of radioautographic techniques (Logan, Ficq & Errera, 1959). This permits the study of incorporation experiments in individual nuclei but does not give any information about the quantitative chemical changes in the nuclei.

It is of interest to know whether there are any common biochemical changes in the nuclei as a result of feeding the two carcinogens. However, the two types of nuclear preparations behaved differently in the incorporation of labelled precursors into nucleic acids and proteins, even though both groups of nuclei, however, showed increases in their protein, nucleic acid and phospholipid content. Thus, although a reasonable correlation is obtained between the biochemical and histological changes in the two groups, there is so far little evidence of any common pattern in the nuclear changes. It is therefore necessary to study more hepatocarcinogens and to compare them with compounds which produce non-malignant hyperplasia of liver and bile-duct cells.

SUMMARY

1. Nuclei were isolated in 0·25M-sucrose from the livers of normal rats and of rats fed with either thioacetamide or *p*-dimethylaminoazobenzene.

2. Nuclear counts, nuclear nitrogen, deoxyribonucleic acid, ribonucleic acid and phospholipids were determined on the nuclei of treated rats at intervals during the 22-week feeding period. In all cases an increase over controls was obtained during the early weeks of feeding.

3. The incorporation *in vitro* of [2-^{14}C]glycine into protein, and [6-^{14}C]orotic acid and [8-^{14}C]-adenine into RNA of the isolated nuclei was studied in control and treated animals over the feeding period. Orotic acid and adenine incorporation fell in thioacetamide-treated animals and rose in *p*-dimethylaminoazobenzene treatment.

4. The results are correlated with the histology of the livers of the treated rats and discussed in relation to carcinogenesis.

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REFERENCES

Bruce, H. M. & Parkes, A. S. (1946). *J. Hyg., Camb.*, **44**, 491.
Firminger, H. I. (1955). *J. nat. Cancer Inst.* **15**, 1427.
Fitzhugh, O. G. & Nelson, A. A. (1948). *Science*, **108**, 626.
Grant, H. C. & Rees, K. R. (1957). *Proc. Roy. Soc.* B, **148**, 117.
Gupta, D. N. (1956). *J. Path. Bact.* **72**, 415.
Logan, R., Ficq, A. & Errera, M. (1959). *Biochim. biophys. Acta*, **31**, 402.
Orr, J. W. (1940). *J. Path. Bact.* **50**, 393.
Rees, K. R. & Rowland, G. F. (1961). *Biochem. J.* **78**, 89.

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Mammalian Fucosidases

1. THE SYNTHESIS OF SUBSTRATES AND INHIBITORS

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A comparative study has already been made of the occurrence of β-D-glucuronidase, β-*N*-acetyl-D-glucosaminidase, α-D-mannosidase and β-D-galactosidase in mammalian tissues (Conchie, Findlay & Levvy, 1959*a*, *b*), and it was suggested that these enzymes could have a common function in the catabolism of mucosubstances. Since L-fucose (6-deoxy-L-galactose) is frequently found in compounds of this class, it was decided to examine the same tissues for L-fucosidase activity, and also to test for D-fucosidase activity. D-Fucose has not been reported in animals, but it does occur in plants, and the enantiomorphs of fucose are related in the same sense as D-glucose is to L-gulose, being theoretically capable of interconversion by end-to-end oxidation–reduction. It was also possible that β-D-galactosidase would hydrolyse β-D-fucosides. The enzyme studies form the subject of subsequent communications. For convenience, the syntheses of the substrates are

collected together here, along with the preparation of the possible enzyme inhibitors, D- and L-fucono-lactone (cf. Conchie & Levvy, 1957).

EXPERIMENTAL

Determinations of carbon, hydrogen and nitrogen are by Weiler and Strauss, Oxford. Melting points are corrected. D-Fucose (L. Light and Co. Ltd.; Aldrich Chemical Company Inc.) and L-fucose (L. Light and Co. Ltd.) are treated together below.

Tetra-O-acetyl-α-fucose. Attempts to prepare β-L-fucose tetra-acetate by the method of Westphal & Feier (1956) were unsuccessful. The α-anomer was prepared by the same general method as that of Wolfrom & Orsino (1934) and Iselin & Reichstein (1944), and an identical procedure was employed for D-fucose.

Fucose (5 g.) was added over 15 min. to a mixture of acetic anhydride (42 ml.) and pyridine (54 ml.), cooled in ice–salt, and the liquid was mechanically stirred for 4 hr. with continued cooling. After 2 days at 0°, the liquid was poured on crushed ice and stirred for 4 hr., more ice being added when necessary. The mixture was extracted with chloroform (250 ml. total), and the chloroform layer was washed six times with an equal volume of water, and dried over $CaCl_2$. Removal of the chloroform gave the mixed α- and β-tetra-acetates as a colourless syrup (10 g.), which was satisfactory for condensation with *p*-nitrophenol. Crystallization from ethanol gave the α-tetra-acetate as colourless prisms.

Tetra-*O*-acetyl-α-L-fucose was obtained in a yield of 7·2 g. (71%); m.p. 92–93°; $[\alpha]_D^{20}$ −113° in chloroform (*c*, 1·5), and −138° in acetone (*c*, 1·5). Wolfrom & Orsino (1934) give m.p. 92–93°; $[\alpha]_D$ −120° in chloroform; Iselin & Reichstein (1944) give m.p. 93°; $[\alpha]_D$ −130° in acetone. The enantiomorph, *tetra-O-acetyl-α-D-fucose*, was obtained in a yield of 4·0 g. (40%); m.p. 92–93°; $[\alpha]_D$ +129° in chloroform (*c*, 1·5) and +143° in acetone (*c*, 1·5) (Found: C, 50·5; H, 6·2. $C_{14}H_{20}O_9$ requires C, 50·6; H, 6·0%.)

p-Nitrophenyl tri-O-acetyl-α-fucoside. The procedure employed by Westphal & Feier (1956) for condensing *p*-nitrophenol with β-L-fucose tetra-acetate in the presence of $ZnCl_2$ catalyst was only slightly modified. Attempts to make the anomeric β-fucoside triacetate by this reaction, with other catalysts, were unsuccessful. *p*-Toluenesulphonic acid, H_2SO_4 and $AlCl_3$ merely gave diminished yields of the α-anomer, whereas $Hg(CN)_2$ gave back the starting material unchanged. Dissolving the $ZnCl_2$ in an acetic acid–acetic anhydride mixture made the procedure unreliable.

Tetra-*O*-acetyl-fucose (5 g. of crystalline α-form or, preferably, αβ syrup), *p*-nitrophenol (9·5 g.) and powdered, anhydrous $ZnCl_2$ (3·2 g.) were fused together under diminished pressure at 120°, and heating was continued for 2 hr. with occasional shaking. After cooling, the melt was poured into chloroform (250 ml.) and washed with water, 0·1 N-NaOH and water, all ice-cold. The chloroform layer was dried over $CaCl_2$ and the solvent was removed under reduced pressure. Several evaporations from methanol gave a crystalline product which was recrystallized from the same solvent to give clusters of colourless needles (2·1 g., 34%).

p-Nitrophenyl tri-*O*-acetyl-α-L-fucoside had m.p. 168–170°; $[\alpha]_D^{20}$ −228° in chloroform (*c*, 0·5). Westphal & Feier (1956) give m.p. 169–170° and $[\alpha]_D$ −222° in chloroform. *p-Nitrophenyl tri-O-acetyl-α-D-fucoside* had m.p. 169–170°; $[\alpha]_D^{20}$ +231° in chloroform (*c*, 0·5) (Found: C, 52·3; H, 5·4; N, 3·4. $C_{18}H_{21}O_{10}N$ requires C, 52·6; H, 5·1; N, 3·4%).

p-Nitrophenyl α-fucoside. The triacetate (5 g.) in methanol (90 ml.) was refluxed with 0·1 N-sodium methoxide in methanol (9 ml.) for 45 min. When the solution was evaporated to one-quarter volume, the fucoside separated as colourless needles which were recrystallized from methanol (1·5 g., 43%). After acid hydrolysis, the fucoside gave a yellow colour with alkali.

p-Nitrophenyl α-L-fucoside had m.p. 194–196°; $[\alpha]_D^{20}$ −317° in acetone (*c*, 0·4). Westphal & Feier (1956) give m.p. 196–197°. *p-Nitrophenyl α-D-fucoside* had m.p. 193–194°; $[\alpha]_D^{20}$ +329° in acetone (*c*, 0·4) (Found: C, 50·5; H, 5·4; N, 5·5. $C_{12}H_{15}O_7N$ requires C, 50·5; H, 5·3; N, 4·9%).

Tri-O-acetylfucosyl bromide. This was made by the one-stage process of Bárczai-Martos & Kőrösy (1950), keeping to their molecular proportions. The product was a colourless gum (1·4 g./g. of D- or L-fucose) which could not be made to crystallize.

p-Nitrophenyl tri-O-acetyl-β-fucoside. To *p*-nitrophenol (6·9 g.) in N-NaOH(69 ml.) was added a solution of aceto-bromofucose (14·6 g. of gum) in acetone (104 ml.). After 5 hr. at room temperature, the acetone was sucked off, and the residue was poured into water (750 ml.) and extracted with chloroform (about 600 ml. total). The chloroform layer was washed with water, 0·1 N-NaOH and water, all ice-cold, and dried over $CaCl_2$. Removal of the chloroform gave an almost colourless glass (6·8 g.) from which crystals could be obtained only of the L-enantiomorph. *p-Nitrophenyl tri-O-acetyl-β-L-fucoside* crystallized from ethanol in small needles (2·25 g.); m.p. 164–166°; $[\alpha]_D^{20}$ +1·5° in chloroform or acetone (*c*, 1·5) (Found: C, 53·2; H, 5·3; N, 3·5. $C_{18}H_{21}O_{10}N$ requires C, 52·6; H, 5·1; N, 3·4%).

p-Nitrophenyl β-L-fucoside. The crystalline triacetate (0·57 g.) was deacetylated as described for the α-anomer, and the methanol solution was dried. Crystallization of the residue from ethanol yielded the *glycoside* (0·28 g., 71%) as clusters of fine needles; m.p. 187–189°; $[\alpha]_D^{20}$ +100° in ethanol (*c*, 1), and +78° in acetone (*c*, 0·8) (Found; C, 50·3; H, 5·6; N, 5·0. $C_{12}H_{15}O_7N$ requires C, 50·5; H, 5·3; N, 4·9%). After acid hydrolysis, the fucoside gave a yellow colour with alkali.

p-Nitrophenyl β-D-fucoside. The triacetate gum (6·8 g.) was deacetylated as above. Crystallization from ethanol gave the *glycoside* (1·35 g.) as clusters of fine needles; m.p. 186–188°; $[\alpha]_D^{20}$ −85° in ethanol (*c*, 1), and −96° in acetone (*c*, 0·8) (Found: C, 50·3; H, 5·6; N, 4·8. $C_{12}H_{15}O_7N$ requires C, 50·5; H, 5·3; N, 4·9%). After acid hydrolysis, the fucoside gave a yellow colour with alkali.

Fuconolactone. [According to Bates *et al.* (1942), L-fuconolactone is the 1→4-lactone.] Fucose (2 g.) in water (3 ml.) was shaken with Br_2 (1·4 ml.) in a stoppered flask until solution was complete, and left for 3–4 days at 37°. After removal of residual Br_2 in a stream of air, the solution was shaken with Ag_2CO_3 and filtered. The filtrate was treated with H_2S and Ag_2S was removed. The solution was kept at 100° for 1 hr. and treated with charcoal. Evaporation of the filtrate to dryness, followed by several evaporations with acetone to remove the last traces of water, gave a gum which crystallized after about 45 min. *in vacuo*. This was recrystallized from acetone with charcoal treatment. Further crops were obtained from the mother liquor on

repeating the drying procedure. The yield of the pure lactone was 0·9 g. (46 %). It titrated entirely as a lactone, with ring opening at pH 7·5–8.

L-Fuconolactone had m.p. 104–106°; $[\alpha]_D^{21}$ + 74° in water (*c*, 2) (Equivalent weight by titration with NaOH, 171. Calc. for $C_6H_{10}O_5$, 162). Müther & Tollens (1904) give m.p. 106–107°; $[\alpha]_D$ + 78° in water. D-Fuconolactone had m.p. 104–106°; $[\alpha]_D^{21}$ – 70° in water (*c*, 2) (Equivalent weight by titration with NaOH, 163. Calc. for $C_6H_{10}O_5$, 162). Votoček (1902) gives m.p. 106°; $[\alpha]_D$ – 76° in water.

SUMMARY

1. The α- and β-D- and L-fucosides of *p*-nitrophenol have been prepared as substrates for enzyme studies, by using the Helferich reaction for the α- and the Koenigs–Knorr reaction for the β-compounds. The corresponding aldonolactones have also been made.

REFERENCES

Bárczai-Martos, M. & Kőrösy, F. (1950). *Nature, Lond.*, **165**, 369.
Bates, F. J. & associates (1942). *Polarimetry, Saccharimetry and the Sugars*. Washington: United States Government Printing Office.
Conchie, J., Findlay, J. & Levvy, G. A. (1959*a*). *Biochem. J.* **71**, 318.
Conchie, J., Findlay, J. & Levvy, G. A. (1959*b*). *Nature, Lond.*, **183**, 615.
Conchie, J. & Levvy, G. A. (1957). *Biochem. J.* **65**, 389.
Iselin, B. & Reichstein, T. (1944). *Helv. chim. acta*, **27**, 200.
Müther, A. & Tollens, B. (1904). *Ber. dtsch. chem. Ges.* **37**, 306.
Votoček, E. (1902). *Z. Zuckerind. čsl. Repub.* **27**, 16. Quoted by Müther & Tollens (1904).
Westphal, O. & Feier, H. (1956). *Chem. Ber.* **89**, 582.
Wolfrom, M. L. & Orsino, J. A. (1934). *J. Amer. chem. Soc.* **56**, 985.

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Mammalian Fucosidases

2. α-L-FUCOSIDASE

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The mammalian glycosidases, β-D-glucuronidase, β-*N*-acetyl-D-glucosaminidase, α-D-mannosidase and β-D-galactosidase, form a group of enzymes with the following features in common. They are present in all mammalian tissues that have been examined (Conchie, Findlay & Levvy, 1959*a*, *b*), and on structural grounds there is reason to believe that they will attack mucosubstances or their degradation products. The activity in sex organs, and to a lesser extent in other organs, is under endocrine control (Conchie & Findlay, 1959), and cancer tissue is usually higher than most normal tissues in its activity (Conchie & Levvy, 1957*a*; Conchie *et al.* 1959*a*). In the liver cell, all four enzymes are largely confined to the cytoplasmic granules (Conchie & Levvy, 1960; Sellinger, Beaufay, Jacques, Doyen & de Duve, 1960), but for β-glucuronidase at least a large part of the enzyme activity is free in the cytoplasm of cancer cells (Conchie & Levvy, 1959). Each enzyme mentioned is subject to powerful and selective competitive inhibition by the aldonolactone that corresponds to the substrate in structure and configuration (Conchie & Levvy, 1957*b*; Findlay, Levvy & Marsh, 1958). β-Glucuronidase hydrolyses the β-glucuronides that are formed *in vivo* from alcohols, phenols and certain carboxylic acids (for review, see Levvy & Marsh, 1959). It thus may have an additional function that the other three enzymes lack, since there are no known natural substrates for them that correspond to the β-glucuronides, in the animal kingdom at least.

L-Fucose is a frequent constituent of mucosubstances (Bettelheim-Jevons, 1958), and is of particular interest in connexion with the serological specificity of the blood-group substances (Kabat, 1956; Watkins, 1959), in which it is believed on indirect evidence to have the α-configuration. The α-L-fucose residue is also found in the human-milk oligosaccharides (Kuhn, Baer & Gauhe, 1955, 1958). It therefore seemed feasible that the glycosidases discussed above are accompanied by an α-L-fucosidase, and this possibility has now been examined, by using materials described in the preceding paper (Levvy & McAllan, 1961).

EXPERIMENTAL

Enzyme preparation. The fresh tissue was suspended in water with the aid of an all-glass homogenizer (Jencons Ltd.) and made 0·1 M with respect to acetic acid–NaOH