the presence of antibodies against tuberculin in the blood serum of animals which have received injections of tubercle bacilli. The test clearly distinguishes sera of normal animals from those obtained from animals infected with tubercle bacilli. This method demonstrates the presence of antibodies against tuberculin in many of the animals tested at a time when the complement-fixing antibodies are not demonstrable, and at a time when many of the animals fail to exhibit skin-sensitivity to tuberculin.

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Isolation of Isoandrosterone from Urine in a Case of Virilism.

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Adrenal virilism of women is frequently associated with an abnormally high titer of urinary androgens. So far two androgenic compounds, dehydroisoandrosterone and isoandrosterone, have been isolated from urine of this type. Dehydroisoandrosterone has been found in several cases of virilism (mostly tumors of the adrenal cortex),[1] but isoandrosterone has been encountered only in one instance (a case of adrenal hyperplasia)[2] and has as yet not been found in any other type of human urine. We, therefore, wish to report the isolation of isoandrosterone from the urine of a woman suffering from severe hirsutism.

S.T., a 27-year-old woman was admitted to the Urological Clinic of the Presbyterian Hospital, New York, in 1937. Examination showed a marked hirsutism of body, face, and limbs, a slightly enlarged clitoris and a hypoplastic uterus. Aerograms taken in 1937 and 1939 gave no evidence of an adrenal enlargement. On biopsy (1940) both ovaries were found to be polycystic. The clinical data do not seem to permit a diagnosis as to the gland responsible for the

1 Crooke, A. C., and Callow, R. K., *Quart. J. Med.*, 1939, **32**, 233; Callow, R. K., *Proc. Roy. Soc. Med.*, 1938, **31**, 841; Dorfman, R. I., Wilson, H. M., and Peters, J. P., *Endocrinology*, 1940, **27**, 1.

2 Butler, G. C., and Marrian, G. F., *J. Biol. Chem.*, 1938, **124**, 237; Marrian, G. F., and Butler, G. C., *Nature*, 1938, **142**, 400.

observed symptoms. The patient did not receive any hormonal treatment either prior to or during the period of urine collections (1937). At that time the urine contained about 100 I.U. of androgens per liter when assayed on the capon.

Urine specimens totaling 38 liters were hydrolyzed according to the procedure of Peterson, Gallagher and Koch[3] and extracted with ether within 2 weeks after their collection. The neutral ketones were obtained in the usual manner and fractionated with digitonin. The ketones precipitable with digitonin (36 mg melting 142-156°)* were acetylated. Upon recrystallization 15.2 mg of colorless needles melting at 89-94° were obtained. The final product (11.3 mg) melted at 95-96°. The melting point of isoandrosterone acetate has been given as 97° [4] and as 116°.[2, 5] It appears that this discrepancy is due to crystal polymorphism. On standing for about 18 months the acetate was partly converted into the higher melting modification (114°). A third modification of the acetate melting at 106.5° was obtained by acetylation of a synthetic preparation of isoandrosterone. While its melting point did not change on recrystallization, a mixture of all three forms was obtained on seeding with the isolated material. This preparation showed 2 points of incomplete fusion (followed by resolidification) at 97° and at 106.5° and melted completely at 116°. When the specimen was finely powdered before heating it melted sharply at 115.5-116.5°. Specimens obtained by resolidification after complete fusion usually melted at 97° and occasionally at 106.5°. A mixture of the isolated and the synthetic material melted at 113.5-115.5°.

The isolated acetate upon alkaline hydrolysis yielded colorless needles melting at 172.5-174.5°. The melting point remained unchanged after admixture with an authentic specimen of isoandrosterone melting at 174.5°. The isolated material was converted into a benzoate which melted at 217.5-219.5°. A mixture with synthetic isoandrosterone benzoate melting at 219.5° melted at 219.5°.

Examination of the mother liquors obtained in the purification of the ketones precipitable with digitonin gave no indication of the presence of dehydroisoandrosterone. This seems to be noteworthy

[3] Peterson, D. H., Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1937, **119**, 185.

* All melting points reported are corrected.

[4] Ruzicka, L., Goldberg, M. W., Meyer, J., Bruengger, H., and Eichenberger, E., *Helv. Chim. Acta*, 1934, **17**, 1395; Butenandt, A., and Cobler, H., *Z. physiol. Chem.*, 1935, **234**, 218

[5] Dorfman, R. I., and Fisch, W. R., *J. Biol. Chem.*, 1940, **135**, 349.

since this compound is considered to be a normal urinary constituent.[6] The ketonic fraction which was not precipitated by digitonin yielded the 2 main 17-ketosteroids which are found in the urine of normal women,[6] androsterone and etiocholanol-3(α)-one-17.

Summary. Isoandrosterone, androsterone, and etiocholanol-3(α)-one-17 have been isolated from the urine of a woman suffering from virilism. No dehydroisoandrosterone has been found.

While this paper was in press Pearlman[7] reported the isolation of isoandrosterone from the urine of normal women and of patients with cancer. The yield which is given only for normal female urine is appreciably lower than that obtained from this case of virilism.

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Enhancement of Certain Toxic Effects of Codeine and Morphine by Sulfapyridine.*

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In a previous paper it has been demonstrated that sulfapyridine enhances the narcotic and toxic effects of papaverine.[1] The increase of the anaesthetic and toxic effects of barbituric compounds on rats when treated with sulfanilamide has been shown by Adriani.[2] It was considered of interest to investigate whether the action of other drugs affecting the central nervous system is also increased by sulfapyridine. The present paper deals with the influence of sulfapyridine on the effects of codeine and morphine.

The codeine experiments were performed on rats, mice, and rabbits, those of morphine only on mice. The weights of the animals ranged from 190 to 240 g for the rats, 20 to 23 g for the mice, and

6 Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

7 Pearlman, W. H., *J. Biol. Chem.*, 1940, **136**, 807.

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1 Glaubach, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 325.

2 Adriani, J., *J. Lab. and Clin. Med.*, 1939, **24**, 1066.