Genetic Control of Nortriptyline Kinetics in Man

A Study of Relatives of Propositi with High Plasma Concentrations*

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Considerable variability has been observed in the steady-state plasma concentrations of nortriptyline in psychiatric patients undergoing treatment with the drug (Hammer, Idestrom, and Sjöqvist, 1967). Some patients have been found to have exceptionally high plasma levels forming a mode which was separated from the great majority (Sjöqvist *et al*, 1968). Nortriptyline (NT) steady-state plasma levels have been shown to be under genetic control (Alexanderson, Price Evans, and Sjöqvist, 1969) in a study of identical and fraternal twins receiving a small daily dose of the drug. The study of Alexanderson *et al* (1969) did not appear, however, to contain persons who developed exceptionally high steady-state plasma levels.

Nortriptyline is easily absorbed from the intestine (Hammer and Sjöqvist, 1967), individual differences in the binding of plasma protein are negligible (Borgå *et al*, 1969), and the renal excretion of unchanged nortriptyline accounts for less than 5% of the daily dose under steady-state conditions (Sjöqvist *et al*, 1968). The variability in steady-state plasma concentration is therefore presumably due to differences in rate of metabolism and apparent volume of distribution (Alexanderson *et al*, 1969). Investigations in human volunteers suggest that rate of metabolism is the quantitatively most important source of variance (B. Alexanderson and O. Borgå, unpublished observations).

The objects of the present study were to attempt to ascertain whether patients achieving very high steady-state NT plasma concentrations do so because of their genetic constitution and whether the manner of genetic control was monogenic or polygenic.

Material and Methods

High Nortriptyline Plasma Level Propositi. Three 'high-level' patient proposti had been ascertained. Two of these (L.S. and H.I.) were retested for plasma NT concentration using the technique described below. The third proposit (L.I.) was not available for retesting. The findings in the proposti are summarized in Table I.

Relatives available for Nortriptyline Testing. Pedigrees were available via proposti L.S. and H.I. and are shown in Figure 1. For the third proposit (L.I.) only two sisters were available. In total 29 relatives were tested.

Random Subjects. Twenty random subjects were tested. These comprised 4 unrelated spouses of relatives, 3 hospital employees such as doctors and nurses, 6 students, 4 patients free of organic disease, and 3 healthy relatives of 3 psychiatric patients whose nortriptyline or desmethylimipramine steady-state plasma levels were in the main mode of Fig. 8 of Sjöqvist *et al* (1968).

Dosage of Nortriptyline and the Taking of Blood Specimens. All subjects were given NT as sugar coated commercial tablets (Sensaval, Pharmacia). The dose was 25 mg three times daily for all subjects and was given for up to 8 days.

Blood specimens were generally taken on days 4, 5, 6, and 8, but sometimes they had to be drawn on different days. Three subjects (one relative and two random subjects) stopped taking the drug within three days due to side effects. Two subjects (one relative and one random subject) who had side effects were unable to continue the drug after day 6.

Clotting of the blood specimens was prevented with heparin and 0.5 ml 0.1-N hydrochloric acid was added to each 2.0 ml of plasma as a preservative. The specimens were stored at -20° C until determinations of plasma NT concentrations were performed, which generally occurred within 2 weeks.

Determination of Plasma Nortriptyline Concentrations. Plasma concentrations of NT were determined by the method described by Hammer and

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TABLE I
'HIGH-LEVEL' PROPOSITI

A Determination of steady-state plasma concentration of nortriptyline

Data derived from	Subject	Sex	Weight (kg)	Dosage of NT (mg t.i.d.)	Concomitant Drug Treatment	Steady-state Plasma Concentration of NT (m μ g/ml)
Sjöqvist <i>et al.</i> , 1969	L.S.	Female	55	50	Pentobarbital (0.1 g)	264
Hammer <i>et al.</i> , 1967	L.I.	Female	48	25	Methaqualone (0.25 g)	138
Sjöqvist <i>et al.</i> , 1968	H.I.	Female	65	25	Oral iron and vitamins	275

B Data obtained on retesting in present study

(Subject L.I. was not available for retesting. Figures within brackets indicate values obtained in connection with the study of Hammer *et al.*, 1967)

Subject	Plasma Concentration of NT on 25 mg t.i.d. (m μ g/ml)					Comments
	Day 4	Day 5	Day 6	Day 8	Day 10	
L.S.				168	185	Interval between first and second test 1 year. No concomitant drug treatment. Free from exposure to other drugs 2 months before testing
L.I. H.I.	(100) 114	129	(127) 129	133	(125)	
						Interval between first and second test two years. The patient had been receiving phenothiazines continuously in the meantime, and her weight on retesting was 83 kg. Free from other drugs 2 weeks before retesting

Brodie (1967) and further described by Sjöqvist *et al.* (1969). Unknowns were processed in duplicate, and standards in triplicate at 30, 50, 100, and 200 m μ g NT per ml were processed in each determination procedure.

Results

Full details of the results are shown in Table II.

Exclusions of Subjects because of Concomitant Disease and Drug Therapy. Nine subjects were either diseased and/or receiving concomitant drug therapy. Details are given in Table III together with the arbitrary decision on inclusion in further calculations. Subjects receiving barbiturates or contraceptive steroids were excluded.

FIG. 1. Pedigrees for two propiti with exceptionally high steady-state plasma levels of nortriptyline.

Barbiturates are known to influence the steady-state plasma levels of tricyclic antidepressants (Hammer *et al.*, 1967), and studies in progress by von Bahr *et al.* (unpublished data from this laboratory) indicate that contraceptive steroids affect the kinetics of other drugs, such as antipyrine.

All other 40 subjects were free of significant disease and had not been exposed to drugs or other chemicals during at least 3 months before the study. Specific enquiries were made concerning all doctor's prescriptions, patent medicines, laxatives, vitamins, tonics, and contraceptive steroids.

The Use of Plasma Concentrations to Characterize an Individual. Two measurements were utilized. First, the plasma NT concentrations on the sixth day; these were available for all subjects. Second, the area under the curve described by plotting NT concentrations (y) against days of NT therapy (x). The advantage of the latter procedure was that it utilized all observations available. One subject (Table II, case 3) could not be used for this procedure because of the paucity of the data available. A difficulty which arose with some subjects was the absence of a plasma NT concentration result on the eighth day, and this was resolved as follows. When the plasma NT concentration appeared to be approaching steady state on day 6, the mean of the values of days 4, 5, and 6 was used as the value for day 8. When the plasma NT concentration appeared to be increasing on day 6,

TABLE II
BASIC DATA ON SUBJECTS STUDIED

Subject No.	Designation in Pedigree	Sex	Weight (kg)	Age	Plasma Nortriptyline Concentration, $\mu\text{g/ml}$ on day							
					1	2	3	4	5	6	7	8
1	A:I:1	m	100	74				72	85	88		103
2	A:II:1	f	63	46				70	91	112		
3	A:II:3	m	74	44	25	42	78			106		
4	A:II:5	f	63	43				60	78	95		114
5	A:II:7	m	76	42				99	112	123		143
6	A:II:8	m	70	40			59	69		107		147
7	A:II:10	m	80	38			59	89		107		146
8	A:II:11	m	80	32			17			28	29	34
9	A:II:12	m	101	29				58	60	62		
10	A:III:1	f	51	18				64	69	70		73
11	A:III:2	f	56	19				134	190	197		203
12	A:III:3	f	50	16				151	152	161		140
13	A:III:4	m	77	25				52	64	67		74
14	A:III:5	m	70	22				72	62	48		119
15	A:III:6	m	72	19				45	53	34		46
16	A:III:7	m	55	15				114	128	121		117
17	A:III:8	f	65	17				39	108	115		96
18	A:III:9	m	67	21				109	127	138		148
19	A:III:10	f	57	17				71	83	84		98
20	A:III:11	m	63	16				169	187	222		267
21	B:I:1	f	44	75				68	85	101		102
22	B:I:2	m	80	71				59	56	62		82
23	B:II:1	m	84	48				77	82	70		91
24	B:II:2	m	75	47				48	58	55		56
25	B:II:3	f	76	44				49	47	51		49
26	B:II:4	f	56	42	30			96	119	108		
27	B:II:6	m	84	35				49	56	48		61
28		f	45	67				170	299	386		428
29		f	55	65				141	110	176		
30	A:II:2	m	70	48				59	72	93		82
31	A:II:4	f	74	44				54	64	64		56
32	A:II:6	m	83	46				40	39	49		66
33	A:II:8	f	62	42				40	49	41		46
34		f	60	25				120	122	110		144
35		m	57	39				40	43	48		57
36		m	54	59				55	55	68		72
37		f	74	61				63	81	77		81
38		m	55	36				68	80	107		92
39		f	56	30				102	126	148		
40		m	60	25				34	57	65		65
41		f	76	26				42	49	57		60
42		f	65	49				55	56	54		71
43		f	62	21				38	35	28		28
44		f	59	21				85	96	105		97
45		f	65	21				49	36	59		29
46		f	62	21				47	51	67		54
47		m	77	25				74	96	90		107
48		m	60	22				90	89	82		133
49		m	77	32				33	38	41		48

the day 6 value was used as the day 8 value. The upper boundaries of the areas were obtained by joining the concentration values with straight lines.

Plasma Nortriptyline Concentrations on Day 6. $\log_{10}$ transformations of these values were approximately normally distributed. There was a significant regression of the $\log_{10}$, 6th-day plasma NT concentration on body weight (Table IV, row 1) and consequently the values were adjusted to a standard weight of 68 kg using the calculated regression coefficient. There was no significant regression of weight-adjusted $\log_{10}$, 6th-day plasma NT concentrations on age (Table IV, row 2).

The mean for 26 males was 1.90 (SE 0.04) and

for 18 females 1.93 (SE 0.04); t to compare means was 0.43. Hence there is no significant influence of sex on the measurement.

The means and standard deviations for relatives and random subjects are shown in Table V. Both the mean and the variance are significantly greater in all the relatives of high-level propositi than in all the random subjects.

Areas under Nortriptyline-day Curves.

The distribution of these values was skewed and could be rendered approximately normal by $\log_{10}$ transformation. A significant regression was found of $\log_{10}$ area on body weight (Table IV, row 3). Hence $\log_{10}$ area values were adjusted to a standard

TABLE III
INDIVIDUALS WITH DISEASE AND ON CONCOMITANT DRUG THERAPY

Subject No.	Designation in Pedigree	Alleged Disorder	Concomitant Drug Therapy	Included in Computations
6	A:II:8	Gout	Phenylbutazone for 2 days before NT test	Yes
11	A:III:2	Nil	Contraceptive steroids	No
17	A:III:8	Nil	Contraceptive steroids	No
19	A:III:10	Nil	Contraceptive steroids	No
21	B:I:1	Convalescing after surgery for simple oesophageal obstruction	Oral iron and folic acid	Yes
22	B:I:2	Cholecystitis	Papaverine 0.8 mg t.i.d. and a tetracycline (Tetralysal Erco) 150 mg 4 times daily	Yes
28		Rheumatoid arthritis	Digoxin 0.25 mg t.i.d. and acetyl salicylic acid 324 mg three times daily	Yes
33	A:II:8	'Run down'	Combination tablet (pentobarbital 50 mg and meprobamate 200 mg) nightly	No
43		Nil	Contraceptive steroids	No

TABLE IV
SOME REGRESSIONS COMPUTED FROM THE BASIC DATA

Row number	x	y	n	b	a	r	t	p
1	Body weight (kg)	Log ₁₀ 6th day plasma NT concentration	44	-0.0081	2.4647	-0.48	3.56	<0.001
2	Age (yr)	Ditto, adjusted for body weight	44	0.0022	1.8323	0.19	1.24	NS
3	Body weight (kg)	Log ₁₀ NT concentration day area	43	-0.0068	3.1272	-0.43	3.05	<0.01
4	Age (yr)	Ditto, adjusted for body weight	43	0.0013	2.6125	0.12	0.77	NS

n = number of pairs of observations
b = regression coefficient
a = intercept (ie, value of y when x = 0 in units of y)
r = correlation coefficient
t = to test for significance of b
p = significance level
NS = not significant at 0.05 level

body weight of 68 kg using the calculated regression coefficient. There was no significant regression of weight-adjusted log₁₀ area on age (Table IV, row 4).

The mean for 25 males was 2.65 (SE 0.04) and for 18 females 2.64 (SE 0.05); t to compare means was 0.32. Hence there is no significant influence of sex on this measurement.

The means and standard deviations for random subjects and relatives are shown in Table V. The mean for all the relatives of high-level propositi is significantly higher than that for all the random subjects.

Further Considerations of Results within Families. The two measurements of NT metabolism used were (1) log₁₀ 6th-day plasma NT concentrations adjusted for body weight and (2) log₁₀ area under plasma NT concentrations curve adjusted for body weight. They give very similar results as can be seen in Figs. 2 and 3, and Table V.

Pedigree A, Fig. 1, gives further relevant information. Full sibs of L.S. lie towards the upper end of the distribution curve for random subjects, while their spouses lie towards the centre of the same distribution. Offspring of the full sibs of L.S. give for measurement (1) a mean of 1.95 (SD 0.26), and for measurement (2) a mean of 2.73 (SD 0.20).

Discussion

Comparison of Results in Random Subjects and Relatives of High-level Propositi. The results of the high-level propositi were approximately at the upper tail of the distribution curve of their relatives and of the distribution curve of the random subjects.

This observation in itself suggested that a polymorphism (ie, variability controlled mainly by alleles at a single locus) was not present. Hence a polygenic control of the measurement under discussion seemed more likely.

TABLE V

SUMMARIZED RESULTS ON VARIOUS CATEGORIES OF SUBJECTS

Category of Subjects	Measurement					
	Log 6th-day plasma NT concentration, adjusted for body weight (C)			Log area under plasma NT concentration curve, adjusted for body weight (A)		
	n	Mean	SD	n	Mean	SD
Random subjects	18	1.8452	0.1319	18	2.5727	0.1546
All relatives of high level propositi	26	1.9648	0.2127	25	2.7263	0.1829
Offspring of full siblings of L.S.	7	1.9512	0.2585	7	2.7346	0.2012

COMPARISONS OF MEASUREMENTS C AND A BETWEEN CATEGORIES OF SUBJECTS

Categories Compared	t	DF	p
<i>Means</i>			
Random subjects, C vs all relatives of high-level propositi, C	2.12	42	<0.05
Random subjects, A vs all relatives of high-level propositi, A	2.89	41	<0.01
Random subjects, C vs offspring of full sibs of L.S., C	1.37	23	NS
Random subjects, A vs offspring of full sibs of L.S., A	2.16	23	<0.05
<i>Variances</i>			
Random subjects, C vs all relatives of high-level propositi, C	2.60	18; 26	<0.05
Random subjects, A vs all relatives of high-level propositi, A	1.40	18; 25	NS
Random subjects, C vs offspring of full sibs of L.S., C	3.84	18; 7	<0.05
Random subjects, A vs offspring of full sibs of L.S., A	1.69	18; 7	NS

n = number of observations
SD = standard deviation
DF = degrees of freedom

Lack of Effect of Age on Plasma Nortriptyline Concentrations. The incidence of adverse reactions in general rises with increasing age, and this observation remains valid when digitalis reactions are deducted (Hurwitz, 1969). This increased susceptibility of the elderly to adverse effects could theoretically be due to a diminished capacity to eliminate or metabolize drugs or to increased susceptibility of 'receptors'. The present work gives no evidence of major alterations in the kinetics of NT with increasing age.

Association of Side Effects with Plasma Concentration of Nortriptyline. The healthy relatives and random subjects tested in this study were given an explanation of the possible adverse effects of NT when their cooperation was obtained before starting drug therapy. During drug therapy and

FIG. 2. Distributions of $\log_{10}$ plasma nortriptyline on the 6th day, adjusted for body weight, for various categories of subjects. FIG. 3. Distributions of $\log_{10}$ area under plasma nortriptyline concentration curve, adjusted for body weight, for various categories of subjects.

before the analyses of plasma NT concentrations, a record was made of whether they volunteered complaints of side effects. Individuals have been categorized as to whether the value of plasma NT concentration on day 6 was larger or smaller than the median of the 44 subjects examined. A test for the association of adverse effects and plasma NT concentration is shown in Table VI and is significant. The exact probability is 0.0309 for one tail and 0.0618 for two tails. Since the biological situation suggests that persons with low plasma levels are not likely to have more side effects than persons with high plasma levels, the one tailed test can be

TABLE VI
ASSOCIATION OF SIDE EFFECTS AND PLASMA
CONCENTRATION OF NORTRIPTYLINE

Side Effects	Plasma NT Concentration		Total
	Below Population Median	Above Population Median	
Not volunteered	17	10	27
Volunteered	5	12	17
Total	22	22	44

$$\chi^2 = 4.7$$

considered appropriate. The finding is in agreement with recent observations in depressed patients treated with nortriptyline (Åsberg *et al*, 1970).

The Nature of the Genetic Control of Nortriptyline Plasma Concentrations. There are two main explanations to be considered. (1) That NT plasma levels are controlled as a polymorphism with 'high-level' propositi being genetically constituted as rare recessives, and (2) that NT plasma levels are controlled by a polygenic system with 'high-level' propositi being selected from the high end of the distribution curve of the population.

In favour of the first explanation was the finding of a separate mode of high-level propositi in the histogram of Sjöqvist *et al* (1968). It must be realized, however, that this distribution is skewed and had not been normalized by $\log_{10}$ transformation. In addition, the analysis of the data is complicated by the fact that the subjects under study were patients and had received various types of psychotropic drugs, including barbiturates, before the study.

In favour of the second explanation are the following: (a) both mean and variance of $\log_{10}$ sixth-day plasma NT concentrations and mean of $\log_{10}$ areas under NT plasma concentration curves are larger in relatives of high-level propositi than in random subjects. (b) The full sibs of high-level propositi studied did not appear to fall into two modes. However, since only 12 full sibs were studied it could be (on the monogenic hypothesis) that only one other recessive phenotype was found; the probability of this occurring is 0.158. (c) The offspring of full sibs of high-level propositi do not appear to form two separate modes. Their mean $\log_{10}$ area under plasma NT concentration curve and the variance of $\log_{10}$ 6th-day plasma NT concentration are larger than those found in random subjects.

There seems to be little definitive evidence against the second explanation. It would therefore appear very probable that NT kinetics in man

is under polygenic control. From the evidence available it is not possible to say either how many loci, or how many alleles at a given locus exercise genetic control of NT plasma concentrations. The results of Alexanderson *et al* (1969) in non-drug treated fraternal twins indicate considerable intra-pair differences in only 4 out of the 11 twinships studied. This might hint that the number of genes involved in the control of NT plasma concentrations is small.

Summary

Twenty-nine relatives of 3 patient propositi developing extremely high plasma concentrations of nortriptyline and 20 random subjects were given 25 mg nortriptyline orally three times daily during 8 days. Plasma concentrations of nortriptyline were determined on days 4, 5, 6, and 8. Forty of the subjects were healthy and had not been exposed to drugs or chemicals 3 months before the study. Of the remaining 9 subjects, 5 were excluded from all further calculations because they had received barbiturates or contraceptive steroids.

Two measurements were used to characterize individuals: (1) plasma concentration of nortriptyline on the sixth day of treatment and (2) the area under the curve obtained by plotting nortriptyline concentrations (y) against days of treatment (x). The distributions of both were skewed but could be rendered approximately normal by $\log_{10}$ transformation. There was a significant regression of both on body weight, hence all values were adjusted to a standard body weight. There was no correlation between weight-adjusted values and age, nor any difference between males and females. There was a significant association between the occurrence of side effects and a plasma concentration above the population median.

There was no tendency towards bimodality in the distributions of all subjects, all relatives or all full siblings of high-level propositi on either of the measurements. The variances were larger in relatives of high-level propositi than in random subjects, and the mean of estimate (2) was larger in the relatives than in the random subjects.

Spouses and offspring of the full sibs of one of the propositi were tested. While the values of the full sibs on both measurements were at the upper tail of the distribution of the random subjects, the spouses were in the centre of the distribution. In the offspring of the full sibs, the variance of measurement (1) and the mean of measurement (2) were larger than in the random subjects.

It is concluded that the manner of inheritance of nortriptyline kinetics is likely to be polygenic.

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