a mean antitryptic index of approximately 7.5, whereas the mean value for the sera of 15 pregnant women was found to be approximately 10.0. This is in accord with the findings of others, who have observed a rise in antitrypsin during pregnancy.[5]

8794 P

Pregnancy in Cholesterol Fed Rats.

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The disturbances in blood cholesterol level known to be associated with pregnancy, and the prevailing impression that pregnancy may increase the incidence of diseases associated with abnormal cholesterol deposition have indicated the desirability for study of the influence of cholesterol feeding during pregnancy.

There is strikingly little record of such a study in the literature. Schönheimer[1] reported that pregnant animals were more subject to the deposition of anisotropic fat than non-pregnant ones. He reports feeding cholesterol to a rabbit which became very sick 14 days after mating and showed resorption of 3 fetuses with one fetus dead at term and abnormal deposition of anisotropic fat in the placenta.

We have used rats for the present study. These were placed at weaning on a diet made up of 20 parts raw casein, 4 parts Osborne and Mendel salts, 4 parts agar, 15 parts Crisco, and 57 parts starch, with one part cholesterol dissolved in Crisco and incorporated in the diet. Vitamin supplements were given separately 3 times a week as yeast, tiki-tiki, or yeast extract for B, and tuna or sea bass liver oil standardized in this laboratory and diluted with corn oil for A and D. With tiki-tiki, raw casein supplied G. After successful mating was demonstrated by the finding of sperm, the protein in the diet was increased to 26% and the starch decreased to 51% and the vitamin B increased 2 to 4 times (in different groups). This diet was modeled on that shown by Morgan and Simson[2] in this laboratory to be adequate to meet the food requirements of the rat during pregnancy.

5 Flexner, L. B., Berkson, J., Winters, H., and Wolman, I., PROC. SOC. EXP. BIOL. AND MED., 1928-29, **26**, 592.

* Deceased, November 16, 1935.

1 Schönheimer, R., *Arch. Path. Anat.* (Virchow), 1924, **249**, 1.

2 Thesis: Catherine M. Cave Simson with A. F. Morgan, Department of Household Science, University of California, Berkeley, 1934.

Vaginal smears were taken daily after the rats were approximately 90 days of age. In general cycles tended to be lengthened by one to 2 days. They were not altered, nor was the mating performance of the animals changed when, at the suggestion of Mrs. O. H. Emerson, a wheat germ oil preparation of tested potency in amounts several times that necessary to supply the vitamin E requirement for normal pregnancy in the rat was given to half the animals. Some difficulty was experienced in obtaining successful matings with cholesterol-fed animals. There seemed, however, to be no difference, either in fertility or in difficulty of mating when cholesterol-fed males or males on stock diets were used. Likewise, cholesterol-fed males showed no loss of fertility when mated with stock females.

Fifteen first and 5 second litters from cholesterol-fed mothers were carried through to term. Thirteen apparently entirely normal animals were killed just before delivery and nine more from the 14th to the 18th days of pregnancy. Three animals showing resorption were killed during pregnancy. Because of the previous study carried out on the same diet in this laboratory, only about half that number of non-cholesterol-fed animals were included in this first series. These were sacrificed during and just at the end of their pregnancies.

The livers of the cholesterol-fed mothers showed the usual fatty appearance. There was also some gross evidence of fatty infiltration of the placentae. Fetuses tended to be slightly smaller than in control and stock animals killed at the same stages of pregnancy. Birth weights of the young were not obtained because we were afraid that handling the animals would induce cannibalism. Two litters were found dead within a day after birth, and one or 2 animals were lost from 3 other litters. Litter size averaged 8.5 for cholesterol-fed mothers, a number not significantly different from the controls but about 4 less than for the stock colony. Eighteen litters were carried to weaning. Weaning weights of babies from cholesterol-fed mothers averaged 20.5 gm., which was more than 15 gm. lower than Morgan and Simson's for the same basal diet, and over 20 gm. lower than the average from our stock colony for litters cut to this size. These animals, however, grew normally after weaning, the average weights of 30 females kept for an additional 6 months on the cholesterol diet was 193 gm. and the average for 15 males, 305 gm.

It would seem, therefore, that we are justified in concluding that, while cholesterol feeding apparently interferes to some extent with

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.

8795 P

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.