STUDIES IN STEROID METABOLISM. XXVI. STEROID ISOLATION STUDIES IN HUMAN LEUKEMIA

By KONRAD DOBRINER,† ATTALLAH KAPPAS,[1] AND T. F. GALLAGHER

(From the Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

(Submitted for publication March 26, 1954; accepted July 15, 1954)

The normal and abnormal physiology of white blood cells can be characteristically influenced by the hormonal environment. Adrenocortical hormones administered as such, or secreted under stimulation by ACTH have been shown to influence the distribution, mass, and functional activity of lymphoid tissue (1–6). The course and incidence of leukemia in test animals are intimately related to alterations in adrenal or gonadal function (7–12) and the striking, albeit temporary, amelioration achieved by adrenocortical or adrenocorticotropic hormones in human leukemia, is well known (13–16). Although decreased urinary ketosteroids have been demonstrated in leukemia (17, 18), excretion of urinary corticoids has been generally unaltered (18). However, few data are available relative to specific gonadal or adrenal hormone metabolites in human leukemia.

We have applied, to the study of this problem, the precise methods of isolation, identification, and quantitation of individual urinary steroids developed within the past decade (19–23). The detailed ketosteroid patterns of six subjects with chronic lymphatic leukemia during control periods and of one subject with acute myelogenous leukemia during a control interval and while on ACTH therapy are reported. The results give evidence of decreased secretory activity of both gonads and adrenal glands in these subjects. The simultaneous development of refractoriness to ACTH therapy in one patient, with evidence of progressive adrenal dysfunction as measured by the urinary steroids, raises, in addition, an interesting question about the relation between clinical relapse in this disease, and the hormonal environment.

† Konrad Dobriner died March 10, 1952.

[1] Research Fellow of the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

METHODS

The urinary ketosteroid patterns of six subjects with chronic lymphatic leukemia were studied. Five of these subjects were male (M45, B58, S59, H62, B68), and one was female (H55). In addition, the urinary ketosteroids of one subject with acute myelogenous leukemia (R29) were studied during the course of two successive therapeutic trials with ACTH. Metabolic balance studies were conducted during these periods and the results have already been reported (Subject R. R. [24]). All urine collections were short term (5 to 16 days) except for that of subject M45 (195 days). The urinary steroid conjugates were hydrolyzed immediately after collection, by a combination of methods employing enzymatic (beef liver β-glucuronidase), cold and/or hot acid hydrolysis (Methods A, B, C, or D [22]).

Neutral steroid extracts were separated into "ketonic" and "non-ketonic," and "α-ketonic" and "β-ketonic" fractions by the usual techniques (19). The "α-ketosteroid" fractions were further analyzed by adsorption and partition chromatography employing silica gel columns. Alumina and/or magnesium silicate columns were employed in certain fractionations. Individual steroid fractions were identified by infrared spectrometry and quantitated by the modified Zimmermann reaction.

The further details of the analytical procedures employed in these studies have already been described elsewhere (19, 22). Variable proportions of 3α, 11β-dihydroxyandrostane-17-one and 3α, 11β-dihydroxyetiocholane-17-one appeared as the $\Delta 9(11)$-analogs after vigorous acid hydrolysis. The values reported for the 11-hydroxylated compounds include these transformation products. Quantitative estimations of 17α-hydroxypregnanolone were made by the Zimmermann reaction. Because the correction factor for this compound is high, the quantitative values may be seriously in error.

RESULTS

The quantitative values for isolated and identified steroids only, are reported in the tables. The age follows the initial of each subject studied.

Chronic lymphatic leukemia

Subject M45: This patient was a white man, admitted to the hospital with an eight-month history of marked weight loss, weakness, adenopathy,

scrotal and ankle edema and ascites. There was marked pallor on examination and adenopathy and splenomegaly were pronounced. The diagnosis of chronic lymphatic leukemia was established from marrow aspiration. Urine collection began two months after admission and continued for 195 days. The only therapy given during this collection period was the administration of repeated blood transfusions. Throughout the collection period, this subject was quite ill.

Urinary ketosteroid data are shown in Table I. The sum of the 11-desoxyketosteroids was well below average for this age group. The sum of the 11-oxygenated C_{19} steroids was within normal limits.

Subject H55: This subject was a white woman who came to the hospital with a proved diagnosis of chronic lymphatic leukemia. She had been an X-ray technician for 25 years. Seventeen years previously at another hospital, radiation menopause had been induced for therapy of uterine fibroids. Except for lymphadenopathy, she was essentially well during the urine collection period.

The sum of the 11-oxygenated C_{19} steroids was within the range observed in a series of normal women (25). The absence of androsterone and etiocholanolone from this subject's urine is very unusual.

Subject B58: This subject was a white man, admitted to the hospital with a two-year history of progressive fatigue. Examination revealed a high white blood cell count, hepato-splenomegaly and generalized adenopathy. The diagnosis of chronic lymphatic leukemia was established by marrow aspiration. Urinary steroids were studied during a 15-day control period preceding hormone therapy.

Levels of 11-oxygenated and 11-desoxy C_{19} steroids during this control period, were considerably lower than those of normal men of similar age.

Subject S59: This subject was a white man, with a 12-month history of adenopathy, admitted to the hospital one year previous to the present study. The diagnosis of chronic lymphatic leukemia had been established at that time. This patient had received several forms of therapy during the year previous to study, including Amethopterin and X-ray, with little effect on his massive adenopathy and little decrease in the size of his liver or spleen. Urinary steroids were studied during a five-day control period preceding ACTH therapy.

The sums of both C_{19} 11-oxygenated and 11-desoxysteroids were below the lowest limits observed in a series of normal men (22).

Subject H62: This subject was a white man, admitted to the hospital with a one-year history of weakness and weight loss and with a reportedly high white blood cell count. Clinical and laboratory examination established the diagnosis of chronic lymphatic leukemia. This subject was somewhat febrile during the control period. The influence of his febrile course upon his steroid pattern is open to question.

The sum of 11-oxygenated C_{19} steroids was at

TABLE I

Ketosteroid patterns in chronic lymphatic leukemia

Compounds	Leukemic patients						Five male leukemics 45 to 68 years	Normal males 50 years and above
	Milligrams per 24 hours						Average values	Average values
C_{19} 11-oxysteroids	1.8	1.5	.4	.3	1.2	1.3	1.0	1.8
C_{19} 11-desoxysteroids	.7	—	.7	.3	.9	1.0	.7	4.4
11-Ketoetiocholanolone	.3	.5	.2	tr.	.6	.3	.3	.7
11-Hydroxyetiocholanolone	1.3	.4	.1	—	.2	.5	.4	—
11-Hydroxyandrosterone	.2	.6	.1	.3	.4	.5	.3	.8
Etiocholanolone	.5	—	.4	.3	.6	.6	.5	2.6
Androsterone	.2	—	.3	tr.	.3	.4	.2	1.5
Hydrolysis	A	D	B	B	C	D		
Sex	M	F	M	M	M	M		
Patient-age	M45	H55	B58	S59	H62	B68		

TABLE II

Subject R29—The qualitative and quantitative steroid response to ACTH in a case of acute myelogenous leukemia

Compounds	Periods I ACTH 100 mg./day 9 days	II ACTH 100 mg./day 9 days	III Control 6 days	IV Control 6 days	V ACTH 100 mg./day 9 days	VI ACTH 200 mg./day 9 days
	Milligrams per 24 hours					
C_{19} 11-oxysteroids	3.0	4.0	.7	.2	1.1	4.2
C_{19} 11-desoxysteroids	6.4	14.2	4.2	3.3	4.4	15.1
17α-Hydroxypregnanolone	2.3	1.7	—	.5	1.3	6.7
11-Ketoetiocholanolone	.9	1.4	.4	.2	.3	.8
11-Hydroxyetiocholanolone	.9	1.4	—	—	tr.	tr.
11-Hydroxyandrosterone	1.2	1.2	.3	tr.	.8	3.4
Etiocholanolone	5.1	11.5	3.0	1.4	2.4	11.4
Androsterone	1.3	2.7	1.2	1.9	2.0	3.7
Hydrolysis	Method C					
Sex	Male					
Patient-age	R 29					

the lower limit of the normal range for men in this age group. The level of C_{19} 11-desoxysteroids was also at the lowest level observed for normal men.

Subject B68: This subject was a white man, admitted to the hospital six months previous to study, with a one-month history of adenopathy and a reportedly high white blood cell count. The diagnosis of chronic lymphatic leukemia was established by marrow biopsy. Five months previous to study, this subject had received P^{32} therapy with a remission of his disease. His adenopathy and hepato-splenomegaly had recurred, however, at the time of study. He appeared well, otherwise.

The urinary ketosteroid values were lower than average although no striking alterations are evident.

Acute myelogenous leukemia

Subject R29: This patient was a white male physician, admitted to the hospital one day previous to study, with a one-month history of muscle and bone pain, and with an abnormally high white blood cell count. The diagnosis of acute myelogenous leukemia was established by marrow aspiration. He was acutely ill and it was impossible to obtain a control period preceding his first course of ACTH therapy. During this first therapeutic period he received 100 mg. per day of ACTH intramuscularly for a total of 24 days. Urinary steroids were studied during the first nine days (Period I) and the last nine days (Period II) of therapy. There was a striking clinical, metabolic and hematologic response to this treatment (24). Twelve days after the withdrawal of ACTH therapy, and during the period of clinical and hematologic remission, a control period (Period III) was obtained and the subject was discharged. Ten days later he was re-admitted because of increasing weakness and adenopathy. Marrow aspiration at this time showed complete relapse of his disease. A six-day control study (Period IV) was obtained following which he was again treated with 100 mg. per day of ACTH intramuscularly for nine days (Period V). During this period, edema of the face developed, although no eosinophile response was evident. Hematologic relapse persisted. The dose of ACTH was increased to 200 mg. per day at the end of this nine-day period. During the succeeding six days, there was an eosinophile response, but no hematologic remission of his disease. Period VI records the urinary steroid levels during the last eight days of ACTH therapy. He received 200 mg. per day of ACTH during this period, except for the last 48 hours during which the dose was increased to 400 mg. per day. Balance studies during this course of ACTH revealed a significantly smaller catabolic response than that observed during the first course of ACTH therapy. Bleeding persisted throughout this period and no hematologic response was evident. He expired in shock from profuse hemorrhage during the last day of this period, 23 days after the beginning of this second and unsuccessful trial of ACTH therapy.

The urinary ketosteroid data obtained during the periods studied are shown in Table II. The control data (Period III) for the first course of ACTH were obtained during a period beginning 12 days after the last dose of adrenocorticotropic hormone. It has already been established that the return to normal levels of urinary ketosteroids occurs within this period (26).

The levels of 11-oxygenated and 11-desoxysteroids during this control period were well below the average found in men in this age group. In this respect, the ketosteroid pattern of this subject is analogous to those of the older subjects described above. There is good evidence of adrenal response during ACTH administration from the clinical, hematologic, and metabolic observations during these periods. This is confirmed by the urinary ketosteroid findings, although it might be noted that the response in 11-oxygenated C_{19} metabolites is smaller than that observed previously in normal men receiving similar treatment for experimental purposes (27).

The second control period (Period IV) followed 10 days upon the first, and was obtained during a period of acute relapse in his disease. Some diminution in ketosteroid excretion is evident. The adrenal response to ACTH during the first nine days of this second course of therapy was smaller than that observed during the first course. The response to intense stimulation with ACTH during the last eight days of therapy was evident. Individual differences in 11-oxygenated steroid levels were noted during this period, as compared with the similar period of the first course of therapy.

DISCUSSION

There appear to be alterations in urinary ketosteroids in the seven subjects studied. In all patients the C_{19} 11-desoxysteroid metabolites were well below the average for the appropriate age and sex. It was not possible, in fact, to identify androsterone and etiocholanolone in the urine of Subject H55. While Subject R29 excreted more of these steroids than the other patients, comparison with a series of normal men in this age group (22) suggests a relative depression of these values. A simliar depression of the 11-oxygenated metabolites was observed in most subjects. Thus, subject R29, S59, H62, and B68 all showed low levels of 11-oxygenated steroid metabolites.[2] A comparison of the average ketosteroid values for the male leukemic subjects, 45 years or older, with those of normal men of similar age, is presented in Table I. A pronounced difference in steroidal excretion exists between these two groups.

While the experimental findings show clearly that the steroid excretion is low in the leukemic subjects, the reasons for this are less clearly evident. From the data, the alterations in steroid excretion may precede or result from physiologic changes induced by the subject's disease or they may be the result of a chronic, debilitating illness. In addition many other credible or specious explanations could be advanced for the results observed. These might include changes in the rate of hormone metabolism, increased "utilization" of hormones or metabolites, alteration in renal clearance, production of unusual, non-chromogenic steroids, intestinal rather than urinary excretion of metabolites, formation of chemically stable, unhydrolyzed conjugates, increased tissue storage, and the like. Regardless of the inherent merit of these possibilities, it is clear from the results reported that at least one of the subjects exhibited a clear response to ACTH by an increased excretion of urinary steroid hormone metabolites. In this subject, then, it has been demonstrated that his metabolic functions with respect to the adrenal steroid hormones were normally operative and qualitatively identical with that of other subjects (27). Furthermore, it has been demonstrated that a patient with chronic lymphatic leukemia (Subject F1) handled a tracer dose of hydrocortisone-4-C^{14} in a manner identical with other patients not afflicted with this disease (28). It seems, therefore, unreasonable to invoke remote possibilities when the consistently decreased excretion of steroid metabolites can reasonably be ascribed to a decrease in the output of the steroid producing glands. Therefore, the interpretation that the urinary steroids reflect the hormonal se-

[2] Relative to this group of metabolites, the application of more precise methods of hydrolysis and separation techniques results in the identification of 3α,11β-dihydroxyetiocholane-17-one, in the urine of most normal subjects, contrary to previous observations (22). Similarly, small amounts of 3α,17α-dihydroxypregnane-20-one are normally identified by these techniques.

cretion of the adrenal and gonads is preferred. It should be emphasized that this is a rational explanation of the facts developed by the methods available when these studies were made and not a rigorous demonstration of proven fact.

With these considerations in mind, it is of interest to consider the studies in subject R29 during the two courses of ACTH therapy. The urinary ketosteroids of R29 were at low levels during the first control period. In other respects the ketosteroid pattern was not unusual. The response to ACTH during the first course of therapy was similar to that observed in normal subjects, although the response in 11-oxygenated metabolites appeared somewhat diminished. Nevertheless, during these ACTH periods, pronounced metabolic, clinical, and hematologic responses were obtained (24). The control period obtained immediately after the acute relapse of his disease shows a steroid pattern different from that obtained during a period of complete remission. In view of the established reproducibility of the ketosteroid pattern in man (26), the steroid changes observed in this subject may be related to the change in his clinical status.

The observed changes in levels of 11-oxygenated (adrenal origin) and 11-desoxy (adrenal and gonadal origin) steroids while not pronounced, are suggestive of decreased adrenocortical activity. The somewhat diminished steroid response to ACTH during the first nine days of the second course of therapy and the diminished catabolic response observed during this same period were perhaps reflections of this alteration. Here again, however, the nature and severity of the illness as well as the kind and route of ACTH administration in this subject cannot be discounted. The question of whether the quantitative steroid and metabolic responses to ACTH are reproducible in all respects limits speculation from these data. It does appear, however, that the physiologic events suggested by the steroidal changes, and the abrupt change in clinical status may be of more than coincidental significance.

SUMMARY

The relationship between gonadal and adrenocortical function and human leukemia has been investigated by means of detailed isolation studies of the urinary ketosteroids in seven patients; six with chronic lymphatic leukemia and one with acute myelogenous leukemia during therapy with adrenocorticotropic hormone. Evidence of diminished gonadal and/or adrenocortical secretory activity in these patients was obtained. In one patient, clinical relapse and the development of refractoriness of the leukemia to ACTH therapy seemed related to disordered adrenal function, as determined by the qualitative and quantitative steroid response to ACTH.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council), the Anna Fuller Fund, the Lillia Babbitt Hyde Foundation, and the National Cancer Institute of the National Institutes of Health of the United States Public Health Service, (C-322).

The authors wish to express their deep appreciation to Dr. Olaf Pearson of the Division of Clinical Investigation, Sloan-Kettering Institute, for his generous cooperation in these studies. The authors also wish to express their appreciation to the large group of devoted Research Assistants and technicians who made much of the work possible. The routine chemical and chromatographic separations were carried out by a group under the supervision of Madeleine Stokem and Ruth Jandorek. The colorimetric analyses were under the supervision of Olga Teager and Denise O'Hara. The infrared spectrometry was under the supervision of Friederike Herling.

REFERENCES

1. Dougherty, T. F., Chase, J. H., and White, A., Pituitary-adrenal cortical control of antibody release from lymphocytes. An explanation of the anamnestic response. Proc. Soc. Exper. Biol. & Med., 1948, **58**, 135.
2. Reinhardt, W. O., and Li, C. H., Depression of lymphocyte content of thoracic duct lymph by adrenocorticotrophic hormone. Science, 1948, **101**, 360.
3. White, A., and Dougherty, T. F., Influence of pituitary adrenotrophic hormone on lymphoid tissue structure in relation to serum proteins. Proc. Soc. Exper. Biol. & Med., 1944, **56**, 26.
4. Dougherty, T. F., and White, A., Effect of pituitary adrenotropic hormone on lymphoid tissue. Proc. Soc. Exper. Biol. & Med., 1943, **53**, 132.
5. Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., Similarity of response of thymus and lymph nodes to administration of adrenocorticotropic hormone in the rat. Proc. Soc. Exper. Biol. & Med., 1943, **54**, 135.

6. Valentine, W. N., Craddock, C. G., Jr., and Lawrence, J. S., Relation of adrenal cortical hormone to lymphoid tissue and lymphocytes. Blood, 1948, **3**, 729.
7. Block, M., and Takano, G., Effect of ACTH upon induced leukemia in DBA mice. Cancer Research, 1942, **12**, 250.
8. McEndy, D. P., Boon, M. C., and Furth, J., On the role of the thymus, spleen, and gonads in the development of leukemia in high-leukemia stock of mice. Cancer Research, 1944, **4**, 377.
9. Levin, L., On the possible relationship between adrenocortical function and the leukemic state. Cancer, 1948, **1**, 413.
10. Arentsen, K., and Hogreffe, G., Influence of the sex hormones upon the development of leukemia in mice. Acta path. et microbiol. Scandinav., 1951, **28**, 201.
11. Murphy, J. B., The effect of castration, theelin, and testosterone on the incidence of leukemia in a Rockefeller Institute strain of mice. Cancer Research, 1944, **4**, 622.
12. Law, L. W., Effect of gonadectomy and adrenalectomy on the appearance and incidence of spontaneous lymphoid leukemia in C58 mice. J. Nat. Cancer Inst., 1947, **8**, 157.
13. Leiken, S., Rice, E. C., Bell, D. F., Jr., and Waters, R. J., The treatment of acute leukemia in children. J. Pediat., 1952, **41**, 40.
14. Darte, J. M. M., Snelling, C. E., Laski, B., Jackson, S. H., and Donahue, W. L., ACTH and cortisone in the treatment of leukaemia in children. Canad. M. A. J., 1951, **65**, 560.
15. Pearson, O. H., and Eliel, L. P., Use of pituitary adrenocorticotropic hormone (ACTH) and cortisone in lymphomas and leukemia. J. A. M. A., 1950, **144**, 1349.
16. Burchenal, J. H., Present status of ACTH, cortisone and the antimetabolites in the treatment of leukemia and related disease. Acta haemat., 1952, **7**, 193.
17. Levin, L., The urinary 17-ketosteroid levels of human leukemic subjects. J. Clin. Endocrinol., 1948, **8**, 487.
18. Hanlon, D. G., Mason, H. L., and Stickney, J. M., The urinary excretion of 17-ketosteroids and corticosteroids in leucemia; the effect of aminopterin (4-aminopteroylglutamic acid) on the excretion of corticosteroids in acute leucemia. J. Lab. & Clin. Med., 1950, **36**, 877.
19. Dobriner, K., Lieberman, S., and Rhoads, C. P., Studies in steroid metabolism. I. Methods for the isolation and quantitative estimation of neutral steroids present in human urine. J. Biol. Chem., 1948, **172**, 241.
20. Lieberman, S., Dobriner, K., Hill, B. R., Fieser, L. F., and Rhoads, C. P., Studies in steroid metabolism. II. Identification and characterization of ketosteroids isolated from urine of healthy and diseased persons. J. Biol. Chem., 1948, **172**, 263.
21. Dobriner, K., Lieberman, S., and Rhoads, C. P., Studies in steroid metabolism. III. The application of infra-red spectrometry to the fractionation of urinary ketosteroids. J. Biol. Chem., 1948, **172**, 297.
22. Dobriner, K., Studies in steroid metabolism. XIX. The α-ketosteroid excretion pattern in normal males. J. Clin. Invest., 1953, **32**, 940.
23. Katzenellenbogen, E. R., Dobriner, K., and Kritchevsky, T. H., Separation of steroid hormones and their metabolites by partition-type chromatography. J. Biol. Chem., 1954, **207**, 315.
24. Pearson, O. H., and Eliel, L. P., Experimental studies with ACTH and cortisone in patients with neoplastic disease. Recent Progress in Hormone Research, 1951, **6**, 373.
25. Dobriner, K., Unpublished data.
26. Dobriner, K., Studies in steroid metabolism. XX. The reproducibility of the urinary steroid pattern in humans. J. Clin. Invest., 1953, **32**, 950.
27. Dobriner, K., Studies in steroid metabolism. XXII. The adrenal response to ACTH in young and old men. J. Clin. Invest., 1954, **33**, 222.
28. Hellman, L., Bradlow, H. L., Adesman, J., Fukushima, D. K., Kulp, J. L., and Gallagher, T. F., The fate of hydrocortisone-4-C^{14} in man. J. Clin. Invest., 1954, **33**, 1106.