ATP (3mM) at 4°. After centrifugation at 76900*g*, the supernatant was concentrated and freeze-dried (complex I). The pellet was dissolved in aq. 33% (v/v) pyridine at 0°, diluted and dialysed against water. After centrifugation at 76900*g*, the supernatant was concentrated and freeze-dried (complex II). Complex I contained 0·2% (w/w) of sialic acid; complex II contained 10% (w/w) of sialic acid and readily gelated.

Zone electrophoresis of the complexes on polyacrylamide at pH 2·2 (Takayama, MacLennan, Tzagoloff & Stoner, 1966) gave a pattern of about 16 zones. Excision and re-running of the zones gave no further resolution and the patterns were unaffected by the freeze-drying of the materials. Both complexes gave similar patterns and there were no differences between batches. Carbohydrate was localized in slow-moving zones and aggregated material in complex II, as was material that bound Ruthenium Red.

Complex II was degraded by alkali above pH 9·0 at 20° and at pH 10·5–11·0 at 0°. Neuraminidase removed certain components of low electrophoretic mobility from it and it was selectively degraded by trypsin in the presence of 10mM-Ca^{2+}. Treatment with 0·1% (w/v) sodium dodecyl sulphate for 12hr. at 4° modified the electrophoretic pattern of complex II. The aggregation of the complex was examined in the analytical ultracentrifuge.

Many of the electrophoretic zones represent distinct species or mixtures of species of proteins, and the glycoproteins form a distinct group among these. Sialic acid may be involved in the aggregation of complex II.

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Blumenfeld, O. O. (1968). *Biochem. biophys. Res. Commun.* **30**, 200.
Harris, J. R. (1968). *Biochim. biophys. Acta*, **150**, 534.
Marchesi, V. J. & Steers, E. (1968). *Science*, **159**, 203.
Rega, A. F., Weed, R. I., Reed, C. F., Berg, G. G. & Rothstein, A. (1967). *Biochim. biophys. Acta*, **147**, 297.
Rosenberg, S. A. & Guidotti, G. (1968). *J. biol. Chem.* **243**, 1985.
Schneiderman, L. J. & Junga, I. G. (1968). *Biochemistry*, **7**, 2281.
Takayama, K., MacLennan, D. H., Tzagoloff, A. & Stoner, C. D. (1966). *Arch. Biochem. Biophys.* **114**, 223.

The Effect of Intraductal Prolactin on Protein and Nucleic Acid Biosynthesis in the Rabbit Mammary Gland

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In a recent report (Falconer & Fiddler, 1968) it was suggested that a stimulation of RNA and protein biosynthesis was required for the increase in activity of the enzyme lipoprotein lipase that occurred during a period of prolactin-induced lactogenesis. The aim of this work was therefore to verify or otherwise this suggestion.

Pseudo-pregnancy was induced in virgin oestrous rabbits by the intravenous injection of 50 i.u. of chorionic gonadotrophin, and lactation was initiated by the intraductal injection of 50μg. of prolactin (N.I.H.-P-S8)/duct. In these experiments individual glands were injected with prolactin at regular intervals. On the third or fourth day after the first injection, [^{14}C]leucine (0·1μc in 0·1ml. of 0·9% NaCl/duct) was intraductally injected into the prolactin-treated glands and also into a control gland. All the glands were removed after 1hr. for determination of the specific radioactivity of total protein (Mayne, Barry & Rivera, 1966).

Parallel experiments that determined the incorporation of [^{3}H]uridine into RNA and [^{14}C]-thymidine into DNA were carried out. The labelled precursors were injected either simultaneously (0·2μc of [^{3}H]uridine and 0·1μc of [^{14}C]thymidine in 0·1ml. of 0·9% NaCl/duct) or separately in different animals. The isolation of DNA and RNA was essentially by the method of Munro & Fleck (1966) with slight modification.

The response to prolactin measured by [^{14}C]-leucine incorporation into protein was characterized by two marked peaks in activity. The specific radioactivity increased dramatically during the first 24hr. after injection of prolactin, then fell to near basal value during the next 24hr. and subsequently increased again to form a second peak of incorporation 72hr. after injection of prolactin, and remained at an elevated value at 96hr.

The patterns of [^{3}H]uridine and [^{14}C]thymidine incorporation into RNA and DNA respectively were similar, and showed two marked peaks preceding in time the peaks in protein biosynthesis. An initial peak of incorporation of bases into both nucleic acids was observed during the first 12hr. after injection of prolactin, this subsequently decreasing to the initial value during the next 12hr. and then increasing again to form a second peak of activity 30–36hr. after the hormonal stimulation. At 48 and 60hr. after injection of prolactin much less incorporation was observed than at 30hr.

Falconer, I. R. & Fiddler, T. J. (1968). *Biochem. J.* **110**, 56P.

Mayne, R., Barry, J. M. & Rivera, E. M. (1966). *Biochem. J.* **99**, 688.

Munro, H. N. & Fleck, A. (1966). In *Methods of Biochemical Analysis*, vol. 14, p. 113. Ed. by Glick, D. New York: Interscience–John Wiley and Sons Inc.

Increased Drug-Metabolizing Activity without Increased Cytochrome *P*-450 in Rats Treated with Phenolic Antioxidants

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The endoplasmic reticulum of the liver contains a cytochrome (*P*-450) that has been implicated in the enzymic oxidation of drugs and other foreign compounds (Cooper, Levine, Narasimhulu, Rosenthal & Estabrook, 1965). Since the activities of several of these oxidative enzymes are increased in rats that have been treated with various phenolic compounds (Gilbert, Martin & Golberg, 1967), the cytochrome *P*-450 content of the livers of similarly treated rats was measured. Most of the compounds that increased the enzyme activities also increased the cytochrome *P*-450 content of the liver, but two compounds caused several-fold increases in the drug-metabolizing enzymes with little or no change in cytochrome *P*-450 content. These two compounds were 2,6-di-*tert.*-butyl-4-methylphenol (BHT; I) and 2,6-di-*tert.*-butyl-4-methoxymethylphenol (II).

The livers of female rats (100g.) were removed 24hr. after an oral dose (1·5m-moles/kg.) of these test compounds. Activities of the microsomal enzyme BHT oxidase (Gilbert & Golberg, 1965) were (expressed as means ± S.E.M., with numbers of values in parentheses): controls, 0·26 ± 0·04 (4), compound I, 0·78 ± 0·07 (3), and compound II, 1·15 ± 0·02 (4) μmoles/hr./g. of liver. The cytochrome *P*-450 contents of the same livers were: controls, 9·2 ± 0·7, compound I, 10·5 ± 0·8, and compound II, 12·4 ± 0·7nmoles/g. of liver.

A similar result was obtained by using the duration of action of hexobarbitone to measure the rate of drug metabolism *in vivo*. At 48hr. after doses of 0·2, 0·5 or 1·5m-moles of compound II/kg. the hexobarbitone sleeping times of female rats (100g.) were significantly decreased (75, 65 and 48% of control respectively; groups of four to six), indicating accelerated hexobarbitone oxidation. The cytochrome *P*-450 contents of the livers of identically treated rats were not significantly changed (107, 121 and 107% of control respectively; groups of six). At 48hr. after a dose of phenobarbitone (100mg./kg.) both the hexobarbitone sleeping time and the cytochrome *P*-450 content were significantly altered (27% and 232% of control respectively; groups of six).

It appears from these experiments that cytochrome *P*-450 may not be rate-limiting for oxidative drug metabolism *in vivo* or *in vitro*.

Cooper, D. Y., Levine, S., Narasimhulu, S., Rosenthal, O. & Estabrook, R. W. (1965). *Science*, **147**, 400.

Gilbert, D. & Golberg, L. (1965). *Fd Cosmet. Toxicol.* **5**, 481.

Gilbert, D., Martin, A. D. & Golberg, L. (1967). *Abstr. 4th Meet. Fed. Europ. biochem. Soc., Oslo*, A-197.

The Haem–Protein Interaction in Cytochrome *P*-450

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In order to understand the many spectroscopic peculiarities associated with cytochrome *P*-450 we have made a series of model studies on protohaem complexes, especially those in which the iron is further bound to a thiol. The ferrihaem complexes with thiols give rise to absorption and electron-proton-resonance spectra that closely resemble those of cytochrome *P*-450 and are compatible with the ferric iron being largely in a low-spin state (Bayer, Hill, Roder & Williams, 1969). However, there is always some high-spin ferric iron present and the amount of this form of the iron is dependent on the substrate added (Williams, 1968). As these substrates can not bind the iron in general it follows that they must modify the iron–protein bonding.

The spectroscopic properties of ferrocytochrome *P*-450 indicate that the iron is high-spin. Such ferrohaem complexes have very little affinity for thiols. We conclude that the curious difference spectra of cytochrome *P*-450, oxidized minus reduced, are due to the unusual spin states of the iron, ferric iron low-spin and ferrous iron high-spin, and to the weakening or breaking of the iron–sulphur bond on reduction. On addition of carbon monoxide, nitric oxide or isonitriles ferrocytochrome *P*-450 has absorption bands at 450 and 550nm. but with no α-band. Model studies show that this type of spectrum is most closely approached when metal atoms in porphyrin complexes become five-co-ordinate and the metal lies out of the plane of the porphyrin by some considerable distance. This state of the metal, should it also arise on reaction with oxygen, would lead to a very open complex that is similar to the five-co-ordinate forms of vitamin B_{12} derivatives. Vallee & Williams (1968) have refered to such unusual co-ordination conditions as entatic states.