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SPECIAL ARTICLES

STEROID HORMONE EXCRETION BY NORMAL AND PATHOLOGICAL INDIVIDUALS

A STUDY is in progress of the abnormalities of the intermediary metabolism of the steroid hormones which are associated with disease. As a part of this program a systematic investigation has been initiated involving the examination of individual urine collections in amounts adequate for extensive fractionation and chemical characterization of the constituent steroids. This note presents the findings to date from the processing of 2- to 6-month collections of urine from six normal persons, six patients with cancer and four patients with clinical evidence of hyperplasia of the adrenal gland.

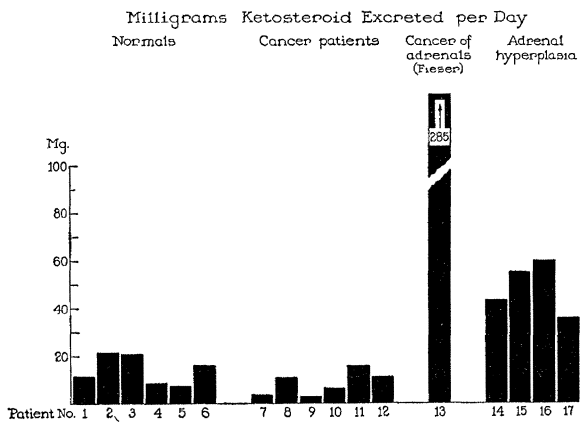

FIG. 1

After hydrolysis of the urine with sulfuric acid and extraction with ether, the extracts were partitioned into acidic, phenolic and neutral fractions. The neutral material next was separated with Girard's reagent (T) into ketonic and non-ketonic fractions. Each of these then was processed with digitonin into the 3-alpha- and 3-beta-ketosteroid fractions.

The 24-hour excretion rate of the total ketosteroids by the individuals studied, as estimated by the Callow modification[1] of the colorimetric method of Zimmermann,[2] are summarized in Fig. 1. Included for comparison are the results of a previous study (Fieser[4]). The cancer patients excreted, in general, smaller amounts than did the normal individuals and the patients with adrenal hyperplasia.

The average contents of digitonin-precipitated material (or 3-beta-hydroxy steroids) in the total ketosteroid fraction were: normals, 0.6–2.2 per cent.; cancer patients, 0.3–6.0 per cent.; patients with adrenal hyperplasia, 3.7–11.4 per cent. (Fig. 2).

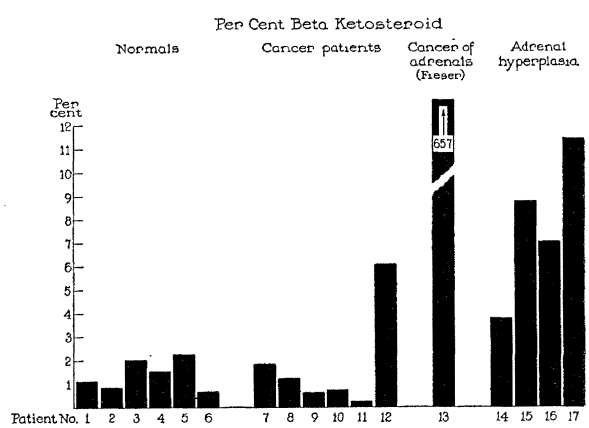

FIG. 2

The material not precipitated by digitonin (3-alpha-hydroxy-ketosteroids and 17-ketosteroids) was adsorbed from ligroin solution onto activated alumina (Brockmann) and separated by systematic fractional elution into the following fractions (Fig. 3):

Fraction I (elution with mixtures of petroleum ether and benzene). The presence in this fraction of $\Delta^{3,5}$-androstadienone-17[3,4] was demonstrated in the material from four of six normals and from all the patients with adrenal hyperplasia. This was done by the isolation of the semicarbazone and oxime and

[1] N. H. Callow, R. K. Callow and C. W. Emmons, *Biochem. Jour.*, 32: 1312, 1938.

[2] W. Zimmermann, *Z. physiol. Chem.*, 233: 1935; 245: 47, 1936.

[3] H. Burrows, J. W. Cook, E. M. F. Roe and F. L. Warren, *Biochem. Jour.*, 31: 950, 1937.

[4] J. K. Wolfe, L. F. Fieser and H. B. Friedgood, *Jour. Am. Chem. Soc.*, 63: 582, 1941.

[5] H. Hirschmann, *Jour. Biol. Chem.*, 136: 483, 1940.

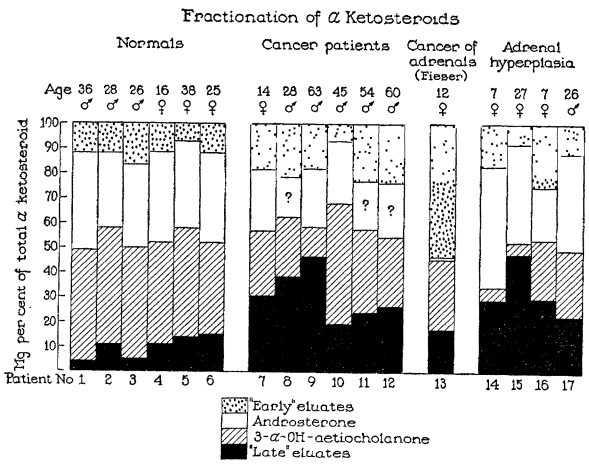

FIG. 3

by spectroscopic characterization. No chemical or spectroscopic evidence was found to suggest the presence of the dienone in urine collections from the two other normal individuals or from the patients with cancer. Androstenone-17[5, 6] was isolated from two normal urines and three hyperplasia urines (m.p. 114–114.5° C., $[\alpha]_D^{38}$ +148° C.). It was identified by analysis and through the oxime. Androstenone-17 was not found in the urine of any cancer patient studied. The unidentified material of Fraction I had no characteristic ultraviolet absorption spectrum and gave no alcohol-insoluble semicarbazone.

The origin of the $\Delta^{3,5}$-androstadienone-17 found in hydrolyzed urines is being investigated, and preliminary results based merely upon the identification of the dienone in suitably processed fractions by its characteristic absorption spectrum indicate that the substance can be detected in extracts of certain unhydrolyzed urines. It also appears, from the spectroscopic evidence, that acid hydrolysis of a urine previously devoid of any detectable amount of $\Delta^{3,5}$-androstadienone-17, but to which dehydroisoandrosterone has been added, results in the production of a certain amount of the dienone.

Fraction II (elution with benzene and benzene-ether). Androsterone appears to be the chief constituent of this fraction. It was isolated from all collections except those from three patients with cancer (m.p. 177–178° C.). The androsterone was identified by analysis and by mixed melting point determinations of the hydroxyketone and its acetate with authentic samples. In Fraction II of the urine collections from the patients with evidence of adrenal hyperplasia certain apparently homogeneous substances were present which were not previously found in urine. One of these melts at 149–150° C., and another, isolated in amounts too small for analysis, melts at 134–135° C.

Fraction III (elution with ether and with ether containing 10 per cent. acetone). The chief constituent was identified as 3-alpha-hydroxyaetiocholanone-17 and was isolated in every instance (m.p. 143–144° C., remelting at 150° C., no depression in melting point on mixing the substance or its acetate with authentic samples).

Fraction IV (elution with ether-acetone, acetone and methanol). This fraction afforded a variety of substances. From two collections of normal urine and one of urine from a patient with evidence of adrenal hyperplasia there was isolated a substance, m.p. 199–200° C., which appears from the analysis to have the formula $C_{19}H_{32}O_3$ (Found: C, 74.30; H, 10.21). A second substance, m.p. 185–186° C., was isolated in traces from the urine of one patient with evidence of adrenal hyperplasia; it depresses the melting point of the 3-alpha-hydroxyandrostenone-17 isolated by Wolfe, Fieser and Friedgood[4] from the urine of a patient with adrenal tumor. A third substance, m.p. 232–234° C., was found in the urine of one cancer patient and, in larger amounts, in the material from three of the patients with adrenal hyperplasia. A fourth substance, m.p. 172–176° C., was isolated from the urine of a patient with evidence of adrenal hyperplasia, and other, so far impure, crystallizates have been encountered in this fraction.

Colorimetric assays were made of the total ketosteroid content of the four fractions of ketonic material not precipitated by digitonin. In the series of six normal urine collections, Fractions I and IV each constituted 5–15 per cent. of the total, and Fraction II (androsterone) and Fraction III (3-alpha-hydroxyaetiocholanone) together made up 70–90 per cent. of the total and were found present in the ratio 4:5. This finding is in confirmation of the observations of Callow and Callow.[7, 8] In the urine collections from patients with cancer the distribution of material was not as constant as in those from normal individuals and ratios between Fractions II and III were irregular. In three instances no androsterone was found in the Fraction II and, furthermore, Fractions I and IV were, with one exception, larger than normal. In the urines from adrenal hyperplasia patients, Fraction I usually contained 10–20 per cent. of the total ketosteroids, as was found in the material from normals, while Fraction IV contained distinctly more material than normal. On the whole, in the urine collections of the patients, both with cancer and adrenal hyperplasia, the ratio of the fractions varied

[6] W. Pearlman, *Endocrinology*, 30: 275, 1942.

[7] N. H. Callow, *Biochem. Jour.*, 33: 559, 1939.

[8] N. H. Callow and R. K. Callow, *Biochem. Jour.*, 33: 931, 1939.

considerably from one individual to another and, as noted above, individual urine collections contained apparently specific and novel ketosteroids in Fractions II and IV. We are now endeavoring to accumulate quantities of these substances sufficient for chemical characterization.

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CONTROL OF FLOWERING WITH PHYTOHORMONES[1]

ATTEMPTS to influence flowering in *Ananas comosus* (L.) Merr. have involved experiments with certain synthetic phytohormones, including α-naphthaleneacetic acid, naphthaleneacetamide, naphthalenethioacetamide and a commercial product known as *Fruitone*. It has now been established that flowering can be induced in advance of the normal period or delayed until much later by the use of appropriate concentrations of these chemicals.

Typical data are given in Tables 1 and 2. Low concentrations of α-naphthaleneacetic acid (the compound used most extensively) applied as foliage sprays induced formation of inflorescences in advance of the normal period, but high concentrations, particularly when applied in solution at the apex, delayed flowering far beyond that of the controls. The fact that natural flowering of a uniform fall planting of slips occurs at a fairly definite season made it possible to schedule applications of the substances at desired intervals prior to normal differentiation of the inflorescence.

Flowering in *Ananas* involves a transition from the differentiation of vegetative structures to the formation of an inflorescence at the apical meristem. Steps in the process follow in succession, as already described.[2] The diameter of the meristem first widens, then flower bud primordia instead of leaf primordia are produced, and the peduncle elongates. The meristem gradually narrows again during the production of floral primordia and finally resumes differentiation of leaf primordia which give rise to the crown or top terminating the main axis of the plant.

An interval of about 2 months elapses from the time that the meristem first widens until the young inflorescence becomes externally visible in the center of the plant. From this fact it appears that the early stages in differentiation of the inflorescence must have followed shortly after the first application of the lowest concentration of naphthaleneacetic acid (Table 1). Conversely, differentiation of floral parts was greatly retarded by the highest concentrations (Table 2),

TABLE 1

EARLY FLOWERING INDUCED BY DILUTE SOLUTIONS OF α-NAPHTHALENEACETIC ACID SPRAYED ON LEAVES FOUR MONTHS PRIOR TO NORMAL DIFFERENTIATION OF INFLORESCENCE

Concentration of solution Per cent.	Weekly applications No.	Plants No.	Plants which had formed flower buds at stated periods after first application	
			2 months No.	3 months No.
Controls	–	20	0	0
.001	6	20	20	20
.006	1	20	18	20
.006	3	20	9	19

TABLE 2

DELAY OF FLOWERING BY MORE CONCENTRATED SOLUTIONS OF α-NAPHTHALENEACETIC ACID POURED IN CENTER OF PLANT ONE MONTH PRIOR TO NORMAL DIFFERENTIATION OF INFLORESCENCE

Concentration of solution Per cent.	Biweekly applications No.	Plants No.	Plants which had formed flower buds at stated periods after first applicaton		
			4 months No.	6 months No.	8 months No.
Controls ..	–	20	19	20	20
.01	3	20	0	16	19
.05	3	20	0	1	7
0.1	3	20	0	0	3
0.1	1	20	0	18	20

although new leaves were formed after the applications which resulted in the longest delay in flowering. When early flowering was induced by the low concentrations of these chemicals there was no external evidence of abnormal development of tissues. When flowering was delayed for a long period of time, however, considerable distortion and constriction was observed in the portion of the stem and in the leaves at the level of the apical meristem at the time of application of the phytohormones.

Since the differentiation of the inflorescence itself was initiated (Table 1) in our experiments, the results differ from earlier uses of the same or similar phytohormones in the production of parthenocarpic fruits,[3,4] the hastening of flowering by seed treatments which accelerated growth[5,6] or premature flowering of tobacco which was said to be due to

[1] Published with the approval of the Acting Director as Technical Paper No. 139 of the Pineapple Research Institute of Hawaii, University of Hawaii.

[2] K. R. Kerns, J. L. Collins and H. Kim, *New Phytol.*, 35: 305–17, 1936.

[3] F. G. Gustafson, *Proc. Nat. Acad. Sci.*, 22: 628–36, 1936.

[4] F. E. Gardner and P. C. Marth, SCIENCE, 86: 246–7, 1937.

[5] K. V. Thimann and R. H. Lane, *Am. Jour. Bot.*, 25: 535–43, 1938.

[6] H. L. Stier and H. G. duBuy, *Proc. Am. Soc. Hort. Sci.*, 36: 723–31, 1939.