and Goodman, L. S., *J. Pharm. and Exp. Therap.*, 1954, v110, 86.

3. Courvoisier, S., Fournel, J., Ducrat, R., Kolsky, M., et Koetschet, P., *Arch. internat. de pharmacodyn. et de thérap.*, 1953, v92, 305.

4. Moyer, J., Kent, B., Knight, R., Morris, G., Huggins, R., and Handley, C. A., *Am. J. M. Sc.*, 1954, v227, 283.

5. Information published by Smith, Kline and French Laboratories.

6. Lehmann, H. E., and Hanrohan, G. E., *Arch. Neurol. and Psychiat.*, 1954, v71, 227.

7. Winkelman, N. W., *J.A.M.A.*, 1954, v155, 18.

8. Finkelstein, M., Spencer, W. A., Hamman, C. S., and Albert, S. N., *Fed. Proc.*, 1954, v13, 354.

9. Melville, K. I., *Fed. Proc.*, 1954, v13, 386.

Received July 9, 1954. P.S.E.B.M., 1954, v86.

Conjugated Steroids V. Hydrolysis of Total Ketosteroids in Urine.* (21215)

IRVING B. ONESON† AND SAUL L. COHEN.

From the Department of Physiological Chemistry and Max H. Hoffman Endocrinological Research Laboratory, University of Minnesota Medical School, Minneapolis.

Numerous attempts have been made to effect a complete nondestructive hydrolysis of ketosteroids without affecting in any way the nature of the steroids. It has been possible to hydrolyze the ketosteroid glucuronides under mild conditions with enzymes prepared from bacteria(1) or spleen(2). Recently, Cohen and Oneson have described the hydrolysis of ketosteroid sulfates with dioxane-trichloroacetic acid(3). About 40% of the total ketosteroids of normal male urine are apparently conjugated as sulfates, of which slightly more than half is of the beta configuration. The sum of the ketosteroid sulfates and glucuronides exceeds the value obtained by routine hydrochloric acid hydrolysis by about 15%. This paper describes the hydrolysis of the total ketosteroids by the use of dioxane-HCl.

Methods. Urine residues are prepared in the same manner as for the hydrolysis of the ketosteroid sulfates(3). This involves extraction with butyl alcohol of acidified (pH 2-3) urine. The extract is washed with water and evaporated to dryness at temperatures not exceeding 60°. Portions of the butyl alcohol residues equivalent to 100 to 200 cc of urine are hydrolyzed by the addition of 10 cc of 1,4 dioxane containing 10% of concentrated hydrochloric acid. The alpha and beta fractions are assayed after their separation with digitonin according to the method of Frame (4).

Results. A concentration of acid which

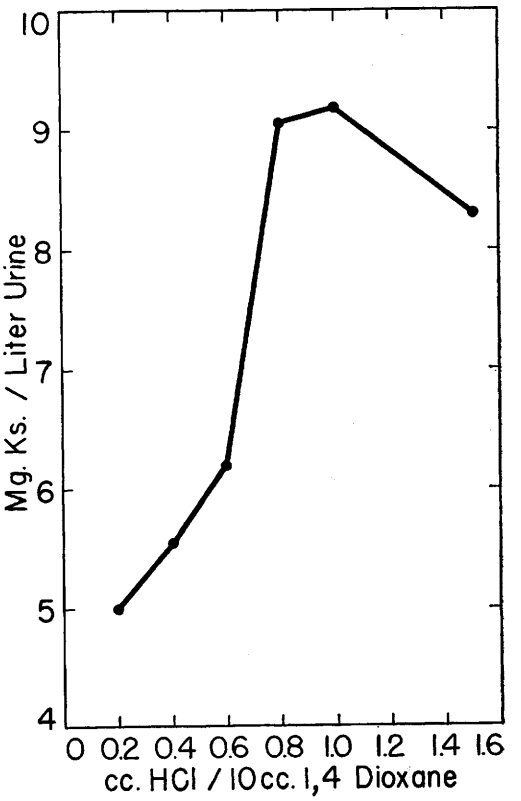

FIG. 1. Release of ketosteroids by dioxane HCl.

* These researches were carried out under grants made by the U. S. Public Health Service and the University of Minnesota.

† Present address, Ethicon, Inc., New Brunswick, N. J.

TABLE I. Standard HCl vs Dioxane, Dioxane-HCl and Glucuronidase.

Urine batch	mg Ks/l urine: (a) Standard HCl total	(a) Dioxane total	α	β	(b) Glucuronidase total	a + b	Dioxane-HCl: Total	α	β
NMU 24	10.2	2.5	1.3	1.2	8.2	10.7	12.0	10.3	.94
27	9.2	3.8	1.5	2.0	7.4	11.2	10.4	8.4	1.9
124	9.2	3.8	1.4	2.3	5.8	9.6	9.5	7.1	2.4
153	10.2	4.2	2.2	1.7	6.8	11.0	11.7	10.2	1.8
168	10.7	4.6	2.2	2.4	7.2	11.6	12.2	9.9	2.2
215A	11.0	4.4	2.1	2.2	7.4	11.8	13.5	9.8	3.0
NMU 216	9.8	4.4	1.3	2.8	7.5	11.9	13.3	10.7	2.4
LPU 20	5.5	2.2	1.6	.6	3.4	5.6	6.1	5.1	.7

effects maximum hydrolysis was found by dissolving the residue in 10 cc of 1,4 dioxane; after adding 0.1-1.4 cc (1-14%) concentrated HCl, the preparation was heated in a steam bath for 10 minutes (see Fig. 1). It can be seen that 8-10% HCl gives a maximum hydrolysis of the urinary ketosteroids. Results of the dioxane-HCl hydrolysis on various urine residues are shown in Table I. It can be seen from these data that about 17% more steroids are formed than by the standard HCl procedure. About 20% of the total steroids were found to be of beta type. Heating with an excess of acid or heating for a long period of time results in the destruction of the beta steroids. It is important to point out that longer heating causes destruction of the ketosteroids. Thus, Urine NMU 153 heated with dioxane HCl for 10 minutes resulted in the release of 2.1 mg of beta steroids. After heating for 15 minutes, the beta steroids fell to 1.97 mg, and after heating for one hour the beta steroids were 0.94 mg.

Summary. The total ketosteroids of urine may be readily hydrolyzed by dioxane-hydrochloric acid. This hydrolysate contains about 20% of the total steroids as beta type.

1. Buehler, H. J., Katzman, P. A., Doisy, P. P., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 297.

2. Cohen, S. L., *J. Biol. Chem.*, 1951, v192, 147.

3. Cohen, S. L., and Oneson, I. B., *ibid.*, 1953, v204, 245.

4. Frame, E. G., *Endocrinology*, 1944, v34, 175.

Received July 13, 1954. P.S.E.B.M., 1954, v86.

Increased Metabolic Rate without Thyroid Participation on Injection of Rats with Pituitary Erythropoietic Fractions.* (21216)

A. N. CONTOPOULOS, E. S. EVANS, S. ELLIS, AND M. E. SIMPSON.

From the Institute of Experimental Biology and the Department of Anatomy, University of California, Berkeley.

As reported recently, extracts from sheep pituitary can be obtained(1) containing an erythropoietic principle which repairs the anemia resulting from hypophysectomy(1-3), prevents the development of the neonatal anemia(4,5), and produces polycythemia in the normal(6), hypophysectomized(7,8), and adrenalectomized rat(2). Reticulocytes and normoblasts appear in the peripheral blood of hypophysectomized rats injected with this erythropoietic factor, and their bone marrow is stimulated. Inasmuch as reports have appeared in the literature indicating that increased activity of the bone marrow is accompanied by an elevated metabolic rate(9-11), the caloric output of rats injected with the pituitary erythropoietic fraction was examined.

* Supported in part by U. S. Public Health Grant.

Methods. Female rats of the Long-Evans