- Cooper, G. F. & Robson, J. G. (1969a). *J. Physiol., Lond.*, **203**, 403.
- Cooper, G. F. & Robson, J. G. (1969b). *J. Physiol., Lond.*, **203**, 411.
- Dalglish, C. E. (1952). *Biochem. J.* **52**, 3.
- Hartley, B. S. (1970). *Biochem. J.* **119**, 805.
- Schmer, G. & Kreil, G. (1969). *Analyt. Biochem.* **29**, 186.
- van Heyningen, R. (1971). *Nature, Lond.* (in the Press).
- Walls, G. L. (1942). *The Vertebrate Eye and its Adaptive Radiations*, pp. 191 & 199. Bloomfield Hills, Michigan: Cranbrook Institute of Science.

Nature and Properties of Retinol-Binding Proteins

By W. KIRBY, G. WHITE and J. GLOVER. (*Department of Biochemistry, University of Liverpool, Liverpool L69 3BX, U.K.*)

Retinol-binding protein was isolated from human serum by using two different procedures. The first, similar to that employed by Kanhai, Raz & Goodman (1968), involves the following stages: (i) fractional precipitation of retinol-binding protein from serum by ammonium sulphate to yield concentrate (preparation C₁), (ii) chromatography of preparation C₁ on (a) DEAE-cellulose to give preparation C₂ and of the latter on (b) a combination of Sephadex G-75 and G-100 gels to yield preparation C₃, followed by (iii) electrophoresis preparation of C₃ on polyacrylamide gel at 5°C, yielding preparation C₄. Retinol (0.5mg) in 0.5ml of ethanol was then added to maintain saturation of the carrier protein, which was finally chromatographed on Sephadex G-75 to remove excess of buffer salts and retinol (preparation C₅).

Preparation C₅ was resolved by disc-gel electrophoresis into three components with α_1 mobility, only two of which fluoresced. The major slower-moving protein was called fraction RBP_s and the other fraction RBP_r. The third component with greatest mobility and present in only small amounts was subsequently found to bind retinol and was called fraction apo-RBP. These correspond to the fractions H₂, H₁ and A respectively reported by Raz, Shiratori & Goodman (1970).

A second, shorter, isolation procedure was developed. This involves chromatography of serum on arginine-Sepharose (6B), prepared as described by (Porath & Fornstedt, 1970), by using a gradient buffer system and concentration of the various fractions by membrane ultrafiltration. The retinol-containing fractions are detected by fluorescence and two major pure retinol-binding protein fractions can be obtained after passage of concentrates through the column three more times with various conditions of pH and salt concentration to be described. The first to be eluted from the final

column contains mainly fraction RBP_s with a trace of fraction RBP_{r1} and the second mainly fraction RBP_{r1} with a trace of a third protein, fraction RBP_{r2}, as revealed by disc-gel electrophoresis and isotachopheresis. Analysis of the pure components shows them to have the same amino acid composition, which is very similar to that reported by Kanhai *et al.* (1968). The molecular weights as determined in the ultracentrifuge and by Sephadex-gel chromatography are also the same at 21900. Both fraction RBP_r and fraction RBP_s contain 1mol of retinol/mol of protein. Evidence from u.v. and fluorescence spectra indicate that fraction RBP_r is derived from fraction RBP_s by transition of the latter to a more stable conformation. Optical-rotatory-dispersion spectra of the latter at pH 8.0 indicate that the molecule is randomly coiled.

The electrophoretic mobilities of fractions RBP_s and RBP_r were α_1 in polyacrylamide gel, in agreement with Kanhai *et al.* (1968). However, on support systems such as paper and cellulose acetate free from molecular-sieving effects and with tris buffer at pH 8.9 the true mobility is α_2 and less than that of caeruloplasmin, as shown by Glover & Walker (1964).

Insufficient evidence has been obtained yet to determine whether fraction RBP_s is the sole component in fresh serum or whether fraction RBP_r is also present or has been produced as an artifact by the isolation procedures. In other experiments with pig serum similar phenomena have been observed.

We acknowledge with thanks the financial support of the Medical Research Council. W. K. and G. W. are in receipt of Science Research Council studentships.

- Glover, J. & Walker, R. J. (1964). *Expl Eye Res.* **3**, 327.
- Kanhai, M., Raz, A. & Goodman, DeW. S. (1968). *J. clin. Invest.* **47**, 2025.
- Porath, J. & Fornstedt, N. (1970). *J. Chromat.* **51**, 479.
- Raz, A., Shiratori, T. & Goodman, DeW. S. (1970). *J. biol. Chem.* **245**, 1903.

Studies on Olfactory Receptor Mechanisms

By G. H. DODD. (*Biophysics Division, Unilever Research Ltd., Colworth/Welwyn Laboratory, The Frythe, Welwyn, Herts., U.K.*)

The olfactory neurones of higher animals, in contrast with the pheromone-receptor cells of insects, exhibit a broad stimulus specificity. The cells respond to a wide range of chemicals, though no two cells appear to have an identical specificity profile (Gestland, Lettvin & Pitts, 1965). Although a large number of speculative mechanisms have been advanced for the olfactory transduction process, few experimental investigations have been

carried out at the molecular level. The isolation of the terminal swelling of olfactory neurones (Koch & Norring, 1969) has now made it feasible to investigate the molecular basis of the receptor mechanisms.

In a typical preparation starting with about 20 large rabbits the yield of purified terminal swellings was about 200 μ g of protein. Neither high-resolution n.m.r. nor electron spin resonance with nitroxide probes was sufficiently sensitive to detect odorant binding at the membrane concentration available.

Fluorescence probe methods were sufficiently sensitive to detect odorant binding to the olfactory terminal swellings. The fluorescent probe 1-anilinonaphthalene-8-sulphonate interacts with the membranes. The binding of this probe to the terminal swellings resembled the binding to other membranes, e.g. erythrocyte membranes (Freedman & Radda, 1969), and differed from most proteins, e.g. glutamate dehydrogenase (Dodd & Radda, 1969), in that an indefinite number of probe sites was found. Aromatic odorants formed a ground-state complex with 1-anilinonaphthalene-8-sulphonate, but most aliphatic and alicyclic odorants did not alter the fluorescence of the probe. Butyric acid was chosen as the most suitable odorant for these experiments. This odorant reproducibly induces, in the 10^{-4} concentration range, characteristic changes in the terminal swellings that are reflected in a 1.2-fold enhancement of the fluorescence of bound 1-anilinonaphthalene-8-sulphonate. Other odorants give similar changes. Similar changes are seen when a sonicated egg-yolk lecithin suspension containing 1-anilinonaphthalene-8-sulphonate is titrated with butyric acid and other odorants.

These observations have been interpreted as evidence of odorant binding to a phospholipid region of the olfactory sensory membranes rather than to any receptor proteins. The results suggest that localized structural changes in membrane lipids give rise to the receptor potential. This interpretation is compatible with our studies on a model system for olfactory transduction (Cherry, Dodd & Chapman, 1970).

Cherry, R. J., Dodd, G. H. & Chapman, D. (1970). *Biochim. biophys. Acta*, **211**, 409.

Dodd, G. H. & Radda, G. K. (1969). *Biochem. J.* **114**, 409.

Freedman, R. B. & Radda, G. K. (1969). *FEBS Lett.* **3**, 150.

Gestland, R. C., Lettvin, J. Y. & Pitts, W. H. (1965). *J. Physiol., Lond.*, **181**, 525.

Koch, R. B. & Norring, N. L. (1969). *J. Neurochem.* **16**, 145.

Trans-synaptic Induction of Dopamine (3,4-Dihydroxyphenethylamine) β -Hydroxylase in Adrenergic Tissues of the Rat

By P. B. MOLINOFF,* S. BRIMIJOIN and J. AXELROD (introduced by L. L. IVERSEN). (*Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20014, U.S.A.*)

A sensitive enzymatic assay for the enzyme dopamine (3,4-dihydroxyphenethylamine) β -hydroxylase was used to study changes in enzyme activity that occur with pharmacologically induced alterations in the activity in the sympathetic nervous system. The assay is based on the enzymic conversion of the β -hydroxylated product of the reaction into its *N*-methyl derivative in the presence of *S*-adenosyl[Me- 14 C]methionine (Molinoff, Weinshilboum & Axelrod, 1971). Endogenous inhibitors of dopamine β -hydroxylase were inactivated with copper sulphate.

The activities of dopamine β -hydroxylase (Molinoff, Brimijoin, Weinshilboum & Axelrod, 1970) and of tyrosine hydroxylase (Mueller, Thoenen & Axelrod, 1969) were increased in adrenergic tissues of the rat by agents, including reserpine, that are believed to cause a reflex increase in sympathetic tone. After reserpine treatment dopamine β -hydroxylase activity increased within 24h in sympathetic ganglia. In the heart and, as previously reported, in the adrenal gland (Viveros, Arqueros, Connett & Kirshner, 1969) the increase in activity was preceded by a fall that occurred within a few hours of the injection. The activities of several enzymes not involved in catecholamine biosynthesis were not affected by reserpine.

When efferent-nerve impulse activity was blocked by either surgical decentralization or by ganglion-blocking agents, the effect of reserpine was abolished. Monoamine oxidase inhibitors, bretylium, which blocks the neurally induced release of noradrenaline, and catecholamines themselves all interfere with the ability of reserpine to release catecholamines and all blocked the induction of dopamine β -hydroxylase in ganglia. The administration of the catecholamines dopa (3,4-dihydroxyphenylalanine) and dopamine also caused a marked decrease in cardiac dopamine β -hydroxylase activity.

The observed increase in dopamine β -hydroxylase activity appears to represent the synthesis of new enzyme and not the activation of pre-existing enzyme. Thus the K_m for phenethylamine, 6×10^{-4} M, and the electrophoretic mobility of dop-

* Present address: Department of Biophysics, University College London, Gower Street, London WC1E 6BT, U.K.