Winrow & Rudney (1969) found that a rat kidney mitochondrial fraction contains the major activity of the *p*-hydroxybenzoate polyprenyltransferase. This has been verified in rat liver mitochondria, and, further, it has been established that the activity resides in the inner mitochondrial membrane (K. Momose & H. Rudney, unpublished work). We have now been able to obtain preparations entirely from rat liver that do not require supplementation with extracts from *M. lysodeikticus*. With a supernatant fraction obtained by centrifugation at 48000g, mevalonate and ATP we have been able to show that polyprenyl-*p*-hydroxybenzoate is formed, by using *p*-hydroxy-[7-¹⁴C]benzoic acid as a marker. An important factor in being able to successfully detect the formation of polyprenyl-*p*-hydroxybenzoate is the rapid treatment and solvent extraction of the preparation after incubation, otherwise all traces of these compounds disappear, indicating their extreme lability. This may explain previous difficulties in observing polyprenyl derivatives of *p*-hydroxybenzoate in mammalian systems (Whistance *et al.*, 1971). Dialameh *et al.* (1970) reported that CoA derivatives are involved in *p*-hydroxybenzoate incorporation into ubiquinone precursors, e.g. 2-nonaprenyl-6-methoxyphenol. In the mitochondrial systems adopted by us we have been unable to show a requirement for CoA.

Recent experiments have shown that with well-washed rat liver mitochondria the requirement for the 48000g supernatant fraction, mevalonate and ATP can be replaced by isopentenyl pyrophosphate alone. This indicates that enzymes are present in mitochondria that can synthesize polyisoprenoid chains from isopentenyl pyrophosphate. A search for the enzymes involved in polyisoprenoid synthesis indicated that mitochondria have isopentenyl pyrophosphate isomerase activity, which is almost as active as that present in the 48000g supernatant fraction. The implications of these findings will be discussed.

Previous studies from this laboratory with *Rhodospirillum rubrum* indicated that the *M. lysodeikticus* and isopentenyl pyrophosphate supplementation of sonicated *R. rubrum* preparations could lead to the synthesis of polyprenyl-*p*-hydroxybenzoate and polyprenylphenol (Raman *et al.*, 1969). Further studies by E. Markianos & H. Rudney (unpublished work) on the fractionation of the *M. lysodeikticus* and isopentenyl pyrophosphate incubation mixture indicated that certain fractions when added to the *R. rubrum* extract synthesized polyprenyl-*p*-hydroxybenzoate only, whereas others were able to synthesize both polyprenyl-*p*-hydroxybenzoate and polyprenylphenol. This would indicate that possibly some alteration in the structure of the polyprenyl side chain was responsible for the differential effect.

It has also been found that polyprenyl pyrophosphate derivatives used as side chains were inactive

unless they were bound to a protein. Fractionation of the polyprenyl pyrophosphate-synthesizing system with ethanol gave fractions that still had protein attached to them. When these fractions were treated with trypsin, all *p*-hydroxybenzoate polyprenyltransferase activity was lost. This indicated that polyprenyl pyrophosphates must be bound to protein for maximal activity, and may thus suggest the necessity of some form of carrier protein. The *Micrococcus* extract did not contain the enzyme for transferring polyprenyl pyrophosphate to *p*-hydroxybenzoate or the decarboxylation system for converting polyprenyl-*p*-hydroxybenzoate into polyprenylphenol. Fractionation of *R. rubrum* extracts with ammonium sulphate have enabled us to separate the transferase from the decarboxylating enzyme.

Use of [3*R*,4*R*-³H]- and [3*R*,4*S*-³H]-mevalonate incubated with a yeast extract to give labelled isopentenyl pyrophosphate, followed by supplementation with the *M. lysodeikticus* and *R. rubrum* extracts, provided polyprenyl-*p*-hydroxybenzoate and polyprenylphenol, which retained the tritium labelling from [3*R*,4*R*-³H]mevalonate only, thereby producing evidence that the polyprenyl pyrophosphate side chains are primarily in the *trans* form. This result indicates that *cis-trans* isomerization may not be a factor in the variability of different fractions of *M. lysodeikticus* in providing side chains for making polyprenyl-*p*-hydroxybenzoate. Further studies on the nature and length of the side chains and the factors controlling their synthesis are continuing.

This work was supported by Grant AM-12463 from the National Institutes of Health, U.S. Public Health Service.

Dialameh, G. H., Nowicki, H. G., Yekundi, K. G. & Olson, R. E. (1970) *Biochem. Biophys. Res. Commun.* **40**, 163

Raman, T. S., Rudney, H. & Buzzelli, N. K. (1969) *Arch. Biochem. Biophys.* **130**, 164

Rudney, H. (1970) *Proc. Symp. Natural Substances Formed Biologically from Mevalonic Acid* (Goodwin, T. W., ed.), p. 89, Academic Press, London

Whistance, G. R., Field, F. E. & Threlfall, D. R. (1971) *Eur. J. Biochem.* **18**, 46

Winrow, M. J. & Rudney, H. (1969) *Biochem. Biophys. Res. Commun.* **37**, 833

1,25-Dihydroxycholecalciferol: a Hormonal Form of Vitamin D Active on Intestine

By H. F. DeLUCA (Department of Biochemistry, University of Wisconsin, Madison, Wis. 53706, U.S.A.)

Dietary vitamin D or that formed in the skin by irradiation of 7-dehydrocholesterol is transported to the liver, where it is hydroxylated to 25-hydroxy-

cholecalciferol (DeLuca, 1969). This reaction is catalysed by a mitochondrial enzyme system that requires molecular O_2 and NADH. This reaction is strongly inhibited by its product 25-hydroxycholecalciferol and represents an important control mechanism for the conservation of vitamin D on the one hand and prevention of vitamin D toxicity on the other. The 25-hydroxycholecalciferol makes its appearance in the kidney tissue, where it is hydroxylated in the 1-position to form 1,25-dihydroxycholecalciferol (Holick *et al.*, 1971). The conversion of 25-hydroxycholecalciferol in vitamin D-deficient rats and chicks into 1,25-dihydroxycholecalciferol is blocked by the prior administration of actinomycin D or cycloheximide, suggesting that 25-hydroxycholecalciferol induces the formation of some factor necessary for 1-hydroxylation (Tanaka & DeLuca, 1971). The 1,25-dihydroxycholecalciferol then makes its appearance in the intestine, where it induces an increased intestinal Ca^{2+} transport by a process that is not blocked by actinomycin D (Tanaka *et al.*, 1971). A protein that is considered to be a precursor of the Ca^{2+} -binding protein has been found in the intestine (Drescher & DeLuca, 1971). It is believed that this protein is converted into the Ca^{2+} -binding protein under the influence of 1,25-dihydroxyvitamin D and that the Ca^{2+} -binding protein makes its appearance at the brush border or microvilli of the intestinal absorption cells (Wasserman, 1970). The Ca^{2+} -binding protein then facilitates Ca^{2+} transfer into the intestinal absorption cells. A Ca^{2+} -dependent adenosine triphosphatase has also been found in the brush borders of intestines from chicks and rats after the administration of vitamin D (Martin *et al.*, 1969; Melancon & DeLuca, 1970). The relationship of the Ca^{2+} -binding protein to the Ca^{2+} -dependent adenosine triphosphatase is unclear at the present time. Both systems are believed to function in the vitamin D-mediated stimulation of intestinal Ca^{2+} transport. Ca^{2+} is then transported across the intestinal mucosal cell and is expelled into the serosal medium by a process that requires the presence of Na^+ (Martin & DeLuca, 1969). It is unknown at the present time whether a vitamin D-mediated process participates in the serosal transfer of the Ca^{2+} .

1,25-Dihydroxycholecalciferol induces bone-mineral mobilization more rapidly than does any other derivative of the vitamin. This action, however, is blocked by actinomycin D, and it is uncertain whether it or a further derivative is the metabolically active form in this process.

Vitamin D can therefore be considered a hormone in the sense that it is converted into 25-hydroxy- and 1,25-dihydroxy-cholecalciferol, which represent the hormonal forms of the vitamin. The process whereby 1,25-dihydroxycholecalciferol influences intestinal Ca^{2+} transport is partially understood,

whereas the mechanism of its action in inducing bone-mineral mobilization is essentially unknown.

- DeLuca, H. F. (1969) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **28**, 1678
 Drescher, D. & DeLuca, H. F. (1971) *Biochemistry* **10**, 2308
 Holick, M. F., Schnoes, H. K. & DeLuca, H. F. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 803
 Martin, D. L. & DeLuca, H. F. (1969) *Amer. J. Physiol.* **216**, 1351
 Martin, D. L., Melancon, M. J. & DeLuca, H. F. (1969) *Biochem. Biophys. Res. Commun.* **35**, 819
 Melancon, M. J. & DeLuca, H. F. (1970) *Biochemistry* **9**, 1658
 Tanaka, Y. & DeLuca, H. F. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 605
 Tanaka, Y., DeLuca, H. F., Omdahl, J. & Holick, M. F. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 1286
 Wasserman, R. H. (1970) in *The Fat Soluble Vitamins* (DeLuca, H. F. & Suttie, J. W., eds.), pp. 21-37. University of Wisconsin Press, Madison

Retinol Transport in Rat Plasma and its Nutritional Regulation

By DEW. S. GOODMAN, Y. MUTO, J. E. SMITH and P. MILCH (Department of Medicine, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032, U.S.A.)

Vitamin A circulates in rat plasma as retinol bound to a specific transport protein, retinol-binding protein. Retinol-binding protein was isolated from rat plasma and its properties were studied. Rat retinol-binding protein has a molecular weight of approx. 19000-20000 as estimated by sedimentation equilibrium, and also by gel filtration on standardized columns of Sephadex G-100 and by disc gel electrophoresis in the presence of sodium dodecyl sulphate. Rat retinol-binding protein has one binding site for one molecule of retinol, and has absorption and fluorescence spectra similar to those previously reported for human retinol-binding protein (Kanai *et al.*, 1968). In rat plasma, retinol-binding protein circulates as a protein-protein complex, together with a larger protein with electrophoretic mobility very slightly greater than that of rat serum albumin. Despite the many similarities between rat and human retinol-binding proteins, there is no immunological cross-reactivity between the two proteins, as indicated by the absence of immunoreactivity when rat retinol-binding protein was tested in the radioimmunoassay for human retinol-binding protein (Smith *et al.*, 1970).

A specific anti-(rat retinol-binding protein) serum was prepared in a rabbit by the injection of purified rat retinol-binding protein emulsified with Freund's adjuvant. A radioimmunoassay for rat retinol-binding protein was developed by using a double-antibody precipitation technique. Nutritional