reference to the amounts injected, since a dosage of 150 mg. nitrogen per kilo brought about serum sickness in 80% of 25 animals. Smaller doses (*i. e.*, from 71 to 100 mg. nitrogen) of this fraction resulted in a decreased occurrence of serum sickness and since none of the animals receiving 35 mg. of nitrogen per kilo developed the disease, it may be inferred that under these conditions the threshold for development of serum sickness in the most susceptible animals lies at a dosage of between 35 and 71 mg. pseudoglobulin nitrogen per kilo. The albumin fraction, though administered in a dosage as large as 150 mg. nitrogen per kilo was without effect in causing serum sickness.

Under these conditions, it may be assumed that the inciting agent of serum sickness is encountered primarily in the pseudoglobulin fraction of horse serum.

6854

Preparation of Prolan, Theelin and Theelol from the Same Urine.

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No doubt the possibility of preparing prolan, theelin and theelol from the same urine has occurred to many investigators interested in sex hormones. In this note we have given the adaptation of processes published from this laboratory which permits the preparation of the 3 substances. The combined methods which have been in constant use for about one year give a satisfactory yield of all 3 compounds.

The prolan is made by the benzoic acid process described by Katzman and Doisy.[1] The filtered urine is strongly acidified with hydrochloric acid and after a few days the supernatant liquid siphoned from the precipitate. The urine is extracted with butyl alcohol in the continuous extraction apparatus (Veler, Thayer and Doisy[2]). The solvent is distilled off, the residue leached with benzene and the benzene removed by distillation. The residue from 100 gallons of urine is dissolved in 1500 cc. of 80% ethyl alcohol

[1] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

[2] Veler, C. D., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.

containing 150 cc. of concentrated hydrochloric acid. The solution is hydrolyzed by boiling for 4 hours, the alcohol removed by distillation and solid NaOH added until the solution is alkaline to phenolphthalein. The solution is transferred to separatory funnels and extracted with butyl alcohol to which an equal volume of petroleum ether is added. The butyl alcohol-petroleum ether solution of theelin and theelol are then worked up according to the process described by Doisy and Thayer[3] beginning, however, at Step 3. We have found that the purification of theelol as the sodium salt (MacCorquodale, Thayer and Doisy[4]) to be quite advantageous.

Though we have no quantitative data on the yield of theelin, the recovery seems to be quite satisfactory. The yield of theelol has ranged from 2 to 5 mg. per gallon of urine.

[3] Doisy, E. A., and Thayer, S. A., *J. Biol. Chem.*, 1931, **91**, 641.

[4] MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, 1933, **99**, 327.