intrauterine growth, we may, nevertheless, carry cholesterol-fed animals through successful pregnancy and lactation studies. Because it is possible to follow cholesterol distribution in tissue, this should represent a useful method for study of the lipid transport.

Tissue analyses from these and later series of pregnant animals will be reported in the future. Mrs. Godfrey's untimely death has necessitated reorganization of our plans for this study and delayed the work to the extent that we have felt that the potential usefulness of her findings has indicated this preliminary report.

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Isolation of a Water-Soluble Pregnandiol Complex from Human Pregnancy Urine.

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A physiologically inactive solid alcohol was prepared from human pregnancy urine by Marrian[1] in 1929. This substance was identified by Butenandt[2] in 1930 and was called by him pregnandiol. It was insoluble in water and showed a melting point of 233-235°C. (uncorrected). Recently O'Dell and Marrian[3] have obtained evidence for the existence of an acid-hydrolyzable form of pregnandiol.

We have been able to isolate a water-soluble complex of pregnandiol from pregnancy urine. The method of preparation was as follows: Pregnancy urine (9th month) was extracted with butyl alcohol. The extract was evaporated to dryness under reduced pressure. The residue was taken up in N/2 NaOH and re-extracted with butyl alcohol, the butyl alcohol fraction washed twice with water and evaporated to dryness. The residue was dissolved in a minimal amount of water and the substance was precipitated with acetone. This was collected by centrifuging and purified by crystallization from hot water and several times from ethyl alcohol.

The white crystalline substance, so obtained, melts at 268-71°C. (uncorrected) with decomposition and evolution of gas. It crystallizes from alcohol in thin plates. It is soluble in water, less solu-

[1] Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

[2] Butenandt, A., *Ber. dtsch. chem. Ges.*, 1930, **63**, 659.

[3] O'Dell, A. D., and Marrian, G. F., *Canadian Chemical Convention*, 1936, June 11th.

ble in ethyl alcohol, insoluble in ether. Qualitative tests for nitrogen, sulphur, phosphorus and halogens were negative. It is precipitated from aqueous solution at room temperature by acid, yielding a crystalline compound of acidic nature, which melts at 179-180°C. (uncorrected) and decomposes with gas formation.

The material melting at 271°C. was subjected to acid hydrolysis in the autoclave, and a crystalline substance was obtained (insoluble in water but soluble in acetone) melting at 218°C., which on recrystallization from acetone and alcohol melted at 233°C. It showed no depression of mixed melting point with pregnandiol. A solution of the complex showed no reduction of alkaline copper, however, the filtrate after hydrolysis caused marked reduction. Both the filtrate and the original material gave a strongly positive naphthoresorcinol test (Tollens) showing the presence of glucuronic acid.

The carbon-hydrogen analyses for the free acid gave the following values: C, 63.65%, 63.52%; H, 8.75%, 8.77%. The theoretical values for pregnandiol-glucuronic acid ($C_{27}H_{44}O_8H_2O$) are C, 63.1%; H, 8.95%. The values for the acid agree reasonably well with those required for pregnandiol-glucuronic acid. The complex extracted directly from the urine contains sodium and is a monosodium salt of pregnandiol glucuronic acid. The yield of the pregnandiol complex obtained by this method as the sodium salt, from pregnancy urine near term, is at least 40 to 50 mg. per liter, which on hydrolysis would give about 20 to 25 mg. of pregnandiol, a greater amount than has previously been reported.

As pregnandiol may be regarded as the excretion product of corpus luteum hormone and also is the initial product in the synthesis of this hormone, the above relatively easy method of extracting and measuring this substance should be of practical significance.

We are at present measuring the amount of this complex excreted during the various stages of pregnancy.*

* Carbon-hydrogen analyses were done by Dr. V. Niederl, New York University.