first dose. A blood culture obtained before treatment was started yielded meningococcus, group I and subsequent blood cultures were negative.

The results in these cases appear to be comparable to those obtained in similar cases treated with penicillin or with effective sulfonamides. There were no toxic effects attributable to aureomycin in any of these cases.

Further experience is needed to determine the optimum dosage and duration of treatment as well as the effect in more severe cases. The present findings, however, suggest that aureomycin may be an effective agent in pneumococcal and meningococcal infections and warrants further clinical trial in these and other types of infections.

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Excretion of Androgens and 17-Ketosteroids in the Cebus Monkey Following the Administration of Testosterone Propionate.

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(Introduced by H. Selye.)

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An increase in urinary 17-ketosteroids and androgens after administration of testosterone has been noted in man,[1] chimpanzee,[2] Rhesus monkey,[3] guinea pig[4] and rat.[5] Normally Cebus monkeys excrete minimal amounts of androgens as compared with other primates,[6] hence we wished to examine their 17-ketosteroids and androgen excretion after administration of testosterone propionate.*

Material and Methods. Six adult male Cebus monkeys, weighing 2.8 - 3.3 kg, were used. After a control period of 6 days, each animal received daily 50 mg of testosterone propionate subcutaneously, in 1 ml of peanut oil solution, for 12 days followed by 5 days during which no treatment was given. Throughout the period of observation (23 days) the urine of each monkey was continuously collected under toluene. Twice daily the urine specimen of all monkeys were pooled, acidified with 1% hydrochloric acid, placed in the refrigerator and extracted the following day. Hydrolysis, extraction and fractionation by Girard's reagent for chemical and biological assays was done as previously described.[7] The urinary extracts from the control period of 6 days (I), the treatment period of 12 days (II) and the post-treatment period of 5 days (III) were kept separate for chemical and biological assays. Colorimetric estimations were done by the Zimmermann method as modified by Callow *et al.*[8] and by the antimon trichloride technic.[9] Assays of androgenic activity were made using the chick comb method.[6]

Results. A. *Androgenic activity.* In a preliminary test we verified that the androgenic activity of the urine collected during periods I, II, and III was approximately the same. Therefore the final assay was done only on

1 Dorfman, R. I., and Hamilton, J. B., *J. Clin. Invest.*, 1939, **18**, 67.

2 Fish, W. R., and Dorfman, R. I., *Endocrinology*, 1944, **35**, 22.

3 Dorfman, R. I., and Hamilton, J. B., *Endocrinology*, 1939, **25**, 28.

4 Dorfman, R. I., and Fish, W. R., *J. Biol. Chem.*, 1940, **135**, 349.

5 Dorfman, R. I., *J. Biol. Chem.*, 1938, **123**, xxx.

6 Valle, J. R., Henriques, S. B., and Henriques, O. B., *Endocrinology*, 1947, **41**, 335.

* Testosterone propionate was furnished through the courtesy of the Ciba Pharmaceutical Company of S. Paulo.

7 Henriques, O. B., and Henriques, S. B., *Mem. Inst. Butantan*, 1946, **19**, 11.

8 Callow, N. H., Callow, R. K., and Emmens, C. W., *Biochem. J.*, 1938, **32**, 1312.

9 Pincus, G., *Endocrinology*, 1943, **32**, 176.

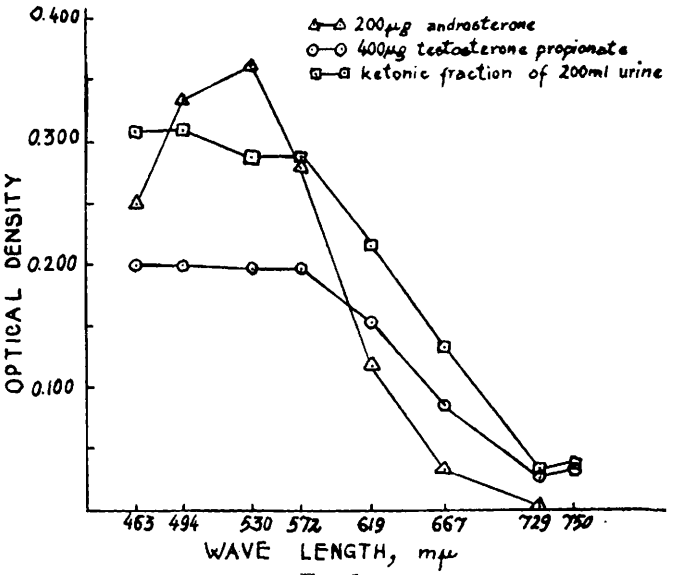

FIG. 1.

Comparison of absorption spectra of androsterone, testosterone propionate and ketonic fraction of urinary extract submitted to Zimmermann's reaction.

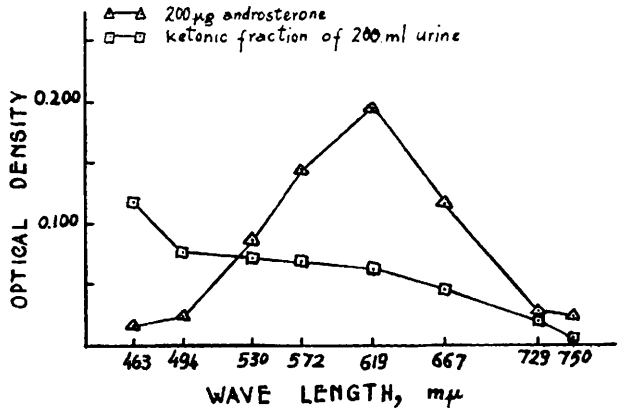

FIG. 2.

Comparison of absorption spectra of androsterone and ketonic fraction of urinary extract submitted to antimon trichloride reaction.

samples I and II. Androgen excretion was found to be 94.2 ± 24.5 μg per liter for sample I and 87.9 ± 39.0 for sample II. This corresponds to 10 and 6.5 μg per 24 hours, respectively, for one monkey.

B. *17-Ketosteroids.* Using Zimmermann's procedure, the color obtained was brownish instead of violet. Therefore we analysed this color with the Pulfrich photometer. Fig. 1 shows the absorption spectra of androsterone, testosterone propionate and the ketonic fraction from the extract of 200 ml of urine of untreated monkeys. Fig. 2 shows the absorption spectra of the same substances with the exception of testosterone, using the antimony trichloride reaction. It will be noted that there is no maximum absorption at 530 mμ for the urinary extract with the Zimmermann's reaction nor at 620 mμ with antimony trichloride procedure. After administration of testosterone propionate, no significant qualitative or quantitative change was detected in

the absorption spectra.

Discussion and Summary. As previously stated, the administration of testosterone to many animals including man and the Rhesus monkey, results in an increased urinary excretion of androgens and 17-ketosteroids. In contradistinction to this we noted no increase in either of these substances in the urine of treated Cebus monkeys. This finding parallels those of Dingemanse and Tyslowitz,[10] Paschkis *et al.*,[11] who found no increase in the urine of dogs similarly treated. In dogs[11] and man[12] increased urinary estrogenic activity has been shown after testosterone treatment. Our results would suggest that in the Cebus monkey as in man and the dog, testosterone can be converted to estrogens, but further work is necessary to elucidate this point.

[10] Dingemanse, E., and Tyslowitz, R., *Endocrinology*, 1941, **28**, 450.

[11] Paschkis, K. E., Cantarow, A., Rakoff, A. E., Hansen, L. P., and Walking, A. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 213.

[12] Dorfman, R. I., and Hamilton, J. B., *Endocrinology*, 1939, **25**, 33.

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Determination of Creatine, Creatinine, and Related Compounds in Urine by Means of Paper Chromatography.*

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A partition chromatographic procedure for the identification and estimation of creatine and creatinine and their demethylated analogues, glycocyamidine and glycocyamine (guanidoacetic acid) was devised during the course of an investigation of aminoaciduria in dystrophic humans.[1] Two different color-reactions were investigated to indicate the location of the various substances on the developed chromatogram. One was based on the Jaffe reaction, the formation of a reddish-orange product when alkaline picrate is added to solutions of creatinine and related compounds. This reaction has been used by Dent[2,3] to identify creatinine on a paper strip following partition chromatography. The other was a modification of the Voges-Proskauer reaction in which diacetyl in alkaline solution forms a pink product with creatine and similar compounds.

Method Using Jaffe Reaction. The Jaffe reaction is the conventional method for the detection of creatinine. It is dependent on a cyclic lactam structure such as is found in creatinine and glycocyamidine and is not given by the hydrated straight chain analogues, creatine and glycocyamine. It is necessary, therefore, to dehydrate the latter compounds *in situ* before applying the color reaction. It was found that heating the dry paper strips for one hour at 100-110°C transformed creatine to creatinine. Glycocyamine was dehydrated to glycocyamidine by heating for one hour at 100-110°C but the conversion was not quantitative. Further heating did not appear to increase the amount of glycocyamidine formed as evidenced by the depth of the color reaction. Therefore, in actual practice a constant heating period at a constant temperature was carefully followed in order to make valid comparisons between the depth of color formed with standard solutions and with urines of unknown composition.

Details of the experimental procedure are as follows: A 0.025 ml aliquot of urine or

* Communication No. 144.

[1] Ames, S. R., and Risley, H. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 131.

[2] Dent, C. E., *Biochem. J.*, 1947, **41**, 240.

[3] Dent, C. E., in Reifenstein, E. C., Conference on Metabolic Aspects of Convalescence, Josiah Macy, Jr. Foundation, New York, 1946, p. 126.