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of Dienone with a chance contaminant was checked for by running 'blank' tests from the Girard step onwards, in which no peak of retention time at all similar to that of Dienone was detected on the gas chromatograms run at very high sensitivity. Verification of this tentative identification of Dienone in plasma by gas chromatography of derivatives (the methyl-oximes, the enol heptafluorobutyrate and the product of reduction with borohydride), and by mass spectrometry, is in progress.

Making allowances for losses determined from recovery experiments the concentration of Dienone in male peripheral plasma is about 0·04–0·06 μg./100ml.

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Glycoproteins in the External Mucous Secretions of the Plaice, *Pleuronectes platessa*, and other Fishes

By T. C. Fletcher and P. T. Grant. (*N.E.R.C. Fisheries Biochemical Research Unit, University of Aberdeen*)

Mucous secretions of fishes have been implicated in a variety of biological functions and chemical analyses have shown that mucins from several genera contain both protein and carbohydrate moieties (Wessler & Werner, 1957; Enomoto, Nagatake & Tomiyasu, 1963; Lehtonen, Kärkkäinen & Haahti, 1966).

Surface mucins from live plaice, cod, dab and sole were dialysed at 4° against water saturated with toluene. The non-diffusible material was soluble in 0·6M-perchloric acid and contained protein (about 60% w/w), ash (about 10% w/w), varying amounts of hexosamines, fucose, galactose, mannose, glucose, sialic acid, ester sulphate, but only trace amounts of uronic acid and phosphate.

The mucin from plaice has been further characterized. One major and at least two other precipitin lines were obtained by immunodiffusion using rabbit antisera to whole mucin. Gel-filtration with Sephadex G-200 or Sepharose 4B resulted in the major fraction (>60% w/w) being recovered in a single peak and two minor and more retarded fractions. The major fraction was further purified by chromatography on DEAE-Sephadex (A-25) and analyses of the dried material by the method of Walborg, Christensson & Gardell (1965), gave fucose 5·8% (w/w), galactose 4·7% (w/w), glucose 1·9% (w/w) and mannose 1·4% (w/w). Galactosamine 8·4% (w/w), glucosamine 2·9% (w/w) and *N*-acetylneuraminic acid <1% (w/w) were also present. The principal amino acids in the protein were threonine (9·8% w/w), serine (5.4% w/w), glutamic acid (5·2% w/w), aspartic acid (4·3% w/w), with only trace amounts of sulphur-containing amino acids.

The two minor fractions had a similar composition to that of the major component, except that they contained all the ester sulphate of the whole mucin and differed in their amino acid composition, notably in that the ratio of hydroxy to acidic amino acids was 0·7.

The results of these studies suggest that the external mucins of plaice contain glycoproteins of similar composition to those of some mammalian epithelial secretions. A further similarity is that the whole mucin contained traces of lysozyme activity. The biosynthetic origin of these fish glycoproteins is of interest since plaice serum appears to contain the major glycoprotein component of the mucin, as judged by immunochemical and other experiments.

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The Metabolism of Psilocin and Psilocybin by Fungal Enzymes

By S. M. Bocks. (*Dyson Perrins Laboratory, Oxford*)

Psilocybin and the corresponding phenol psilocin were isolated by Hofman, Heim, Brack & Kobel (1958) as the active principles from the psychotropic mushrooms of the genus *Psilocybe* and in *Stropharia cubensis*. The oxidation of psilocin was found to be catalysed by purified *p*-diphenol oxidase (laccase) obtained from *Polyporus versicolor* (Bocks, 1967). The product of the enzymically catalysed reaction was blue in colour and its ultraviolet spectrum showed absorption maxima at 610mμ and 400mμ and was similar in colour and in its ultraviolet sprectrum to the blue product obtained by the chemical oxidation of psilocin with ferric chloride. The apparent K_m for psilocin at pH 3·6 was found to be 8×10^{-7}M when the reaction was catalysed by laccase from *P. versicolor*. Under identical conditions, psilocybin, the phosphorylated ester of psilocin was not oxidized and hence it appears that the presence of the free hydroxyl group on the aromatic ring is essential.

The blue product was found to be readily reduced

to a colourless compound by ascorbic acid and appears to be quinonoid in nature. Oxygen uptake was found to occur during the oxidation of psilocin by laccase and a study of the effect of pH showed a broad pH optimum below pH 4·0.

The presence of a laccase type enzyme was detected in homogenates of the mycelium of *Psilocybe cubensis* and as an extracellular enzyme in the culture medium, which was found to catalyse the oxidation of 2,6-dimethoxyphenol, *p*-cresol, pyrogallol and psilocin by methods previously described (Benfield, Bocks, Bromley & Brown, 1964; Bocks, 1967).

The crude homogenates of the mycelia of *Polyporus versicolor* and *Psilocybe cubensis* were able to oxidize psilocybin at a very slow rate to give the blue compound and it was concluded that the homogenates contained an enzyme which catalysed the hydrolysis of the phosphate group of psilocybin and permitted the oxidation of the psilocin so formed to the blue product.

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Interaction of the Carcinogen *N*-Methyl-*N*-nitrosourethane with Various Rat Tissues and their Constituents after a Single Carcinogenic Dose, Administered Intragastrically or Intraperitoneally

By R. Schoental. (*Toxicology Research Unit, Medical Research Council Laboratories, Carshalton, Surrey*)

Using *N*[^{14}C]-methyl-*N*-nitrosourethane the distribution was studied of the radiocarbon in various rat tissues and their constituents at various times after a single intragastric or intraperitoneal dose of this compound. The dose used is known to induce cancer in certain organs after an interval of one year or longer, without any further treatment (Schoental, 1960, 1963; Schoental & Magee, 1962, Schoental & Benstead, unpublished results).

The results will be demonstrated and the development of tumours in certain organs in relation to the route of administration will be discussed.

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The Effects of Actinomycin D and Cycloheximide on Nucleic Acid and Protein Synthesis in the Rat Kidney after Folic Acid Injection

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Parenteral administration of folic acid to rats induces an increase in DNA synthetic activity in the kidney commencing at about 18hr. and rising to approximately twenty times the normal level by 26hr. after a single injection of folate. Prior to the onset of increased DNA synthesis, nuclear RNA synthetic activity rises to about twice the normal level by 12hr. after folate (Threlfall, Taylor, Mandel & Ramuz, 1967). Similar changes have been observed in regenerating rat liver (Fujioka, Koga & Lieberman, 1963; Bucher & Swaffield, 1965; Doly, Ramuz, Mandel & Chambon, 1965).

We have used actinomycin D (AMD) to establish whether increased RNA synthesis is obligatory for the subsequent rise in DNA synthesis, and cycloheximide (CHX) to examine the relationship between nuclear protein and DNA synthesis in the kidney. Male August rats were injected with folic acid as described previously (Threlfall, Taylor & Buck, 1966).

Actinomycin D (0·25μg. per g. body wt.) was strongly inhibitory to nuclear RNA synthesis, measured by *in vivo* uptake of [^{3}H]-uridine. At times up to 18 hr. after folate administration AMD depressed DNA synthesis, measured by *in vivo* incorporation of [^{3}H]-thymidine, by more than 70% of the value found for rats given folic acid only. At times between 20 and 23hr. AMD was ineffective, suggesting a requirement for new RNA synthesis up to the time at which increased DNA synthesis begins, but not subsequently. Similar observations on the salivary glands following isoproterenol stimulation have been reported (Baserga & Heffler, 1967).

In contrast, CHX (1μg. per g. body wt.) was strongly inhibitory to DNA synthesis at 26hr. when given at any time between 0 and 25hr. after folate. The inhibition was almost complete during the period prior to increased DNA synthesis and about 80% at 23 and 24hr. when acceleration of DNA synthesis had already become established. CHX given 23hr. after folate completely suppressed the increased incorporation of [^{14}C]-lysine into basic nuclear protein associated with DNA synthesis at 26hr. There appears to be a continuing requirement for protein synthesis during both the pre-DNA synthetic phase and the period of increased DNA synthesis itself, though the possibility of direct interference of CHX with DNA replication during the latter period cannot be entirely excluded. These