(Herzog, Johnston & Lauler, 1967) and an arterio-lar dilator in the cat and dog (Horton & Jones, 1969) at blood concentrations of 0.1–10 ng/ml. During passage through the pulmonary circulation of the cat or dog substantial loss of the biological activity of the prostaglandins E but not the prostaglandins A occurs, and consequently the possibility that the prostaglandins A may act as circulating hormones has been suggested (Horton & Jones, 1969; McGiff *et al.* 1969).

The metabolism of prostaglandin A₁ by cat blood plasma has been investigated. Prostaglandin A₁ was incubated with cat plasma at 37°C for 1 h and the plasma extracted for polar fatty acids. Examination by u.v. spectrophotometry of fractions from silicic acid chromatography revealed a compound with the same elution volume as prostaglandin A₁ and with an extinction maximum at 278 nm. This compound was separated from any residual prostaglandin A₁ by g.l.c. and identified by mass-spectrometric analysis as prostaglandin B₁ [15-hydroxy-9-oxoprost-8(12),13(*trans*)-dienoic acid], a structural isomer of prostaglandin A₁.

Isomerization of prostaglandin A₁ to B₁ is known to occur rapidly under mild alkaline conditions (Bergström, Ryhage, Samuelsson & Sjövall, 1963). However, prostaglandin B₁ was not formed when prostaglandin A₁ was incubated with Krebs-Henseleit solution at pH 7.4. Further, the isomerization system present in the plasma was destroyed by heating at 70°C but not at 55°C for 20 min. These experiments suggested that the conversion was enzymic.

To obtain a purified preparation, cat plasma was subjected to ammonium sulphate precipitation and the isomerase activity of the dialysis residues was estimated by measuring the rate of increase of extinction at 283 nm after the addition of prostaglandin A₁. Some 80% of the isomerase activity and 30% of the protein content of the plasma was found in the 50–75% saturation fraction. Ion-exchange chromatography of this fraction on DEAE-Sephadex A-50 resulted in a further fivefold purification. The isomerase has an optimum pH in the region of 8.5.

The isomerization of prostaglandin A₁ to B₁ results in the considerable loss of biological activity. For example, prostaglandin B₁ has only 2% of the potency of prostaglandin A₁ on the peripheral blood vessels of the cat.

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The Role of Haemocytes in the Biosynthesis of Protocatechuic Acid in the Cockroach Colleterial System

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L-Tyrosine is the precursor of protocatechuic acid found in the oothecae of *Periplaneta americana* (Brunet, 1963). Haemocytes from this cockroach have been shown to contain tyrosine decarboxylase (Whitehead, 1969). Unexpectedly, tyramine can also be converted into protocatechuic acid *in vivo* by colleterial glands. The monoamine oxidase responsible for this deamination is located in haemocytes (see Shambough, 1969) and not in the gland cells. The products of monoamine oxidase activity found in the haemolymph of adult female cockroaches 1 h after a 'pulse' of [1-¹⁴C]tyramine or [G-³H]tyramine have been separated by high-voltage electrophoresis and chromatography.

Protocatechuic acid is found in the gland but not in the haemolymph. Of the substances in the blood that are derived from tyramine, an electrically neutral (pH 6.5) fraction gives rise to protocatechuic acid when injected into female *P. americana*.

Inhibitors of tyrosine decarboxylase and monoamine oxidase have been used with [U-¹⁴C]tyrosine 'pulses' *in vivo* to show the dependence of the tyrosine- to -protocatechuic acid pathway on these haemocyte enzymes.

The results of an experiment with allotectomized cockroaches 'pulsed' with [U-¹⁴C]tyrosine suggest that the pathway in the colleterial system is controlled by juvenile hormone (see Willis & Brunet, 1966) and inhibited by α -ecdysone. Isolated haemocytes treated with juvenile hormone and [U-¹⁴C]-tyrosine shown an enhanced ability to deaminate tyrosine.

Control of protocatechuic acid biosynthesis is therefore most likely exerted in the haemocytes associated with the colleterial glands. In the absence of the haemocytes, protocatechuic acid cannot be synthesized from tyrosine by the isolated gland.

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A Possible New Sclerotization Agent in the Cockroach Cuticle

By J. K. KOEPPE and R. R. MILLS (introduced by D. L. Whitehead). (*Department of Zoology, University of Oxford, Oxford OX1 3PS, U.K.*)

A variety of sclerotization agents exist in insects (Brunet, 1967). Among these are *N*-acetyldopamine (Karlson & Sekeris, 1962) which has been shown to play an important role in the 'tanning' of the cockroach cuticle (Mills, Lake & Alworth, 1967). Further investigation has shown that [*carbinol*-¹⁴C]-noradrenaline [2-amino-1-(3,4-dihydroxyphenyl)-ethanol] is also incorporated into the cuticle of the American cockroach, *Periplaneta americana* L.

By using conditions both *in vivo* and *in vitro*, it has been found that a number of acidic, basic and neutral compounds arise from noradrenaline. These substances have been separated by high-voltage electrophoresis and paper chromatography. Co-chromatography and comparison with known metabolites has led to their identification, which suggests *N*-acetylation may be an important step.

I propose that β -hydroxylation of dopamine followed by *N*-acetylation may be involved in the synthesis of a previously unreported sclerotizing substance.

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Synthesis of Unstable Haemoglobins

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In subjects heterozygous for Hb-A* and a β -chain-variant unstable haemoglobin, the proportion of the unstable haemoglobin usually comprises 25–35% of the total haemoglobin. In three such cases the synthesis rates of the unstable β -chain and the β^A -chain have been shown to be equal (White & Dacie, 1970; Steadman, Yates & Huehns, 1970). The observation that the unstable haemoglobin

* Abbreviation: Hb, haemoglobin.

comprises less than 50% of the total haemoglobin in these cases suggests that a proportion of this haemoglobin is being removed as insoluble inclusion (Heinz bodies).

There are four unstable haemoglobins, however, present in smaller amounts, 15% or less of the total haemoglobin: Hb-Borås, F4 Leu $\rightarrow$ Arg (Hollender, Lorkin, Lehmann & Svensson, 1969); Hb-Santa Ana, F4 Leu $\rightarrow$ Pro (Opfell, Lorkin & Lehmann, 1968); Hb-Sabine, F7 Leu $\rightarrow$ Pro (Schneider, Veda, Alperin, Brimhall & Jones, 1969); Hb-Köln, FG5 Val $\rightarrow$ Met (Carrell, Lehmann & Hutchinson, 1966). In two patients heterozygous for Hb-Köln the relative rates of synthesis of the $\beta^{Köln}$ -chain and β^A -chain have been measured by incubation of their reticulocytes with [³H]leucine and measurement of the total incorporation of [³H]leucine into the two chains. The ratio of β^A - to $\beta^{Köln}$ -chain synthesized was found to be approx. 4:1 (White & Brain, 1970). The low proportion of Hb-Köln found in heterozygotes is therefore due to defective synthesis of the $\beta^{Köln}$ -chain as well as to its denaturation. This explanation may also be valid for the other three haemoglobins.

In all of these four haemoglobins the region of the amino acid substitutions are close to each other and near the haem-like histidine residue, F8. It is postulated that if an amino acid substitution in this region of the β -chain disrupts the normal structure then it may impede assembly of that chain.

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Thermal Denaturation of Human Haemoglobins

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The thermal denaturation of dilute solutions of various human haemoglobins has been studied by following fluorescence changes at 45°C, and from 'melting' curves by using the percentage change in optical rotation at 233nm with temperature.