metabolism was markedly decreased as compared with that of control rats, and (b) the response of this metabolism to phenobarbital and 3-MC was not prevented. If pituitary hormones (somatotropin, corticotropin, and prolactin) secreted by the MtT were responsible for the effects on liver drug metabolism described here and elsewhere (2,3,4), then the administration of these rat hormones (when available) to rats may provide information about the normal regulation of this liver drug-metabolizing enzyme activity.

Summary. The administration of phenobarbital to rats bearing a pituitary mammotropic tumor (MtT) produced an increase in the hepatic metabolism of hexobarbital which was greater than that of similarly treated control rats. The formation of formaldehyde from aminopyrine by liver from MtT-bearing rats, however, was not increased by an amount comparable to that of control rats following phenobarbital pretreatment. The metabolism of benzpyrene was increased more than that of zoxazolamine by liver from MtT rats after an injection of 3-MC, and the metabolism of both compounds was increased more than that found after control rats were injected with 3-MC. An increase in the liver metabolism of four drugs which followed phenobarbital or 3-MC pretreatment of control rats, therefore, was not prevented by growth in rats of a corticotropin, somatotropin, and prolactin producing pituitary tumor.

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The Excretion of the 3-Oxo-Conjugate of Aldosterone* by Normal Adolescent Boys† (33035)

JOSEPHINE B. GARST, PAUL J. VIGNOS, JR., DEAN N. MATTHEWS, AND MAGDALENA HADADY

Division of Medical Research, Veterans Administration Hospital[1] and The Department of Medicine, Western Reserve University, Cleveland, Ohio 44106

Considerable information is available in the literature concerning the excretion of the 3-oxo-conjugate of aldosterone by normal adults, but only a small amount of data has been published for normal children. Of the latter data, relatively little has to do with adolescent boys. Mattox *et al.* (1) have reported 24-hour excretion values of 0.7–6.5 $\mu g/m^2$ of body surface area for four boys

* Now also known as aldosterone 18-glucuronide.

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[1] Address: 10701 East Boulevard, Cleveland, Ohio 44106.

aged 12–14 years. Minick and Conn (2) obtained values of 5–6 μg/24 hours for three normal boys aged 12–14 years, and New *et al.* (3) found the excretion rate for one boy 11 years of age to be 6 μg/24 hours. No information, however, is available for boys at the age (16 years) when body potassium concentration is the highest for males (4). This fact, coupled with the paucity of data for adolescent boys in general, made it seem desirable to obtain further data on the excretion of the 3-oxo-conjugate of aldosterone by adolescent boys.

Materials and Methods. The amount of the 3-oxo-conjugate of aldosterone in the 24-hour urine samples was determined by the method of Garst *et al.* (5) with one modification. Trace amounts of aldosterone-4-^{14}C were added to the residue of the chloroform extracts before any chromatography was done. After chromatography, the location of the samples on the chromatographic strips was verified by determining the position of the radioactive material by means of a strip counter.[2]

Further validation of material isolated. Chromatographic evidence. In addition to the routine methods used for the location of aldosterone during chromatography by this laboratory, viz., R_f in two different chromatographic systems and the location of a radioactive tag, further verification has been done from time to time on the isolated material from random samples as follows, (i) *chromatography of aliquots mixed with authentic aldosterone diacetate in four systems;* (a) hexane-propylene glycol, (b) Bush A, (c) Bush B_3, and (d) cyclohexane, benzene, methanol, water (4:2:4:1) with a single spot being obtained in each instance; (ii) *chromatography of aliquots mixed with aldosterone*-4-^{14}C *in* (a) Bush B_3, (b) cyclohexane, benzene, methanol, water (4:2:4:1), and (c) Bush A with agreement of the band of ultraviolet absorption with that of radioactivity.

Infrared spectrophotometric evidence. The infrared spectrum of a pool of chromatographically homogeneous aldosterone diacetate isolated from randomly selected urine samples was matched with the infrared spectrum of the diacetate of authentic crystalline aldosterone[3] (Fig. 1) with satisfactory results.

Reliability of R_f *hexane-propylene glycol chromatography.* Fifteen random samples of isolated aldosterone diacetate fulfilling the criteria specified in our method had an R_f of 0.687 ± 0.014 (SE) in terms of the standard, 11-dehydrocorticosterone acetate, compared to an R_f of 0.665 ± 0.028 (SE) for 13 samples of authentic aldosterone diacetate against the same standard. The aldosterone diacetate peaks were well separated from the two adjacent peaks; one was well ahead [R_f 0.994 ± 0.007 (SE)] and one, well behind [R_f 0.476 ± 0.026 (SE)].

Recovery studies. Urine samples (12- or 24-hour collections) were hydrolyzed and divided into two aliquots. To one, aldosterone was added. Both aliquots were processed simultaneously for the isolation of aldosterone diacetate. The results are shown in Table I. These results compare favorably with those of Kliman and Peterson (6) obtained by the use of the double isotope derivative method [mean of 93.9% ± 2.09 (SE) with a range of 89–110%.

Reproducibility of the method. Each of three 24-hour urine samples was divided into two equal aliquots and analyzed for the 3-oxo-conjugate of aldosterone. Each pair of aliquots was processed by a different technician. On pair of aliquots showed values of 12.6 and 12.9 μg with a difference of 2.4% based on the smaller value. The second pair showed 12.45 and 12.48 μg with a difference of 0.24%. The third pair showed values of 1.23 and 1.65 μg with a difference of 34% demonstrating that the method is not reproducible for samples smaller than a certain

[2] In routine practice, the aldosterone fraction obtained demonstrates the expected 238–240 mμ maximum, and the presence of an acceptable ultraviolet spectrum is a requisite for quantitative estimation. Rigid adherence to the details of the technique, i.e., thorough washing of the paper strips prior to use, handling of the strips with forceps, use of thoroughly purified reagents, protection of the extracts against undue exposure to light, heat, air and moisture, etc., is necessary in order to consistently obtain reproducible results.

[3] The free aldosterone was generously supplied by Dr. Robert Neher, Basle, Switzerland.

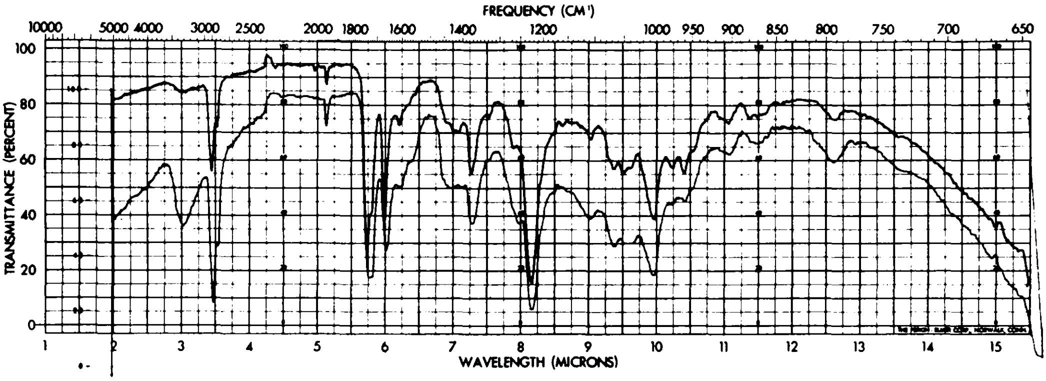

Fig. 1. Comparison of the infrared spectral curve for isolated 3-oxo-conjugate of aldosterone diacetate (bottom curve) with that of authentic aldosterone diacetate. The absorption at 3.0 μ observed in the isolated sample is variable, and appears to be due to the presence of trace amounts of propylene glycol used in the chromatography.

size. The limitations of the spectrophotometric assay and the size of the sample in relation to the amount of background do not permit accurate assay of samples smaller than 3 μg.

Duplicate estimations of the amount of the 3-oxo-conjugate of aldosterone by Ayres *et al.* (7) varied from a difference of 11–56%, and others done by Flood *et al.* (8) showed differences varying from 3.5–36%. The smallest quantity measured in either case was 4.3 μg.

Comparison of result obtained by method of Garst et al. *with result obtained by method of Bravo and Travis* (9). Recently, the amount of the 3-oxo-conjugate of aldosterone was determined for equal aliquots of the same urine sample by our laboratory using the foregoing method, and by another laboratory using a recently published method employing gas chromatography. The results obtained were 6.5 and 5.5 μg, respectively.

Table I. Recovery of Aldosterone Added to Urine Samples.

Experiment	Aldosterone Added (μg)	Aldosterone Isolated (μg)	Recovery (%)
1a	0	14.7	88
1b	6.5	20.4	
2a	0	12.2	86
2b	6.2	17.5	
3a	0	5.7	87
3b	6.0	10.9	
4a	0	3.7	116
4b	15.0	21.1	
5a	0	4.3	99
5b	18.0	22.1	
		Mean = 95.2 ± 6.4 (SE)	

Subjects studied. Four normal boys, 14 to 16 years of age, were studied for 1 or 2 days during which time they lived at home and followed their ordinary routine. Six normal boys, 13–15 years, were studied at the Clinical Research Center (metabolic ward) of University Hospitals for 20 days. Although confined to the ward, these boys were not bed patients, and they engaged in normal activities within the limitations of the ward. The nonhospitalized boys were maintained on their customary *ad libitum* intake of sodium and potassium, whereas those boys in the hospitalized group were given diets in which the sodium and potassium content was controlled as shown in Fig. 2. The amounts of sodium and potassium varied somewhat from boy to boy because each was allowed to select the foods for his own diet. The boys studied on the metabolic ward were given a general physical examination and the following laboratory tests were performed: RBC, Hb, hematocrit, WBC, differential cell count, serum electrolytes, BUN, serum creatinine, urea clearance, and 24-hour urinary protein excretion. All findings were within normal limits. The boys studied on an

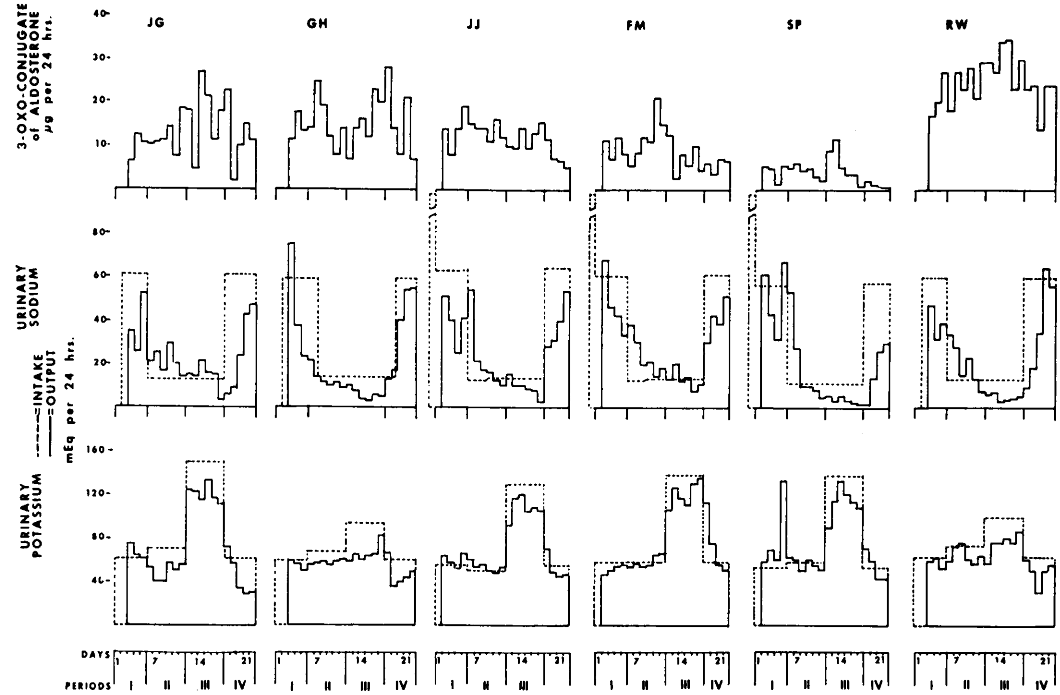

FIG. 2. Excretion of the 3-oxo-conjugate of aldosterone, sodium, and potassium by normal adolescent boys when on a controlled, but varied, intake of sodium and potassium.

outpatient basis had no known physical disabilities.

Twenty-four-hour urine samples were collected daily without preservative and frozen immediately after the collection was finished. The amounts of sodium, potassium, and creatinine, as well as the amount of the 3-oxo-conjugate of aldosterone, were determined on these samples. Serum electrolyte determinations and electrocardiograms were done intermittently.

Results. The 24 hour excretion values for the 3-oxo-conjugate of aldosterone for the four nonhospitalized boys are shown in Table I. The excretion values obtained during an *ad libitum* intake of sodium show wide variation; the values for two of the four boys were significantly elevated over normal adult values obtained by this method (see Table II).

The daily excretion values for the 3-oxo-conjugate of aldosterone, sodium, and potassium for the six boys studied on the metabolic ward are shown in Fig. 2. The mean excretion values for aldosterone are given in Table II. Considerable variation was found in the absolute values regardless of the intake of sodium and potassium. The excretion rate of aldosterone for one boy (RW) during the normal sodium intake was significantly elevated according to our adult standards, and that for another boy (SP) was consistently at the lower limit of normal. Values for the other four boys fell within the normal adult range. Low sodium intake produced a small rise in aldosterone excretion rate for two of the six boys (JC, FM). When low sodium was accompanied by elevated potassium, a further rise occurred for one of these (JC), and modest initial rises were found for two others (RW, SP).

A significant positive correlation was found to exist between urinary aldosterone values and the corresponding potassium–sodium ratios for the entire study done on the metabolic ward (Fig. 3 and Table III). No correlation was observed between the aldosterone excretion values obtained during constant potassium intake (Periods I and II) when they were paired with the corresponding urinary sodium values (Table III). Nor was any

TABLE II. Mean Excretion Values for the 3-Oxo-Conjugate of Aldosterone for Normal Adolescent Boys.[a]

Uncontrolled intake of Na and K (nonhospitalized)			Controlled intake of Na and K (hospitalized on a metabolic ward)					
					Aldosterone (μg/24 hours) (mean values)			
Subjects	Age (years)	Aldosterone (μg/24 hours) (mean values)	Subjects	Age (years)	Normal Na[c] and K	Low Na, normal K	Low Na, high K	Normal Na and K
D.S.	16	30.9 (1)[b]	J.C.	14	10.0 (3)[b]	12.9 (6)	16.7 (6)	12.1 (5)
R.M.	15	15.5 (2)	G.H.	14	14.5 (3)	15.3 (6)	15.3 (6)	14.6 (5)
J.K.	15	10.1 (2)	R.W.	13	21.3 (3)	24.4 (6)	29.7 (6)	19.8 (5)
W.C.	14	24.9 (1)	S.P.	14	4.3 (4)	4.2 (6)	5.4 (6)	1.0 (4)
			J.J.	13	13.8 (4)	13.6 (6)	12.0 (6)	7.5 (4)
			F.M.	15	9.7 (4)	12.1 (6)	6.6 (6)	5.6 (4)
			Mean	12.3 ± 5.7 (SD)				

[a] Mean value for the 3-oxo-conjugate of aldosterone for normal adult males for this laboratory is 9.1 μg ± 2.8 (SD)/24 hours (5).

[b] No. of 24-hour urine samples analyzed is given in parentheses.

[c] See Fig. 2 for exact values of electrolyte intake.

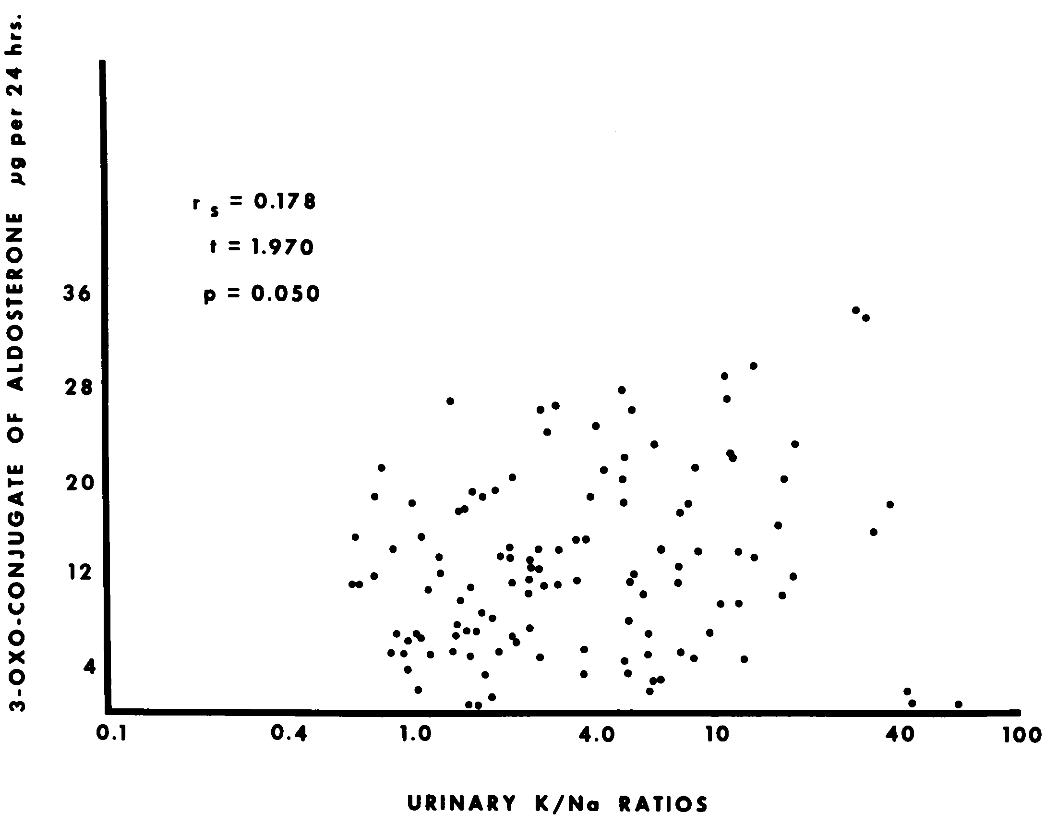

FIG. 3. Positive correlation of urinary aldosterone levels and potassium–sodium ratios for normal adolescent boys.

TABLE III. Correlation from Ranks for Urinary Aldosterone, Sodium, and Potassium for Adolescent Boys.

	No. of persons	No. of pairs of values	r_s[a] found	t found	Probability level
Aldosterone vs Na (intake: Na varied, K constant)	6	57	—0.107	0.798	>0.40
Aldosterone vs K (intake: Na constant, K varied)	6	72	—0.020	0.167	>0.50
Aldosterone vs K/Na (intake: Na varied, K constant)	6	120	+0.178	1.970	0.05

[a] The $r_s = 1-[6\Sigma(d^2)]/[N(N^2-1)]$; (Spearman's formula).

correlation found between the urinary aldosterone values and the corresponding urinary potassium values obtained during constant sodium intake (Periods II and III) shown in Table III.

Discussion. The elevated isolated values for the 3-oxo-conjugate of aldosterone found for normal adolescent boys on an *ad libitum* intake of sodium, and the elevated mean values for some of the boys maintained on a controlled sodium intake would appear to be characteristic of this period of physiological development. The data in Table IV show that the mean value which we obtained for the 3-oxo-conjugate of aldosterone for 10 normal adolescent boys has a considerably greater standard deviation (52% of mean) than does that which we obtained for 18 normal adults (31% of mean). We feel that this difference is not due to methodology. The results obtained by our method for normal adults appear to compare favorably with those obtained by other methods which are currently accepted (see Table IV). The data in Table IV for normal adolescent boys shows disagreement in the results in the literature. A small standard deviation (11% of mean) was found for the excretion values obtained by

TABLE IV. Variation in the Excretion of the 3-Oxo-Conjugate of Aldosterone.

Sources of literature		No. of subjects	Range of values (μg per 24 hours)	Mean ± SD
		Normal adults[a]		
Nowaczynski *et al.*	(10)	9	2.2–10.0	5.1 —[b]
Wettstein	(11)	10	1.0–13.0	—[b]
Dyrenfurth and Venning	(12)	14	1.0– 9.1	4.0 ± 2.6
Mattox and Lewbart	(13)	34	2.0–16.0	5.6 —[b]
Jones *et al.*	(14)	10	4.1–14.5	8.2 ± 3.2
Lieberman and Luetscher	(15)	16	0.8– 7.8	2.3 —[b]
Kliman and Peterson	(6)	26	5.0–19.0	10.0 —[b]
Garst *et al.*	(5)	18	5.4–14.5	9.1 ± 2.8
Flood *et al.*	(8)	14	4.7–10.0	7.6 ± 2.1
		Normal adolescent boys		
Mattox *et al.*	(1)	4[a]	0.7– 6.5	4.1 ± 2.5[c]
Minick and Conn	(2)	3[a]	5.0– 6.0	5.3 ± 0.6
Present work				
Data for all		10	4.3–30.9	15.5 ± 8.0
Data for boys on metabolic ward		6	4.3–21.3	12.3 ± 5.7

[a] Uncontrolled sodium intake.
[b] Data not available.
[c] Values estimated from graph (μg/m^2 body surface).

Minick and Conn for 3 boys, whereas the standard deviation for the data reported by Mattox *et al.* for 4 boys was large (61% of mean). Our standard deviations for the adolescent boys are 52% of mean for all 10 boys and 46% for the 6 boys. It is possible that larger samples in all cases might have resulted in better agreement of the data. Although no final conclusion can be drawn concerning the validity of the wide variation in our results until more data become available, the bulk of the evidence at present suggests that the wider range of values for adolescent boys than normally found for adults may be a real physiological finding.

The variation found in the day to day values for individuals on a normal sodium intake in this study is not unusual. The following instances of such variation reported by others might be quoted: change (rise or fall) in excretion values for two successive 24-hour periods; (a) 6-250% (16); (b) 80-500% (17); (c) 0-75% (18); and (d) 5-300% (19). In the present study, this type of change shows a range of 40-337% which is similar to the range found by others.

The results obtained in our study of adolescent boys for the relationship of urinary aldosterone values to urinary sodium and potassium values suggest that the excretion of these three entities does not follow the reported normal adult pattern (20). Furthermore, the amount of the 3-oxo-conjugate of aldosterone excreted by normal adolescent boys tends to be greater than the amount excreted by normal adults. The reasons for these differences are at present not apparent.

Summary. Excretion of the 3-oxo-conjugate of aldosterone by three of the ten normal adolescent boys studied was found to be substantially greater than that of normal adults. No correlation was observed between the values for urinary aldosterone and urinary sodium, nor between urinary aldosterone and urinary potassium for the group. A barely significant correlation was found between the values for urinary aldosterone and urinary potassium-sodium ratios.

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