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Androsterone/Etiocholanolone Ratios in Male Homosexuals

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Summary

Analyses of 24-hour specimens of urine from healthy adult males for androsterone and etiocholanolone produced values which, when calculated as discriminant scores, discriminated between heterosexual and exclusively homosexual individuals. This confirms a previous study.

No significant differences were found between heterosexuals and homosexuals in parental ages, secondary sex characteristics, genitalia, anthropometry, 17-ketosteroids, and 17-ketogenic steroids.

A significant difference was found between the heterosexual group and homosexual group in the number of homosexual relatives in the immediate and extended families.

Introduction

In the past few years the concept of a biological basis for homosexual behaviour has been reconsidered as a consequence of more precise investigative techniques. A previous study by Margolese (1970) showed that healthy adult males excreted androsterone and etiocholanolone in ratios that clearly discriminated between heterosexual and homosexual subjects. This was followed by a study by Lorraine (1970) who found low urinary testosterone in two homosexual males and raised urinary testosterone in four homosexual females. The idea that changes in endocrine function might contribute to the genesis of homosexuality in humans was then given further impetus by Kolodny *et al.* (1971) who showed that plasma testosterone levels of a homosexual group were significantly lower than those of a control group. When investigating several biochemical parameters Evans (1972) found that homosexuals had a lower androsterone/etiocholanolone (A/E) ratio, and higher 11-ketoetiocholanolone than heterosexuals.

Testosterone, the major natural androgen, is largely secreted by the testis as a result of stimulation by the luteinizing hormone from the anterior pituitary. The adrenal cortex secretes a small amount of testosterone and dehydroepiandrosterone (DHA), which may also be a precursor of testosterone. The intermediate metabolism of testosterone

has not been fully clarified. The liver converts testosterone by reduction to androsterone and etiocholanolone, which are stereo isomers, and they are conjugated mainly as glucuronides, and excreted in the urine. These two compounds plus DHA are the principal compounds making up the urinary 17-ketosteroids (17-KS), and are included in the analysis of urinary 17-KS. Of the total 17-KS about two-thirds come from the adrenal cortex chiefly as DHA. When tested by biological assay almost all of the androgenic activity is found to be due to androsterone. The average normal adult 17-KS excretion is 14 mg/24 hr for men with a range of 9-22 mg by the assay method used.

The present study was undertaken to investigate a larger number and greater variety of subjects than previously, and to provide a more detailed psychological evaluation.

Subjects and Methods

A total of 63 male subjects were studied, which included two sets of identical twins. The heterosexual controls were obtained from college student populations. The homosexual subjects were obtained from four homosexual organizations (see acknowledgements).

One of us (M.S.M.) was responsible for the physical evaluation and endocrine studies. This consisted of a brief metabolic history, determination of blood pressure and pulse, and notations of sexual hair distribution, genitalia, and anthropometric measurements. A blood sample was drawn for determination of the SMA 12 blood chemistry profile and free thyroxine. Each subject submitted a 24-hour specimen of urine which was analyzed for 17-ketosteroids, androsterone, and etiocholanolone. In the case of six homosexuals the urines were also analyzed for 17-ketogenic steroids. All blood and urine determinations were done by Bio-Science Laboratories of Van Nuys, California. The 17-ketosteroids were determined by a modification (Sobel *et al.*, 1958) of the method of Dreker *et al.* (1952). Androsterone and etiocholanolone were determined by the method of Brooks (1958).

In the previous study a statistical analysis was done on the androsterone and etiocholanolone values by linear discriminant analysis. This provided a factor for androsterone (0.231) and for etiocholanolone (0.190) and their difference (0.231A-0.190E) gave a discriminant score. These factors are being used in the present study as a test of their ability to discriminate between two new populations.

Each subject was interviewed by one of us (O.J.) for one and a half to two hours, using a modified Kinsey sexual inventory. The Kinsey heterosexual-homosexual rating scale (Kinsey *et al.*, 1948) was used for classifications, as follows: 0 exclusively heterosexual; 1 predominantly heterosexual, only incidentally homosexual; 2 predominately heterosexual, but more than incidentally homosexual; 3 equally heterosexual and homosexual; 4 predominately homosexual, but more than incidentally heterosexual; 5 predominately homo-

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sexual, but incidentally heterosexual; 6 exclusively homosexual. Data were also obtained in the interview to test Slater's (1962) hypothesis regarding the relation of homosexuality to parental age at birth, and notations were made of the incidence of homosexuality in the immediate and extended families. The Kinsey classifications and the laboratory discriminant scores were arrived at independently. Each subject was asked to estimate the frequency of sexual activity during the three preceding months. Sexual activity was defined as any action that resulted in orgasm. They were also asked to state the time interval between last orgasm and the urine collection. This information was elicited to determine if sexual activity in the control and homosexual groups was comparable, and if the time of orgasm before urine collection might alter the A/E ratio. In addition each subject included in the study, on close questioning, denied the use of drugs, particularly barbiturates or psychotropic drugs. The one transsexual additionally denied the use of oestrogen.

If the physical or psychiatric examination revealed any abnormality the subject was placed in the "non-healthy" category. The subjects were classified as follows: 24 controls (Kinsey 0-1); 23 homosexuals (Kinsey 5-6); 9 intermediate (Kinsey 2-3-4); 1 transsexual. Five heterosexual subjects were classified as non-healthy, three of these because of depression, and two for physical reasons—hypothyroidism and hypercorticoadrenalism. One homosexual (case 62) was classified as non-healthy because of a raised SGOT level (205 units/ml).

Results

Physical examinations showed no significant difference in pubic hair distribution, genital size, or anthropometry between controls and homosexuals.

The free thyroxine values of all subjects were within normal limits with the exception of one heterosexual (subject 58) who was in the hypothyroid range and was therefore classified as non-healthy. Hellman *et al.* (1959) has shown that reversal of the A/E ratio may be associated with thyroid deficiencies.

It has been suggested that the psychological stress that many homosexuals undergo may account for the change in A/E ratio. Kreuz (1972) has shown that psychological stress may result in suppression of plasma testosterone. Since this involved a simultaneous rise in 17-hydroxycorticosteroids, the latter levels were determined as 17-ketogenic steroids in the urine of six homosexuals. All six values were within normal limits. Since urinary 17-ketogenic steroids are generally raised as a result of severe stress, these normal values would not necessarily exclude minor or moderate degrees of stress in the homosexuals as a consequence of their psychosocial situation.

Biographical data obtained during the psychiatric interview, when analyzed statistically by Pearson product-moment correlations, showed no significant relation between Kinsey ratings and mother's age, father's age, or parental age difference at the time of the subject's birth. There was, however, a very significant difference ($P < 0.001$) in the incidence of consanguineous homosexual relatives. Of 24 heterosexuals (Kinsey 0-2) two reported homosexual relatives. Of 28 homosexuals (Kinsey 4-6) 17 reported homosexual relatives, five of whom had two each.

The data obtained at two different times for a homosexual and on four occasions for a heterosexual are given in table I. Though the androsterone and etiocholanolone values fluctuate the variation in A/E ratios is small, and the difference in discriminant scores not significant. Statistically, the data of all healthy subjects show a significant relation between androsterone and etiocholanolone ($P < 0.001$). That

is, subjects having higher androsterone values tend to have higher etiocholanolone values. Therefore the use of single determinations for androsterone and etiocholanolone is valid for a given state of health.

The data for androsterone and etiocholanolone values, A/E ratios, and discriminant scores are presented in table II. The

TABLE I—Variations in Values of Androsterone (A), Etiocholanolone (E), A/E Ratios, and Discriminant Scores (D.S.) with Time

Interval	17-KS	A	E	A/E	D.S.
	(mg/24 hr)				
Homosexual					
0	17	2.4	2.5	0.96	0.079
11 months	20	3.2	3.5	0.91	0.074
Heterosexual					
0	17	3.3	2.4	1.4	0.31
3 weeks	20	3.5	3.1	1.1	0.22
18 months	13	2.2	1.4	1.6	0.24
21 months	16	2.8	1.9	1.5	0.28

TABLE II—Values of 17-Ketosteroids (17-KS), Androsterone (A), Etiocholanolone (E), A/E Ratios, and Discriminant Scores (D.S.) (K. = Kinsey Scale)

Subject No.	Age	17-KS	A	E	A/E	D.S. (0.231A-0.190E)
		(mg/24 hr)				
Male Controls K.O-1 ("Healthy")						
1	45	13	3.0	0.9	3.3	0.52
2	24	20	3.9	2.5	1.6	0.42
3	21	20	3.3	1.9	1.7	0.40
4	22	21	3.3	1.9	1.7	0.40
5	21	14	3.7	2.6	1.4	0.37
6	25	17	2.6	1.3	2.0	0.37
7	18	11	2.4	1.0	2.4	0.31
8	19	13	2.9	1.9	1.5	0.31
9	28	13	2.9	1.9	1.5	0.31
10	24	17	2.1	1.0	2.1	0.30
11	24	16	2.0	1.1	1.8	0.28
12	18	20	3.5	2.8	1.2	0.28
13	39	11	2.5	1.6	1.5	0.27
14	23	11	2.4	1.8	1.3	0.27
15	25	19	1.8	0.8	2.2	0.26
16	28	30	3.7	3.2	1.1	0.24
17	27	8	1.6	0.8	2.0	0.22
18	30	21	2.1	1.5	1.4	0.19
19	34	10	1.6	1.0	1.6	0.18
20	19	15	1.9	1.4	1.3	0.17
21	19	14	2.1	1.8	1.1	0.15
22	53	15	1.8	1.4	1.2	0.15
23	22	12	1.7	1.5	1.1	0.12
24	25	19	3.4	3.5	1.0	0.12
Male K.2-3-4 ("Healthy")						
25	22	18	4.8	3.8	1.2	0.38
26	16	11	2.2	0.7	3.1	0.36
27	39	21	2.8	1.7	1.6	0.31
28	39	21	2.4	1.4	1.7	0.29
29	25	16	2.7	1.9	1.4	0.28
30	16	11	1.7	0.9	1.9	0.22
31	17	15	1.8	1.1	1.6	0.21
32	20	11	0.8	0.7	1.1	0.06
33	26	12	1.4	1.5	0.9	0.05
Male K.5 ("Healthy")						
34	21	13	2.8	1.7	1.6	0.32
35	32	18	2.1	1.2	1.7	0.26
36	23	24	2.4	1.7	1.4	0.23
37	32	10	1.9	1.5	1.2	0.15
38	20	10	2.0	1.7	1.2	0.14
39	46	8	1.2	0.8	1.5	0.12
40	19	16	3.1	3.2	1.0	0.11
41	35	15	1.3	1.2	1.1	0.07
42	18	12	1.6	1.1	1.4	0.15
43	29	7	0.5	1.1	0.4	0.10
44	32	21	2.3	2.3	1.0	0.09
45	33	31	2.4	2.5	1.0	0.08
46	26	15	2.2	2.3	0.9	0.07
47	30	24	2.0	2.1	0.9	0.07
48	28	16	1.3	1.8	0.7	0.04
49	20	7	1.3	1.4	0.9	0.03
50	50	18	1.5	1.7	0.9	0.03
51	50	25	1.8	2.8	0.6	-0.14
52	26	20	3.5	6.8	0.5	-0.05
53	39	16	1.6	2.8	0.6	-0.16
54	28	11	1.7	3.0	0.6	-0.19
55	28	8	1.7	3.5	0.5	-0.26
56	19	12	1.2	3.4	0.3	-0.37
"Non-healthy" Males						
57	26	17	2.6	2.7	1.0	0.09
58	26	17	2.4	2.5	1.0	0.07
59	25	14	1.2	1.2	1.0	0.05
60	29	21	3.9	3.2	1.2	-0.002
61	17	15	1.3	3.6	0.4	-0.38
62	29	21	1.6	4.3	0.4	-0.43

total 17-KS were within normal limits, or in a few subjects had values slightly below the lower limit or slightly above the upper limit of the normal range. Two subjects had values significantly above the upper limit of the normal range, one homosexual with a value of 31 mg/24 hr, and one heterosexual control with a value of 30 mg. Of the 24 controls 23 had androsterone values greater than etiocholanolone, whereas of the 15 Kinsey scale 6 subjects 14 had etiocholanolone values equal to or greater than androsterone. It may be significant that of the two sets of twins (subjects 27, 28 and 50, 51) each individual had a discriminant score similar to his twin brother.

In fig. 1 the discriminant scores are plotted against the Kinsey ratings. The mean discriminant scores (S.D.) were as follows (table III): control group 0.28 (0.10); Kinsey 2-3-4 group 0.24 (0.11), which was not significantly different from the control group; Kinsey 5 group 0.18 (0.08), which was significantly different from the control group ($P < 0.02$); and Kinsey 6 group -0.03 (0.14), which was highly significant ($P < 0.001$). It is also of interest to note that the non-healthy group had a mean score of -0.10 (0.22) and this was not significantly different from the Kinsey 6 group. The one transsexual had an androsterone/etiocholanolone ratio of 1.2, and a discriminant score of 0.09, which could be categorized as either a homosexual or a healthy heterosexual female (preliminary data on females).

FIG. 1—Relation of discriminant scores (0.231A-0.190E) to Kinsey ratings of "healthy" and "non-healthy" males. Horizontal bars denote mean for each group.

TABLE III—Mean Values (S.D.) of 17-Ketosteroids (17-KS), Androsterone (A) Etiocholanolone (E), A/E Ratios, and Discriminant Scores (D.S.). K. = Kinsey Scale

	Age	17-KS (mg/24 hr)	A	E	A/E	D.S. (0.231A-0.190E)
Controls	26	15.8	2.6	1.7	1.6	0.28 (0.10)
Healthy K.2-3-4	24	15.1	2.3	1.5	1.6	0.24 (0.11)
Healthy K.5	28	14.2	2.1	1.6	1.2	0.18 (0.08)
Healthy K.6	30	16.2	1.7	2.6	0.7	-0.03 (0.14)
Non-healthy	25	17.5	2.1	2.9	0.8	-0.10 (0.22)

Androsterone values plotted against etiocholanolone values for the control group and the Kinsey 6 group are

shown in fig. 2. While this ratio is not as good a discriminator as the A/E ratio, there is an obvious difference between the two groups.

The mean (S.D.) weekly frequency of sexual activity of the 24 healthy controls was 4.1 (2.2), of the 32 healthy homosexuals 3.7 (2.2), of the six non-healthy subjects 1.5 (0.8). Statistical analysis of the the control and homosexual data showed $t = 0.795$, D.F. = 56; not significant. The non-healthy and control groups showed $t = 2.758$, D.F. = 28; $P < 0.025$, and the non-healthy and homosexual groups showed $t = 2.257$, D.F. = 36; $P < 0.05$. Thus there was no significant difference in sexual activity between the control and homosexual groups. The non-healthy group had a significantly lower frequency of sexual activity than both control and homosexual groups.

The mean interval between last orgasm and urine collection was two days for the control group, two days for the homosexual group, and five days for the non-healthy group. Thus sexual activity before urine collection was the same for both the control and homosexual groups.

FIG. 2—Relation of androsterone (A) to etiocholanolone (E) in male homosexual and heterosexual subjects.

Discussion

This study confirms the results of the original investigation that healthy male homosexuals have a significantly different A/E ratio and discriminant score than healthy heterosexuals. While this correlation may be a necessary condition for homosexuality, the data for the non-healthy subjects show that it is not sufficient since it does not distinguish between homosexuality and other conditions having the same end metabolic result. Zumoff *et al.* (1971) has shown that there is a decreased conversion of androgen to normal 17-ketosteroid metabolites as a consequence of non-specific illness. He also showed that the recovery of androsterone was decreased to a greater extent than that of etiocholanolone and that the sum of androsterone and etiocholanolone was also decreased. In the present study the mean of the sums of androsterone and etiocholanolone of the control group (4.30) was not significantly different from that of the Kinsey 6 group (4.36), and this was also true in the previous study. This may be interpreted to mean that the decreased A/E ratio in male homosexuals is not a non-specific illness effect, but may be due to a shift in metabolic pathway. Further studies would be needed to establish this. Hatotani *et al.* (1962) found in subjects with atypical periodic psychoses that during the psychotic episode there was a reversal of the A/E ratio due to the relative decrease of androsterone and a return to the normal range during the lucid interval.

There is a parallel between the finding of Kolodny *et al.* (1971) of a decrease in plasma testosterone and the finding

of this study of a reversal of A/E ratio, in that each was significantly different from its respective control group only in the exclusive homosexual (Kinsey 5-6). The significance of this remains to be determined.

While we do not yet have a clear answer as to the relation of the endocrine changes to the direction of sexual drive, this study lends further support to the hypothesis previously proposed: that the metabolic pathway which results in a relatively high androsterone value is associated with sexual preference for females by either sex, whereas a relatively low androsterone value is associated with sexual preference for males by either sex.

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MEDICAL MEMORANDA

Hepatitis after Tattooing: A Fatal Case

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Tattooing as a means of transmitting serum (long incubation) hepatitis is well known and has been reported by Roberts and Still (1950) in Canada, by Smith (1950) in the U.S.A., and in Britain by Robertson (1951) who referred to the unpublished work of Hendry, by Mowat *et al.* (1973), and by Hobson *et al.* (1952) describing cases in Hong Kong. We wish to report a fatal case of hepatitis after tattooing.

Case Report

A bricklayer, aged 22 years, was notified to one of us (G.B.H.) by a member of the Liver Unit at King's College Hospital (I.H.) as a case of serum hepatitis. There was circumstantial evidence in-

criminating tattooing of a crucifix on the forearm as the probable source of the infection, though tattooing preceded onset by only about 30 days and obvious jaundice by only about 43 days. He had been tattooed with three companions on 6 May 1972, a date which was verified by association with the F.A. Cup Final. He was admitted to Salisbury Hospital with obvious jaundice on 19 June with a history of about two weeks' illness (D.P.M.) and he died on 10 August. Serum taken from him on 20 and 24 July contained Australia antigen but no antibody (gel and electrophoresis, J.V.T.G.).

There was no history of any other recent injections or tattoos, though he had been tattooed previously during the summer of 1971. The pathological diagnosis at necropsy (E.A.W.) was staphylococcal septicaemia associated with massive hepatic necrosis due to serum hepatitis. The liver (1,540 g) was of about normal size but showed a severely atrophied and shrunken left lobe and a nodular surface over the right lobe. Histologically the chief appearance was of almost complete necrosis with some regenerating nodules. There were also some areas of normal pattern with pronounced centrilobular necrosis. There were numerous acute purulent abscesses in the lungs, kidneys, and heart. The left antecubital vein contained a laminated organizing thrombus, in which Gram-positive cocci in grape-like clusters were present. Shortly before death a blood culture had been found positive for *Staphylococcus aureus*.

On 4 August one of us (D.J.N.M.) traced the three men tattooed in company with the victim and took blood from each which was examined at the Central Public Health Laboratory, Colindale (gel and electrophoresis). The two men first and second in order of tattooing had not previously been tattooed and were negative for Australia antigen and antibody. The third man, who immediately preceded the victim in order of tattooing, was Australia antigen positive and antibody negative. He had been tattooed nine times in all at Portsmouth, Southampton, Brighton, London, and Dorset. He gave no history of viral hepatitis and was in good health, but stated that he had been heavily jaundiced at birth. The tattooist himself was heavily tattooed, the most recent one two years previously, but his blood, taken on 4 October 1972, was Australia antigen negative and antibody negative (gel and electrophoresis, Central Public Health Laboratory, Colindale).

TATTOOIST'S PREMISES

The tattooist involved uses four electrically operated machines, one for each colour. They vibrate through an amplitude of about $\frac{1}{16}$ in (1.6 mm), a solid detachable needle projecting a maximum of about $\frac{1}{4}$ in (3 mm) through a fixed conical metal collar with a small clearance to avoid friction and allow free flow of pigment. The solution of pigments forms a small reservoir by capillary

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