Shimane J. Med. Sci. Vol. 1, pp. 49—59, 1977

Changes in Urinary Neutral 17-Ketosteroid Excretion Patterns with Age in Normal Subjects and Changes in Patients with Peptic Ulcer

(urinary neutral 17-KS fractions/aging/peptic ulcer)

SHINYA NOTE
Department of Internal Medicine, Shimane Medical University, Izumo, Japan

MIYOJI OHASHI
Yoro Central Hospital, Ogaki, Japan

YUJI WAKAYAMA
Department of Internal Medicine, Shimane Prefectural Hospital, Izumo, Japan

(Received October 15, 1977)

(1) The average daily excretion values in dehydroepiandrosterone (DHA), androsterone (A) and 11-deoxygenated 17-KS were very lower in children and the levels proved to become higher at onset of puberty, to reach the maximum in young adults and then to decrease gradually by aging. But the value of 11-oxygenated 17-KS, which were metabolized mainly from adrenal steroids, was not so much decreased with advancing age after young adulthood. The endogenous secretion of androgens, which contributes to the accelerating of protein anabolic process in the body, appeared to be depressed at the senile stage.
(2) In patients with gastric ulcer of simple or multiple type, average daily excretion value of DHA, A and A+etiocholanolone (E) was decreased accompanying with the diminished value of A/E ratio and the abnormal findings were normalized after wound healing. In spite of decreased A excretion value, the elevation of DHA (%) was recognized in the group of refractory ulcer type. A characteristic picture in patients with duodenal ulcer was found in the increase in total 17-OHCS with decreased DHA and A excretion values. These data indicate that a decrease in endogenous androgen secretion may play an important part in the peptic ulcer formation.

Improvements in the technique of fractionation have made it possible to use urinary 17-KS excretion to determine more accurately androgenic activities in both physiological and pathological conditions (1, 2).

This study was performed first to investigate changing androgenic activities with aging in healthy human subjects and second to clarify the influence of androgens in the pathogenesis of peptic ulcer. Although it is more common at the present time to determine plasma androgenic steroids, such as dehydro-

epiandrosterone sulfate (DS) and testosterone, by means of radioimmunoassay (RIA) (3, 4) as indexes of androgenic activity in the human body, in our studies we measured urinary 17-KS fraction patterns according to Ohashi's method.

MATERIALS AND METHODS

(A) Changes in androgenic activity with aging were studied by determining the 17-KS excretion patterns of five age groups of human subjects : A (6-7 yr, 3 males and 3 females), B (17-24 yr, 5 males and 5 females), C (45-60 yr, 5 males and 5 females), D (63-79 yr, 5 males and 6 females), E (80 yr and over, 10 subjects).

Gastric ulcer patients were divided into three groups, simple, multiple and refractory ulcers, on the basis of endoscopic, X-ray and clinical findings. Urinary steroids, especially the 17-KS fraction, were measured in each group. There were eight patients with only one ulcer (simple ulcer type) and five patients with two or more ulcers (multiple ulcer type). Five patients whose ulcers could not be cured by continuous medical treatment for six months or more comprised the refractory ulcer group. Two patients with duodenal ulcer were also examined, and seven subjects without any ulcers were selected as control subjects.

(B) Aliquots of 24-hr urine specimens from subjects in each group were pooled. Neutral 17-KS were extracted after enzymatic hydrolysis, solvolysis in ethyl acetate and hot acid hydrolysis (5). Chromatographic fractionation of urinary 17-KS was performed by means of gradient elution chromatography on an alumina column using a modification of the technique of Lakshmanan and Lieberman (6). After the fractionation, aliquots of 1.0 ml were taken from each tube and tested by the Zimmermann reaction. Plotting of the optical density values against the fraction gave a graphic representation. Standards were run on a line parallel to the fraction being tested. The reference standards used were : dehydroepiandrosterone (DHA), androsterone (A), etiocholanolone (E), 11-ketoetiocholanolone (11-O-E), 11-hydroxyetiocholanolone (11-OH-E). By this procedure the sum of 17-KS was separated by fractions as shown in Fig. 1. In our laboratory the first and second fractions contain various unidentified steroids : the third (III) consists mainly of DHA ; the fourth (IV) contains A ; the fifth (V) has E ; the fractions after the sixth (VI) are said to contain corticoid metabolites such as 11-hydroxy or 11-keto forms of 17-KS. Our method appears to separate androsterone (which accounts for most of the androgenic activity of urine) from its isomer, etiocholanolone (which has practically no androgenic activity). Dehydroepiandrosterone, the second most active androgen normally present in large quantities in urine, is set apart in the third peak ; 11-hydroxyetiocholanolone, the bulk of which is derived from cortisol secreted by the adrenal cortex, is contained in the ninth fraction.

Total neutral urinary 17-ketosteroids (17-KS) were measured by Miyake method and total urinary 17-hydroxycorticosteroid (17-OHCS) determination accorded on a modification of the method of Reddy using Porter-Silber colour reaction (7).

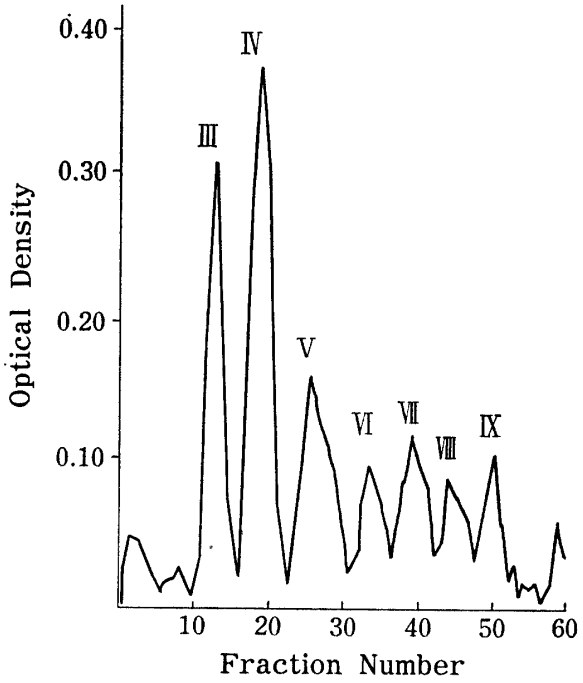

Fig. 1. Graphic representation of optical density values plotted against the fraction number.
III : Dehydroepiandrosterone (DHA)
IV : Androsterone (A)
V : Etiocholanolone (E)
VI : 11-ketoandrosterone (O-A)
VII : 11-ketoetiocholanolone (O-E)
VIII : 11 β-hydroxyandrosterone (HO-A)
IX : 11 β-hydroxyetiocholanolone (HO-E)

RESULTS

A. Data in Normal Subjects

(1) Group A

The average daily excretion values of individual 17-KS in normal male and female children were 0.28 mg and 0.26 mg as DHA ; 0.31 mg and 0.25 mg as A ; 0.31 mg and 0.38 mg as E ; 0.15 mg and 0.19 mg as 11-ketoandrosterone (11-O-A) ; 0.12 mg and 0.18 mg as 11-ketoetiocholanolone (11-O-E) ; 0.12 mg and 0.18 mg as 11-hydroxyandrosterone (11-OH-A) ; 0.17 mg and 0.19 mg as 11-hydroxyetiocholanolone (11-OH-E) and 1.66 mg and 1.88 mg as the sum of the fractions (Table I).

The ratio of 5α-metabolite to 5β-metabolite of 11-oxygenated 17-KS was 0.84 in males and 0.91 in females. Sex differences in the excretion of each 17-KS and in the ratios between the individual 17-KS were not significant in children.

(2) Group B

The average daily excretion values of individual 17-KS in young males and females were 2.37 mg and 1.65 mg as DHA ; 2.37 mg and 1.50 mg as A ; 1.81 mg and 1.66 mg as E and the sum of 11-oxygenated 17-KS (Table II).

In group B, a high excretion of 11-deoxygenated 17-KS was observed, and

TABLE I. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Normal Subjects (8–10 yr)*

Mean±S. D.		DHA	A	E	O-A	O-E	HO-A	HO-E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
M (n=3)	mg/24 hr	0.28 ±0.16	0.32 ±0.20	0.31 ±0.16	0.15 ±0.07	0.15 ±0.07	0.12 ±0.05	0.17 ±0.09	0.91 ±0.51	0.59 ±0.27	0.70 ±0.11	0.97 ±0.24	1.16 ±0.88	0.96 ±0.09
	mg/24 hr/kg	0.011 ±0.004	0.012 ±0.007	0.013 ±0.004	0.006 ±0.002	0.006 ±0.002	0.004 ±0.003	0.007 ±0.003	0.035 ±0.017	0.030 ±0.019			0.064 ±0.026	0.037 ±0.006
F (n=3)	mg/24 hr	0.27 ±0.12	0.25 ±0.05	0.38 ±0.04	0.19 ±0.06	0.18 ±0.04	0.18 ±0.04	0.26 ±0.11	0.90 ±0.18	0.81 ±0.21	0.89 ±0.07	0.65 ±0.13	1.89 ±0.43	1.15 ±0.38
	mg/24 hr/kg	0.010 ±0.006	0.009 ±0.002	0.015 ±0.003	0.008 ±0.003	0.007 ±0.001	0.007 ±0.002	±0.011 0.006	0.035 ±0.007	0.032 ±0.010			0.076 ±0.019	0.046 ±0.011

Abbreviation : M=males ; F=females ; S. D.=standard deviation DHA=dehydroepiandrosterone ; A=androsterone ; E=etiocholanolone ; O-A=11-keto-androsterone ; O-E=11-keto-etiocholanolone ; HO-A=11-hydroxy-androsterone ; HO-E-11=hydroxy-etiocholanolone ; 11-deoxy=11-deoxygenated, 17-KS ; 11-oxy=11-oxygenated, 17-KS ; oxy/deoxy=ratio of 11-oxygenated, 17-KS to 11-deoxygenated, 17-KS ; A/E=ratio of androsterone to etiocholanolone.

TABLE II. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Normal Subjects (17–23 yr)*

Mean±S. D.		*DHA	A	E	O-A	O-E	HO-A	HO-E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
M (n=5)	mg/24 hr	2.37 ±0.42	2.73 ±0.58	1.81 ±0.31	0.53 ±0.05	0.76 ±0.12	0.66 ±0.13	0.77 ±0.15	6.91 ±1.16	2.72 ±0.42	0.39 ±0.03	1.51 ±0.25	10.11 ±1.65	4.34 ±0.53
	mg/24 hr/kg	0.041 ±0.004	0.047 ±0.005	0.031 ±0.004	0.009 ±0.002	0.013 ±0.002	0.011 ±0.003	0.013 ±0.004	0.117 ±0.016	0.047 ±0.005			0.174 ±0.023	0.075 ±0.008
F (n=5)	mg/24 hr	1.65 ±0.32	1.50 ±0.24	1.66 ±0.19	0.33 ±0.03	0.63 ±0.16	0.38 ±0.08	0.60 ±0.15	4.82 ±0.67	1.93 ±0.35	0.40 ±0.04	0.90 ±0.06	7.59 ±1.08	3.85 ±0.68
	mg/24 hr/kg	0.032 ±0.007	0.030 ±0.004	0.033 ±0.003	0.006 ±0.003	0.012 ±0.004	0.007 ±0.003	0.012 ±0.002	0.095 ±0.009	0.038 ±0.006			0.149 ±0.019	0.075 ±0.010

* See Table I for abbreviations.

both DHA and A were higher in males than in females. The ratio of A to E was 1.52 in males and 0.90 in females. Sex differences in both DHA and A excretion and A/E ratio were significant in group B.

(3) Group C

In the five males and five females of group C, the average daily excretion values of each 17-KS were 1.67 mg and 1.10 mg as DHA ; 2.11 mg and 1.35 mg as A ; 1.50 mg and 1.58 mg as E and 2.67 mg and 1.97 mg as the sum of 11-oxygenated 17-KS (Table III). A lower excretion of 11-deoxygenated 17-KS, especially DHA and A, was observed in group C than in group B. The decrease in both 17-ketosteroids was greater in males than in females.

(4) Group D

In group D, the average daily excretion of 11-deoxygenated 17-KS, especially A and DHA, was lower than in groups B and C, DHA being 1.41 mg in males and 0.73 mg in females, and A being 1.65 mg in males and 0.89 mg in females (Table IV). Sex differences were not significant for DHA but were for A at this age.

(5) Group E

The decrease in each 17-KS fraction was still greater in group E. However, 11-oxygenated 17-KS were less markedly decreased than 11-deoxygenated 17-KS (Table V). Accordingly, in group E the ratio of 11-oxygenated 17-KS to 11-deoxygenated 17-KS was higher than in group B, but the ratio of A to E was lower than in the males of group B. In group E, the average daily excretion values of each 17-KS fraction were 0.50 mg as DHA, 0.72 mg as A, 0.83 mg as E, 0.30 mg as 11-OH-A, 0.36 mg as 11-O-E, 0.41 mg as 11-OH-A, 0.54 mg as 11-OH-E, 2.05 mg as total 11-deoxygenated 17-KS, and 1.60 mg as the sum of 11-oxygenated 17-KS. Sex differences in each fraction had disappeared completly by this age.

B. Data in Patients with Peptic Ulcer (Table VI, VII, Fig. 2)

(1) Single Ulcer

In patients with simple ulcer, the urinary excretion of DHA, A and A+E was decreased. Accordingly, the A/E ratio of patients was lower than in non-ulcer subjects.

(2) Multiple Ulcer

In subjects with multiple gastric ulcers, both fractions III (DHA) and IV (A), were greatly decreased along with a marked decreased in the A/E ratio as compared with that in simple ulcer patients. During the course of healing of the ulcer, the decreased excretion of androsterone increased gradually and the high value of etiocholanolone fell to normal levels (Fig. 2).

(3) Refractory Ulcer

In patients with gastric ulcer, the most striking difference between the refractory and multiple ulcer groups was in the excretion of DHA (%) : that is, the value was higher in refractory patients and lower in the other groups.

TABLE III. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Normal Subjects (45–58 yr)*

Mean±S. D.		*DHA	A	E	O-A	O-E	HO-A	HO-E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
M (n=5)	mg/24 hr	1.67 ±0.75	2.11 ±0.68	1.50 ±0.28	0.64 ±0.09	0.71 ±0.20	0.59 ±0.16	0.73 ±0.17	5.28 ±1.58	2.68 ±0.46	0.53 ±0.14	1.41 ±0.40	8.67 ±2.13	4.57 ±0.53
	mg/24 hr/kg	0.030 ±0.011	0.038 ±0.010	0.027 ±0.003	0.012 ±0.002	0.013 ±0.003	0.011 ±0.003	0.013 ±0.002	0.095 ±0.020	0.049 ±0.006			0.157 ±0.025	0.083 ±0.006
F (n=5)	mg/24 hr	1.10 ±0.18	1.35 ±0.16	1.58 ±0.28	0.43 ±0.07	0.49 ±0.10	0.49 ±0.09	0.56 ±0.02	4.04 ±0.56	1.97 ±0.24	0.49 ±0.02	0.86 ±0.07	6.49 ±0.72	4.41 ±0.29
	mg/24 hr/kg	0.023 ±0.003	0.028 ±0.002	0.033 ±0.004	0.009 ±0.001	0.010 ±0.002	0.010 ±0.001	0.012 ±0.001	0.084 ±0.007	0.041 ±0.003			0.135 ±0.008	0.091 ±0.002

* See Table I for abbreviations.

TABLE IV. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Normal Subjects (63–79 yr)*

Mean±S. D.		*DHA	A	E	O-A	O-E	HO-A	HO-E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
M (n=5)	mg/24 hr	1.40 ±0.81	1.63 ±0.51	1.52 ±0.35	0.41 ±0.20	0.40 ±0.16	0.41 ±0.11	0.39 ±0.17	4.55 ±1.59	1.61 ±0.56	0.46 ±0.14	1.06 ±0.18	6.78 ±1.97	3.54 ±0.84
	mg/24 hr/kg	0.029 ±0.018	0.034 ±0.011	0.032 ±0.009	0.008 ±0.004	0.008 ±0.003	0.008 ±0.003	0.008 ±0.003	0.094 ±0.036	0.033 ±0.012			0.139 ±0.045	0.073 ±0.016
F (n=6)	mg/24 hr	0.73 ±0.36	0.89 ±0.26	1.15 ±0.48	0.41 ±0.12	0.40 ±0.15	0.46 ±0.16	0.52 ±0.17	2.77 ±0.97	1.78 ±0.46	0.52 ±0.21	0.83 ±0.21	4.97 ±1.51	3.19 ±1.07
	mg/24 hr/kg	0.016 ±0.005	0.020 ±0.006	0.025 ±0.009	0.009 ±0.003	0.008 ±0.004	0.011 ±0.004	0.012 ±0.002	0.062 ±0.013	0.039 ±0.010			0.111 ±0.026	0.071 ±0.016

* See Table I for abbreviations.

TABLE V. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Normal Subjects (80 yr and over)*

Mean±S. D.		*DHA	A	E	O-A	O-E	HO-A	HO-E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
M (n=5)	mg/24 hr	0.47 ±0.12	0.68 ±0.16	0.77 ±0.08	0.26 ±0.06	0.34 ±0.06	0.41 ±0.09	0.57 ±0.10	1.92 ±0.23	1.58 ±0.18	0.82 ±0.05	0.89 ±0.05	3.87 ±0.49	3.03 ±0.40
	mg/24 hr/kg	0.011 ±0.004	0.015 ±0.003	0.017 ±0.002	0.006 ±0.002	0.008 ±0.001	0.010 ±0.003	0.013 ±0.002	0.044 ±0.006	0.037 ±0.005			0.089 ±0.001	0.069 ±0.009
F (n=6)	mg/24 hr	0.52 ±0.08	0.76 ±0.14	0.88 ±0.18	0.34 ±0.15	0.37 ±0.13	0.40 ±0.14	0.51 ±0.04	2.17 ±0.34	1.63 ±0.35	0.75 ±0.12	0.89 ±0.09	4.19 ±0.66	2.51 ±0.64
	mg/24 hr/kg	0.013 ±0.004	0.019 ±0.004	0.022 ±0.005	0.009 ±0.003	0.009 ±0.003	0.010 ±0.004	0.013 ±0.001	0.055 ±0.005	0.041 ±0.007			0.105 ±0.016	0.063 ±0.019

* See Table I for abbreviations.

TABLE VI. *Urinary Nentral 17-Ketosteroid Excretion Patterns in Patients with Gastric Ulcer (Mean±*S.D.)*

		**DHA	A	E	A+E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
Simple (17-70 yr, M. 8 cases)	%	9.65 ±3.57	23.6 ±9.49	40.0 ±8.22			15.84 ±5.34				
	mg	0.50 ±0.26	1.18 ±0.57	2.00 ±0.54	3.18 ±0.75	3.49 ±0.89	0.79 ±0.32	0.24 ±0.11	0.63 ±0.32	5.02 ±1.08	5.95 ±2.46
Multiple (35–82 yr, M. 5 cases)	%	4.90 ±3.08	14.98 ±6.40	48.34 ±13.08			24.62 ±11.79				
	mg	0.20 ±0.22	0.58 ±0.44	1.81 ±0.90	2.38 ±1.26	2.59 ±1.44	0.82 ±0.30	0.42 ±0.33	0.33 ±0.16	3.62 ±1.42	3.80 ±1.41
Refractory (55–71 yr, M. 4 cases)	%	23.27 ±7.30	23.78 ±2.23	22.28 ±8.08			20.35 ±5.70				
	mg	1.09 ±0.39	1.12 ±0.34	1.06 ±0.34	2.11 ±0.55	3.20 ±0.71	0.96 ±0.36	0.30 ±0.09	1.17 ±0.37	4.65 ±1.16	8.52 ±5.23
Healthy (17–55 yr, M. 10 cases)	%	14.39 ±1.84	27.68 ±3.48	29.81 ±3.07			18.71 ±2.33				
	mg	1.03 ±0.36	1.97 ±0.55	2.13 ±0.63	4.10 ±1.10	5.14 ±1.42	1.40 ±0.44	0.28 ±0.06	0.94 ±0.18	7.58 ±1.06	6.59 ±1.27

*, ** See Table I for abbreviations.

TABLE VII. *Urinary Neutral 17-Ketosteroid Excretion Patterns in Patients with Duodenal Ulcer*

		*DHA	A	E	A+E	11-deoxy	11-oxy	Oxy/deoxy	A/E	17-KS	17-OHCS
No. 1 (20 yr, M.)	%	7.5	21.4	43.9			17.9				
	mg	0.59	1.63	3.34	4.97	5.54	1.35	0.24	0.49	7.60	8.70
No. 2 (32 yr, M.)	%	9.3	21.0	45.2			19.70				
	mg	0.57	1.28	2.76	4.04	4.61	1.21	0.26	0.46	6.10	9.90
Healthy (17–55 yr, M. 10 cases)	%	14.39 ±1.84	27.68 ±3.48	29.81 ±3.07			18.71 ±2.33				
	mg	1.03 ±0.36	1.97 ±0.55	2.13 ±0.63	4.10 ±1.10	5.14 ±1.42	1.40 ±0.44	0.28 ±0.06	0.94 ±0.18	7.58 ±1.06	6.59 ±1.27

* See Table I for abbreviations.

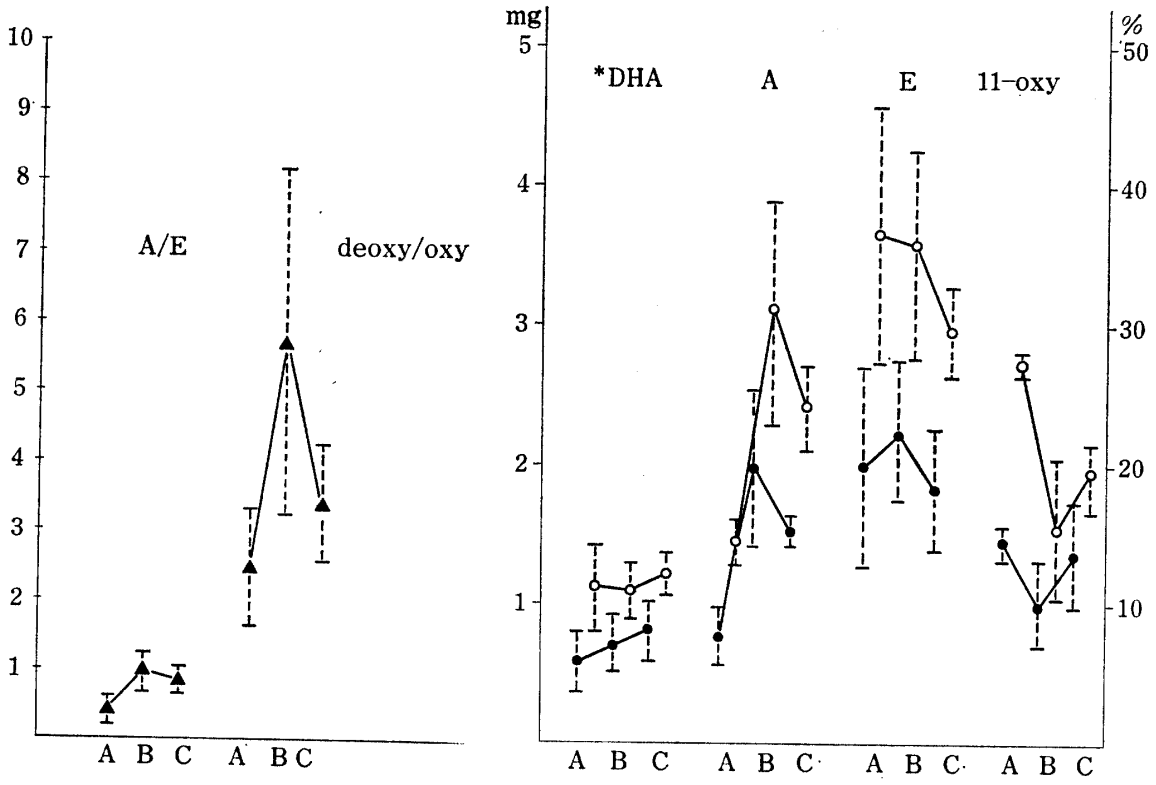

Fig 2. Changes in urinary 17-KS fractions occurred with healing process of wound in patients with gastric ulcer.
* See Table I for abbreviations. A=early stage, B=recovering stage, C=after wound healing, ●——● mg/day, ○——○ %, ▲——▲ ratio. Each point represents the mean value and the vertical bars, S. D.

(4) Duodenal Ulcer

In patients with duodenal ulcer, urinary 17-KS fraction studies showed decreased DHA, A and A/E values, resembling simple or multiple ulcer patients, but total 17-KS excretion not decreased and total 17-OHCS was elevated, unlike gastric ulcer patients.

DISCUSSION

The most abundant urinary 17-KS are A and E, which appear in the urine principally as water soluble conjugates (largely sulfates). The main precursor of urinary 17-KS is dehydroepiandrosterone, but testosterone, androstenedione, and cortisol are included among a large number of minor precursors of this group of hormone metabolites. Although A and E are derivatives of hormones originating either in adrenal or gonadal tissue, their 11-oxygenated analogues are derived mainly from adrenal steroids (8).

It has been suggested recently that unconjugated DHA is a secretory product of the human testes (9) and is in equilibrium with DS in the plasma. However, it appears that the contribution of the gonads to peripheral plasma DS is a

minor one and almost all DS in human plasma originates from the adrenal cortex. The data showing that almost all DS in peripheral plasma is derived from the adrenal cortex, and the evidence that DS is the most abundant C_{19}-steroid secreted by the adrenal cortex and that the peripheral metabolism of C_{19}-steroids does not produce any appreciable DS would indicate that the estimation of plasma DS values could be a more valuable indicator of adrenal secretion of C_{19}-steroids than 17-KS (10).

Dorfman *et al.* have characterized the 17-KS present in the urine following the administration of large doses of cortisol or cortisone, and their findings indicate that the 11-oxygenated etiocholanolone derivatives constitute the major part of the 17-KS originating from 17-OHCS (11). Even though the foregoing studies pointed strongly to the probability that the 17-KS do, in fact, originate from 17-OHCS, definite proof of their origin has awaited verification by isotopic studies. In this communication, Sandberg *et al.* (11) presented evidence that human subjects convert a significant amount of intravenously injected 4-^{14}C-cortisol to ^{14}C-17-KS and the majority of these 17-KS to the 11-oxygenated etiocholanolone groups. Moreover, evidence that 17-hydroxy etiocholanolone might be a major 17-KS metabolite of cortisol has been reported by Dorfman *et al.* (12).

Testosterone and other androgens affect the musculature. They promote amino acid incorporation into the muscle of castrated animals and elevate polymerase activity. These activities belong to protein anabolism (13). Moreover, anabolic steroids can counteract the inhibitory influence of glucocorticoid on protein synthsis in muscle. The glucocorticoids, such as cortisol, are not generally considered anabolic. When large doses are given to experimental animals, rapid weight loss occurs, and the rates of amino acid incorporation into protein are decreased in the muscle.

It is very significant for our studies that the hormone secretion from each originating endocrine organ in regared to both androgen and cortisol metabolites could be measured simultaneously by means of urinary 17-KS fraction pattern measurements on the same specimen of urine in human subjects. For this reason we did not use the determination of related steroids in plasma, but of 17-KS fractions in urine in the present study.

In the literature there are several publications (2, 15) concerning the chromatographic fractionation of urinary 17-KS in adults, but only a few reports deal with the separation of the individual components of urinary 17-KS in children. Indeed, the published values for urinary DHA in children are contradictory, and a comparison is almost impossible because of the different methods of hydrolysis and separation used. With the technique used by Beas *et al.*(14)., no DHA was found in the urine of normal boys and girls. These results are in agreement with those reported by Paulsen and Sobel (15) using similar techniques, but our study by the method modified by Ohashi showed the exsistence of urinary DHA in both boys and girls (see Table I). The plasma DS level was 15 ± 15.0 μg/100ml in males and 14 ± 14.0 μg/100ml in females aged 3—10, as determined by gas chromatography. The mean concentration of plasma DS

in different age groups has been reported by Yamaji and Ibayashi (10). They investigated the plasma DS value in subjects around puberty extensively and found that it rose at the onset of puberty and reached its peak between the end of the second decade and early in the third decade, then decreased gradually with advancing age. The daily urinary excretion values of DHA were lower in children than in young adults in our studies. It is noteworthy that plasma DS and urinary DHA levels are closely related to sexual development.

Beas *et al.* (14) have reported that A and E values in pooled urine from men and women are very similar to data published by others (15). The A/E ratio in their studies was 0.84 for men and 0.79 for women. In children in the same study, urinary A was very low : 0.08 mg/24 hr in both sexes. Very low results were also found for urinary E in both boys and girls. The urinary values of A and E in our studies proved to be very low in boys and girls, then they rose to reach their maximum in young adults. Moreover, urinary A levels were significantly higher in males than in females, and the A/E ratio was 1.52 in males and 0.89 in females. The important components of fractions III and IV are the isomers, A and E, the bulk of which derive from DHA secreted by the adrenal cortex as described above ; both hormones also contain the chief metabolites of testicular secretions. Accordingly, our data showing that the sum of A, E and DHA increased remarkably in both sexes at the onset of adulthood indicate that androgenicity is strongly stimulated at this stage.

The data by Beas *et al.* (14) on urinary 11-oxysteroids in boys and in girls were similar except that boys excreted twice as much 11-OH-A as girls. They showed that the urinary 11-deoxysteroid/11-oxysteroid ratio was quite different in adults and children, and that it was 4.8 and 3.3 in men and women, respectively. On the contrary, in children the total urinary 11-oxysteroid level was higher than the 11-deoxysteroid level. In our studies, the 11-deoxy, 17-KS/11-oxy, 17-KS ratio was undoubtly higher in young adults than in children. It has been suggested that C_{19}-steroids with ketone or hydroxy groups on carbon 11 are of adrenal origin, although some 11-oxysteroids may be derived from the testis and the ovary.

These results may give additional support to the conclusion that during infancy there is a preponderance of steroids originating from the adrenal. During adolescence and adulthood the steroids originating in the ovary and the testis become proportionally more prominent. Ours are the first published data to compare the urinary levels of 61-79 year-olds to those in subjects over 80. Although a study of plasma DS (10) showed a decrease after the age of 60, the mean concentration of plasma DS in different age groups over 60 was not determined. Therefore, our study seem to be the only one to compare urinary 17-KS excretion in healthy 60-79 year-olds and over 80 year-olds.

In our investigation, the peak levels of urinary DHA and A occurred in young adulthood in both sexes, then declined progressively with advancing age. The urinary levels of DHA and A were low between the ages from 61 and 79, but were lowest after the age of 80. There were significant differences in urinary DHA and A levels between the former and the latter age groups.

Moreover, the sex differences in both DHA and A excretion disappeared completely after the age of 80.

Although the daily urinary excretion rate remained unchanged, the percentage of urinary 11-oxygenated, 17-KS (%) was greater in those over 80 than in young adults. The average daily excretion values of 11-hydroxyetiocholanolone, which appears to be metabolized mainly from cortisol produced by the adrenal cortex, were 0.013 mg/kg in males and 0.012 mg/kg in females aged 17-23, and 0.013 mg/kg in males and0. 013 mg/kg in females in those over 80. (i.e., no difference in urinary 11-hydroxyetiocholanolone between these two groups). It is interesting that despite a significant decrease in urinary androgens, the urinary vales of the main metabolized component from cortisol is unchanged in advanced age.

In the literature there are some reports (16, 17) of Japanese studies on urinary 17-KS fractions in patients with peptic ulcer. Inayama (16) used a method in which urinary 17-ketosteroids were fractionated by stepweise elution chromatography different from that used in our study. She reported that, fraction IV (A), and V (E) decreased and the sum of fractions VI and VII (11-oxygenated, 17-KS) increased immediately after excessive bleeding in patients with peptic ulcer. On the other hand, the urinary 17-KS fraction patterns remained unchanged in patients whose ulcers bled less severely. Uemura (17) presented evidence that in peptic ulcer patients fractions V (E), VI+VII (11-oxygenated, 17-KS) increased and the IV/V ratio was depressed, and that these abnormal patterns became normal after healing of the ulcer.

In patients with either simple or multiple ulcers the urinary excretion of III (DHA), IV (A) and IV+V (A+E) fractions was depressed and the IV/V ratio was lower than normal. It is interesting, however, that in patients with multiple ulcers fractions III and IV (DHA and A) and the IV/V ratio were greatly decreased as compared with those in patients with simple ulcer, and that fraction IV (A) increased gradually and the high value of fraction V (E) fell to normal during the course of ulcer healing. These data indicate that a decrease in endogenous androgen secretion may result in simple or multiple ulcer formation.

Moreover, the evidence that in peptic ulcer patients the increased value of the sum of 11-oxygenated 17-KS (%) fell and the decreased DHA+A+E/11-oxygenated 17-KS ratio rose as healing progressed, suggest that a decrease in endogenous cortisol secretion may contribute to cure of the disease.

In patients with refractory ulcer, the most striking difference was in the excretion of the DHA (%) as compared with that in patients with simple or multiple ulcers. The reason for this difference is not known.

As shown in Table VII, in patients with duodenal ulcer, urinary 17-KS fraction studies indicated decreased DHA, A and A/E values, resembling those in simple or multiple ulcer patients, but total 17-KS excretion was not decreased and total 17-OHCS was elevated, unlike gastric ulcer patients. It appears to us that hypersecretion of cortisol by the adrenal cortex contributes mainly to the formation of duodenal ulcer.

REFERENCES

1) Note, S. (1964) Clinical significances on determination of urinary 17-ketosteroid fractions. Folia Endocrinol. Jpn. **40**, 1190 (in Japanese)
2) Endo, J. (1964) Studies on the fractionation of using neutral 17-KS by means of gradient elution chromatography on alumina column. Part I, Urinary neutral 17-ketosteroid excretion patterns in normal young and elderly subjects and their responses to the methopyrapone test and to the zinc-corticotropin test in both groups, 40 : 1190–1202 (in Japanese)
3) Hopper, B. R. and Yen, S. S. C. (1975) Circulation concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate during puberty. J. Clin. Endocrinol. Metab. **40**, 458–461
4) Horton, R., Hsieh, P., Barberia, J., Pages, L. and Cosgrove, M. (1975) Altered blood androgens in elderly men with prostate hyperplasia. J. Clin. Endocrinol. Metab. **41**, 793–796
5) Burstein, S. and Lieberman, S. (1958) Hydrolysis of ketosteroid hydrogen sulfates by solvolysis procedures. J. Biol. Chem. **233**, 331–335
6) Lakshmanan, T. K. and Lieberman, S. (1954) An improved method of gradient elution chromatography and its application to the separation of urinary ketosteroids. Arch. Biochem. Biophys. **53**, 258–281
7) Note, S. (1967) Endocrine disease. In : Diagnosis of internal medicine (G. Wakizaka and E. Kimura, ed.) pp. 631–723, Igaku Shoin, Tokyo (in Japanese)
8) Rivarola, M. A., Saez, J. M., Meyer, W. J., Kenny, E. M. and Migeon, C. J. (1967) Studies of androgens in the syndrome of male pseudohermaphroditism with testicular feminization. J. Clin. Endocrinol. Metab. **27**, 371–378
9) Vande, R. N., Mac Donald, P. C., Bolte, E. and Lieberman, S. (1962) Precursors of the urinary 11-deoxy-17-ketosteroids: Estimation of the secretory rate of dehydroepiandrosterone. J. Clin. Endocrinol. Metab. **22**, 1207–1221
10) Yamaji, T. and Ibayashi, H. (1969) Plasma dehydroepiandrosterone sulfate in normal and pathological conditions. J. Clin. Endocrinol. Metab. **29**, 273–278
11) Sandberg, A. A., Chang, E. and Slaunwhite, W. R. (1957) The Conversion of 4-^{14}C-cortisol to ^{14}C-17-ketosteroids. J. Clin. Endocrinol. Metab. **17**, 437–440
12) Dorfman, R. I. (1954) Neutral steroid hormone metabolites. In : Recent progress in hormone research (G. Pincus, ed.) vol. 9, p. 5, Academic Press, New York.
13) Manchester, K. L. (1974) Protein metabolism and hormone. In : Textbook of endocrinology (R. H. Williams, ed.) pp. 881–889, Saunders, Philadelphia
14) Beas, F., Zurbrugg, R. P., Cara, J. and Gardner, L. I. (1962) Urinary C_{19} steroid in normal children and adults. J. Clin. Endocrinol. Metab. **22**, 1090–1094
15) Paulsen, E. P., Sobel, E. H. and Shfron, M. S. (1967) Urinary steroid metabolites in children, I. Individual 17-ketosteroids in children with normal sexual development. J. Clin. Endocrinol. Metab. **26**, 329–339
16) Inayama, A. (1961) Clinical application of urinary 17-ketosteroid fraction determination and its valuation, P. 4, Changes in urinary 17-ketosteroid fraction patterns in patients with peptic ulcer. Kitano Hosp. J. Med. **6**, 236–241 (in Japanese)
17) Uemura, J. (1962) Significance of urinary 17-ketosteroid fraction determination in normal subjects and patients with cancer of digestive tract and peptic ulcer. Tohoku Igaku Zassi **66**, 397–413 (in Japanese)