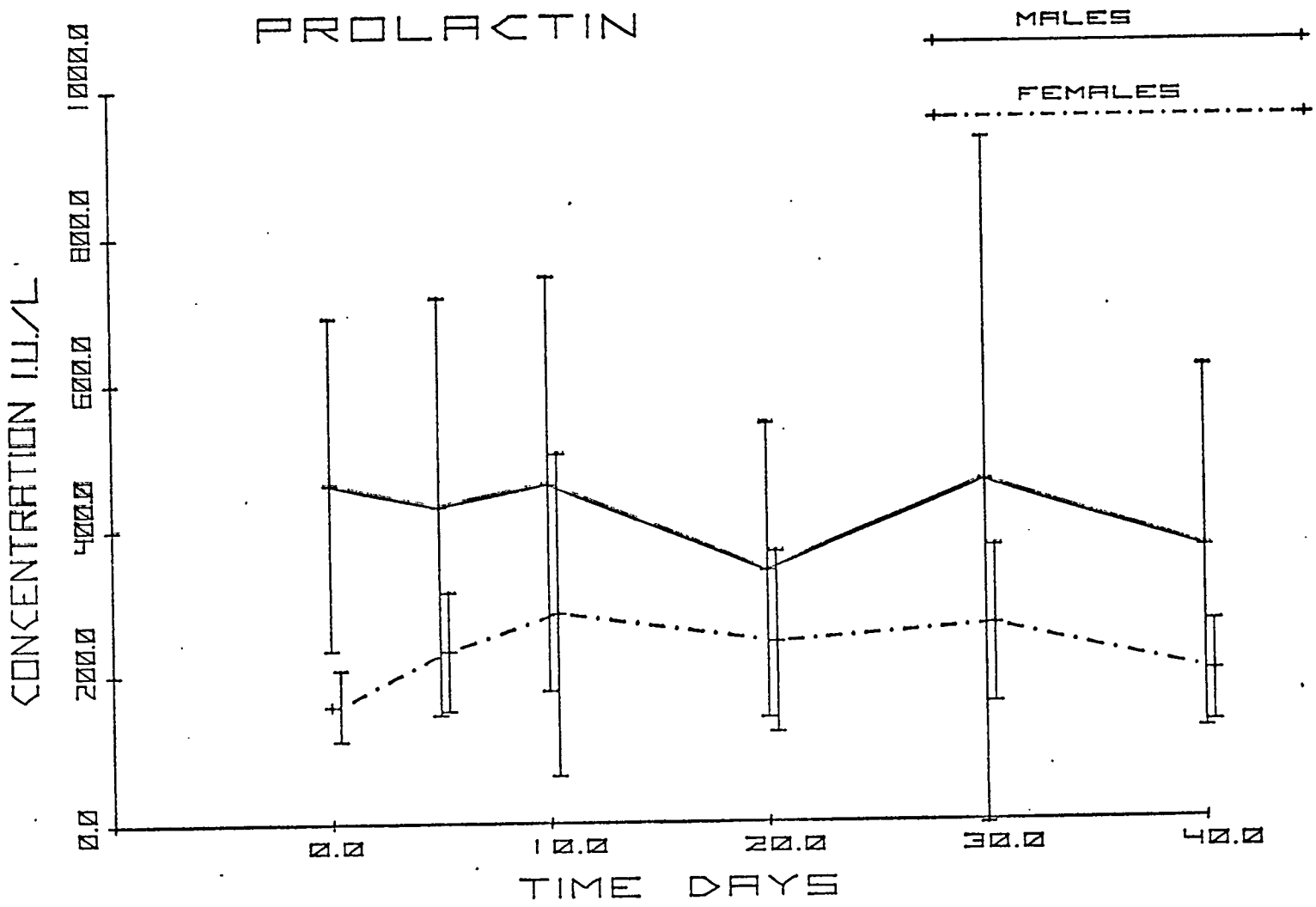


Figure 17: Mean prolactin serum levels (± S.D.) for men and women separately on each evaluation day during treatment with clozapine.

Figure 16 illustrates a graphical representation of mean prolactin serum concentrations (± S.D.) prior to treatment and on each subsequent evaluation day after treatment with clozapine was instituted.

Contrary to expectations, no rise in the serum levels of prolactin occurred after the institution of treatment with clozapine. As was discussed in the introduction (section 2.3) most neuroleptics cause a rise in serum prolactin concentrations and it has been shown that clozapine also caused a rise in prolactin serum levels in rats[44]. No explanation for this observation can be offered.

Figure 17 illustrates the differences between mean prolactin serum concentrations for men and women separately on each of the evaluation days. It can be seen that the men had slightly higher mean values than the women but that no rise in prolactin levels is evident in either group of patients.

4.10 5-hydroxytryptamine-induced platelet aggregation

It was found that clozapine in the range of 200 ng/ml to 600 ng/ml inhibits 5-HT-induced platelet aggregation. This is in keeping with the findings of Boullin et al[45] who demonstrated the same effect with chlorpromazine and its metabolites. However, contrary to expectations and contrary to the findings of Boullin et al[46], no increased platelet aggregation responses to 5-HT occurred in the 15 patients treated with clozapine in this study.

This test does not, therefore, appear to be of any value as an index of therapeutc blood concentrations, clinical improvement, or patient compliance, with regard to clozapine.

4.11 Plasma cholinesterase and red blood cell acetylcholinesterase activity.

In table 14 is tabulated the pre-treatment plasma cholinesterase and red blood cell acetylcholinesterase activity and the activity of both on completion of the study.

	PLASMA ChE		RED BLOOD CELL AChE	
Patient No.	Pre-treatment	Day 40	Pre-treatment	Day 40
1	1737	2525	2352	2950
2	2119	2405	2539	2876
3	2856	2672	1242	2437
4	2939	2038	2351	2634
5	2404	2004	2187	2234
6	2048	2430	2351	2806
7	1737	2725	2398	2496
8	3149	2565	2539	2469
9	3264	2405	2163	2407
10	1658	2075	2743	2656
11	2037	1523	3567	2900
12	2806	1683	2217	2015
13	2138	1546	2822	2376
14	1559	1844	2449	2728
15	2404	2605	2693	3010
Mean (S.D.)	2324 ± 562	2203 ± 414	2440 ± 481	2599 ± 285

Figure 14 Plasma cholinesterase and red blood cell acetylcholinesterase activity prior to treatment and on day 40. Plasma cholinesterase expressed as mU/ml serum. Red blood cell acetylholinesterase expressed as mU/µmol serum.

In keeping with the findings of Domino et al[48,49] plasma cholinesterase and red blood cell acetylcholinesterase activity was found to be within the normal range in these fifteen acute schizophrenic patients. There was also no significant difference between the activity prior to treatment and on day 40 after medication had be instituted. It can therefore be concluded that plasma cholinesterase activity and red blood cell acetylcholinesterase activity are of no use as diagnostic aids in making the diagnosis of acute schizophrenia.

5.1 SUMMARY

In this study, fifteen Black patients suffering from acute schizophrenia were treated with clozapine for a period of 40 days in order to ascertain whether certain laboratory parameters could be utilized to give an indication of the clinical efficacy of clozapine treatment in these patients.

5.1.1 Therapeutic efficacy

Utilizing the B.P.R.S. and F.C. rating scale as indication of the clinical improvement of the patients, it was found that significant clinical improvement occurred upto day 30 whereafter clinical improvement was only slight. Clozapine was well tolerated by all the patients while more than half the patients (53,3%) were fit for unconditional discharge on completion of the study. On completion of the study the working capacity of the majority (80%) was satisfactory.

5.1.2 Side effects

The most common side effects encountered were daytime sedation which was especially prominent during the early stages of the study and hypersalivation which occurred with equal frequency throughout the study period. Other side effects encountered in descending order of frequency were nausea and vomiting, dizziness, headache, disturbance of visual

accomodation, constipation and diarrhoea, disturbed sleep, sweating, inhibition of micturition, and collapse.

5.1.3 Blood pressure and pulse rate

Clozapine had no significant prolongated effect on blood pressure while a significant and sustained rise in pulse rate during the treatment period was noted. It is suggested that this rise in pulse rate could be utilized as a convenient clinical aid in checking patient compliance in patients being treated with clozapine.

5.1.4 Serum concentration of clozapine, clozapine plus metabolites, and metabolites only.

5.1.4.1 No correlation was found between serum levels of clozapine, clozapine plus its metabolites, or its metabolites only and clinical improvement.

5.1.4.2 It was found that on treatment day 5 steady state serum levels of clozapine and of clozapine plus it metabolites had been reached.

5.1.4.3 No auto-induction of the metabolism of clozapine appeared to occur during the treatment period.

5.1.4.4 No accumulation of clozapine or its metabolites appeared to occur during the treatment period.

5.1.4.5 It can be concluded that a certain period of exposure to a more or less constant serum level of clozapine and/or its metabolites is necessary to effect clinical improvement.

5.1.4.6 A significant correlation was found between the lying pulse rate and serum levels of clozapine plus its metabolites. The lying pulse rate can thus offer a reasonable indication of the expected serum levels of clozapine plus its metabolites.

5.1.5 Prolactin serum levels

No rise in serum prolactin levels occurred in these patients after institution of treatment with clozapine. Therefore no correlation between clinical improvement and prolactin serum levels could be ascertained.

5.1.6 5-hydroxytryptamine-induced platelet aggregation

No enhancement of 5-HT-induced platelet aggregation could be determined in these patients undergoing treatment with clozapine. Therefore no correlation could be established between clinical improvement and enhancement of 5-HT-induced platelet aggregation.

5.1.7 Plasma cholinesterase and red blood cell acetylcholinesterase activity.

Both the plasma cholinesterase and red blood cell

acetylcholinesterase activity fell within the normal range prior to the institution of treatment with clozapine. These parameters can therefore not be used as diagnostic aids in the diagnosis of schizophrenia. The activity of both parameters also fell within the normal range on conclusion of the study. It would thus appear that treatment with clozapine did not significantly affect these parameters.

5.2 OPSOMMING

Tydens hierdie studie is vyftien Swart pasiënte wat gely het aan akute skisofrenie behandel met klosapien vir 'n periode van 40 dae om vas te stel of sekere laboratorium parameters gebruik kon word om 'n aanduiding te gee van die kliniese bruikbaarheid van klosapien behandeling by hierdie pasiënte.

5.2.1 Terapeutiese bruikbaarheid

Deur gebruik te maak van die Verkorte Psigiatriese Beoordelingskaal (B.P.R.S.) en die F.C. beoordelingskaal om die kliniese verbetering van die pasiënte te beoordeel, is gevind dat betekenisvolle kliniese verbetering plaasgevind het tot op dag 30 waarna slegs 'n geringe verbetering plaasgevind het. Klosapien is goed verdra deur al die pasiënte terwyl meer as die helfte van die pasiënte (53,3%) geskik

gevind is vir onvoorwaardelike ontslag ten voltooiing van die studie. Na voltooiing van die studie was die werksvermoë van die meeste pasiënte (80%) gevind om bevredigend te wees.

5.2.2 Newe-effekte

Die mees algemene newe-effekte wat voorgekom het was sedasie (wat veral voorgekom het tydens die eerste deel van die studie) en hipersalivasie die voorkoms waarvan dieselfde gebly het gedurende die hele studie tydperk. Ander newe-effekte wat voorgekom het in volgorde van frekwensie (algemeen tot seldsaam) is naarheid en braking, duiseligheid, hoofpyn, versteuring van visuele akkommodasie, hardlywigheid en diaree, verstoorde slaap, sweet, inhibisie van urinering en kollaps.

5.2.3 Bloeddruk en polsspoed

Klosapien het geen betekenisvolle uitwerking op bloeddruk gehad nie terwyl 'n betekenisvolle en volgehoue styging in polsspoed gedurende die behandelingstydperk waargeneem is. Dit word voorgestel dat hierdie verhoging in polsspoed gebruik kan word as 'n kliniese hulpmiddel om vas te stel of die pasiënt wel die medikasie neem in die geval van pasiënte wat met klosapien behandel word.

5.2.4 Serumkonsentrasie van klosapien, klosapien plus metaboliete en metaboliete alleen.

5.2.4.1 Geen korrelasie is gevind tussen serum-

vlakke van klosapien, klosapien plus metaboliete of die metaboliete alleen en kliniese verbetering nie.

5.2.4.2 Daar is gevind dat gelykvlak van klosapien en klosapien plus sy metaboliete reeds op dag 5 voorgekom het.

5.2.4.3 Geen outoinduksie van die metabolisme van klosapien blyk om voor te kom tydens die behandelings tydperk nie.

5.2.4.4 Geen akkumulasie van klosapien of sy metaboliete kom voor tydens die behandelingstydperk nie.

5.2.4.5 Die afleiding kan gemaak word dat 'n sekere tydperk van blootstelling aan 'n min of meer konstante serumvlak van klosapien en/of sy metaboliete nodig is om kliniese verbetering teweeg te bring.

5.2.4.6 'n Betekenisvolle korrelasie is gevind tussen die liggende polsspoed en die serumvlakke van klosapien plus sy metaboliete. Die liggende polsspoed kan dus 'n aanduiding gee van die verwagte serumvlakke van klosapien plus sy metaboliete.

5.2.5 Prolaktien serumvlakke

Geen verhoging in prolaktien serumvlakke het voorgekom nadat die pasiënte met klosapien behandel is nie, derhalwe kon geen korrelasie tussen kliniese verbetering en prolaktien serumvlakke aangetoon word nie.

5.2.6 5-hidroksitriptamiengeïnduseerde plaatjiekleefbaarheid

Geen versterking van 5-HT-geïnduseerde plaatjiekleefbaarheid kon vasgestel word in hierdie pasiënte wat met klosapien behandel is nie. Derhalwe kon geen korrelasie vasgestel word tussen kliniese verbetering en versterkte 5-HT-geïnduseerde plaatjiekleefbaarheid nie.

5.2.7 Plasmacholienesterase en rooibloedselasetielcholienesterase aktiwiteit

Beide die plasmacholienesterase en rooibloedselasetielcholienesterase aktiwiteit was binne die normale perke voordat 'n aanvang geneem is met klosapienbehandeling. Hierdie parameters kan dus nie as diagnostiese hulpmiddels gebruik word by die diagnose van skisofrenie nie. Die aktiwiteit van beide parameters het ook binne die normale perke geval na voltooiing van die studie. Dit blyk dus dat behandeling met klosapien nie hierdie parameters betekenisvol beïnvloed het nie.

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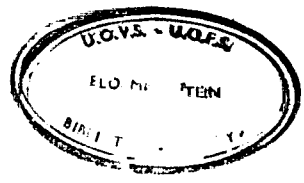

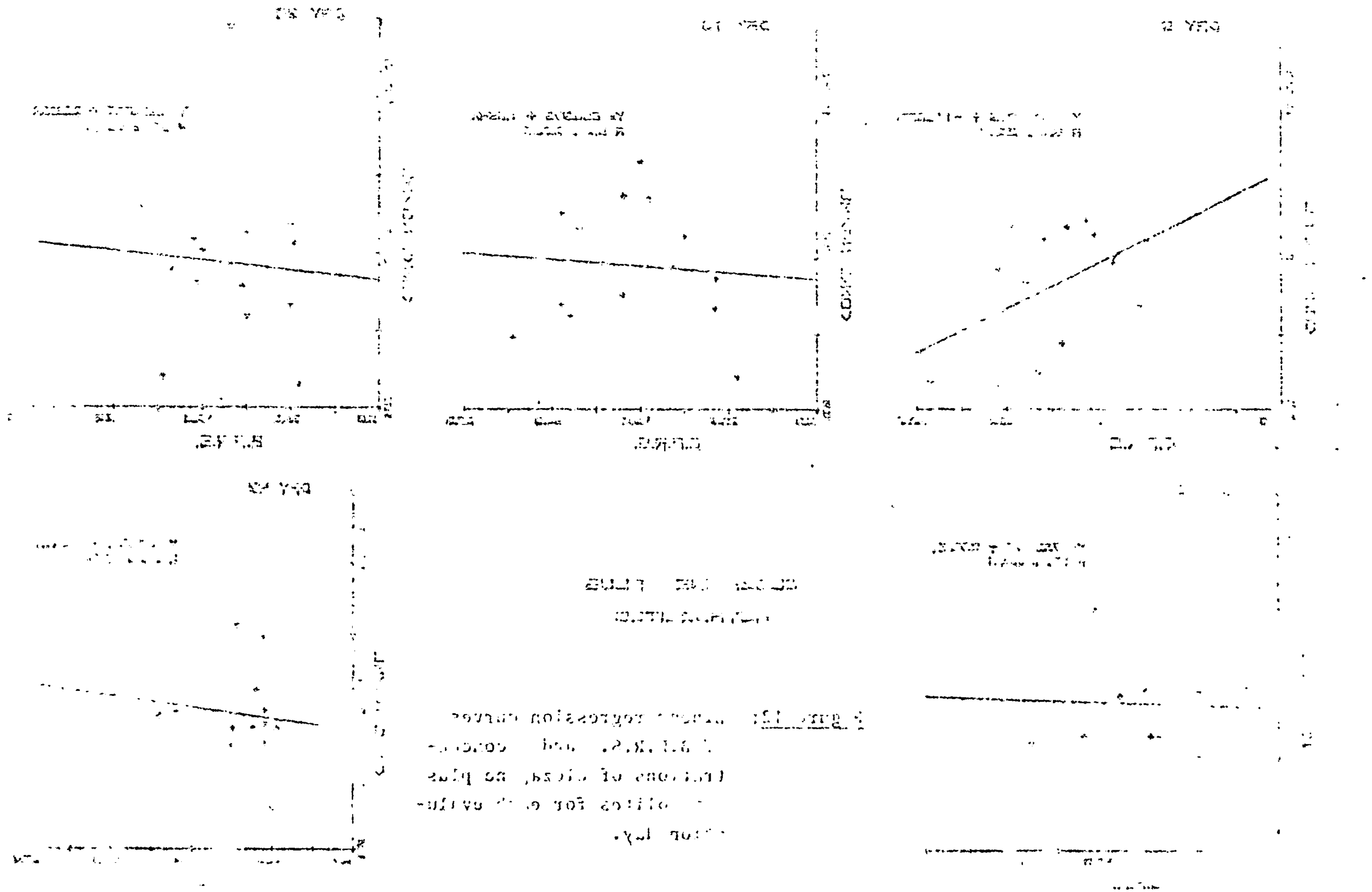

Figure 13: Linear regression curves of [illegible] and concen- trations of [illegible], no plus [illegible] for each evalu- ation day.

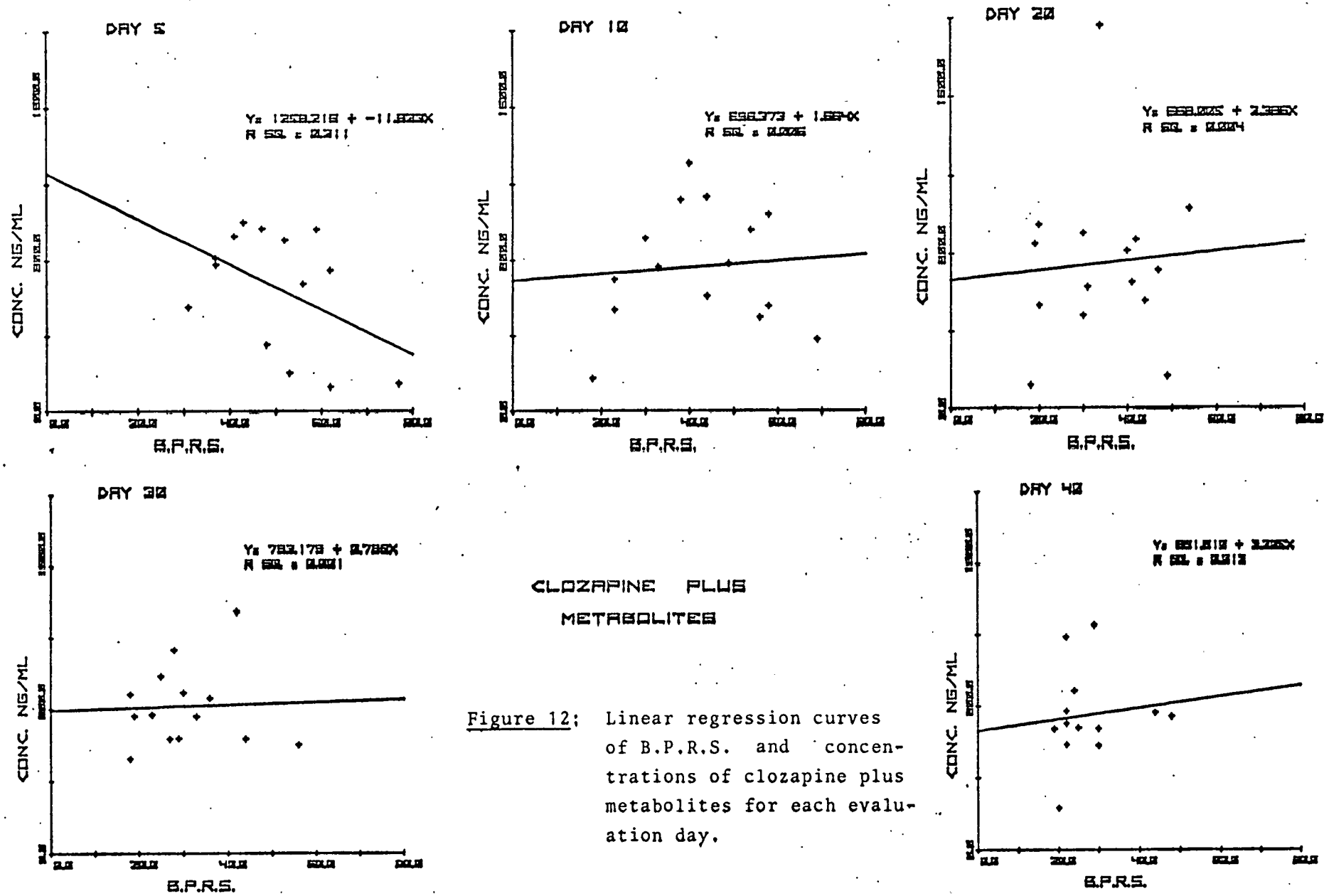

Figure 12: Linear regression curves of B.P.R.S. and concentrations of clozapine plus metabolites for each evaluation day.