rendered heat-labile. This was demonstrated by post-incubation at 20 or 37°C of microsomes hydrolysed by phospholipase at 5°C, EGTA being added before post-incubation. Heat-inactivated enzyme could not be reactivated by sonicated phosphatidylcholine, though this completely restored both activity and heat-stability to glucose 6-phosphatase from microsomes with 95% of the phosphatidylcholine and 70% of the phosphatidylethanolamine hydrolysed at 5°C. Lecithin probably failed to reactivate glucose 6-phosphatase for Duttera, Byrne & Ganoza (1967) because they treated with phospholipase at 20°C.

Preliminary conclusions are that glucose 6-phosphatase is not wholly lecithin-dependent, since 40–60% of the activity remains when 90–100% of the lipid is hydrolysed. Superimposition of heat-inactivation prevents quantitative definition of the phospholipid-dependence of glucose 6-phosphatase, so this must be investigated by low-temperature techniques.

- Beaufay, H. & de Duve, C. (1954). *Bull. Soc. Chim. biol.* **36**, 1551.
 Duttera, S. M., Byrne, W. L. & Ganoza, M. C. (1967). *J. biol. Chem.* **243**, 2216.
 Hallinan, T., Nagley, P., Murty, C. N., Bennett, J. & Grant, J. H. (1969). *Biochim. biophys. Acta*, **173**, 554.
 Nagley, P. & Hallinan, T. (1968). *Biochim. biophys. Acta*, **163**, 218.

Effect of Vitamin A Status of the Content of some Steroids in the Ovarian-Vein Blood of Pregnant Rats

By J. GANGULY (*Indian Institute of Science, Bangalore, India*) and G. S. POPE, S. Y. THOMPSON, JOYCE TOOTHILL and J. EDWARDS-WEBB. (*National Institute for Research in Dairying, Shinfield, Reading, U.K.*) and H. B. WAYNFORTH. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London W.1, U.K.*)

Female rats given a vitamin A-deficient diet with retinoic acid maintain normal oestrous cycles and conceive when mated with normal males, but pregnancy is terminated by resorption at about day 14 (Thompson, Howell & Pitt, 1964; Juneja, Murthy & Ganguly, 1964). Juneja, Moudgal & Ganguly (1969) showed that the resorption is prevented by injections of pregnenolone or oestradiol-17 β , whereas progesterone is only partially effective. Further, the specific activity of 3 β -hydroxy Δ^5 -steroid dehydrogenase is less in the ovaries of retinoate-fed rats during pregnancy.

Groups of rats maintained on a vitamin A-deficient diet supplemented with either retinyl acetate or retinoic acid grew and conceived normally,

but after day 12 of pregnancy those receiving retinoate ceased to increase in body weight and no pups were born; pregnancy in the other group was normal.

To investigate steroid secretion rates, ovarian-vein blood was taken at days 9 and 15 of pregnancy. Tracers of ^3H -labelled steroids were added to pooled extracts of blood each obtained by the addition of acetone to the sample. Extracts were fractionated by partition between NaHCO_3 and ether, column chromatography (silica-gel adsorbent) and partition between toluene–light petroleum and 0.4M-NaOH, giving fractions containing neutral and phenolic steroids. Oestradiol-17 β and oestrone, separated by t.l.c. on silica gel, were finally purified by paper chromatography. Each oestrogen was dissolved in propane-1,2-diol–water (1:10, v/v), for bioassay by the intravaginal tetrazolium-reduction method of Martin (1960, 1964). Progesterone and 20 α -hydroxy-4-pregnen-3-one were determined by g.l.c. after further purification by solvent partitioning and t.l.c.

With both supplements there were marked increases in the secretion rates of progesterone and 20 α -hydroxypregnenone as pregnancy advanced, as has been reported for normal rats by Eto, Masuda, Suzuki & Hosi (1962), Fajer & Barraclough (1967) and Hashimoto, Hendricks, Anderson & Melampy (1968). However, the secretion rates of these steroids were about one-third less in the retinoate-fed rats. No differences in the secretion rates of oestradiol-17 β and oestrone were found. It is suggested that the decreased secretion of progesterone and 20 α -hydroxypregnenone reflected the lowered specific activity of 3 β -hydroxy Δ^5 -steroid dehydrogenase in the ovaries of the retinoate-fed rats and may have been a cause of the pregnancy disorder.

- Eto, T., Masuda, H., Suzuki, Y. & Hosi, T. (1962). *Jap. J. Anim. Reprod.* **8**, 34.
 Fajer, A. B. & Barraclough, C. A. (1967). *Endocrinology*, **81**, 617.
 Hashimoto, I., Hendricks, D. M., Anderson, L. L. & Melampy, R. M. (1968). *Endocrinology*, **82**, 333.
 Juneja, H. S., Moudgal, N. R. & Ganguly, J. (1969). *Biochem. J.* **111**, 97.
 Juneja, H. S., Murthy, S. K. & Ganguly, J. (1964). *Indian J. Exp. Biol.* **2**, 153.
 Martin, L. (1960). *J. Endocr.* **20**, 187.
 Martin, L. (1964). *J. Endocr.* **30**, 21.
 Thompson, J. N., Howell, J. McC. & Pitt, G. A. J. (1964). *Proc. R. Soc. B*, **159**, 510.