CCXXXVIII. LIVER GLYCOGEN.

III. THE MOLECULAR UNITS OF FISH AND RABBIT GLYCOGENS.

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IN continuation of investigations in progress in this laboratory, glycogens from rabbit and fish livers, purified by the method of Bell and Young [1934], have been subjected to methylation according to Haworth and Percival [1932]. Although preliminary acetylation is thus involved, Bell and Kosterlitz [1935] have shown that it is unaccompanied by degradation, and it is therefore concluded that true methylated glycogens are obtained in this way. Hydrolysis of these derivatives, followed by quantitative separation and investigation of the cleavage products, revealed complete chemical identity of the two glycogens derived from widely different sources, although Bell and Young previously found a distinction in respect of the coloration developed with iodine.

Both glycogens, when fully methylated, showed OMe 45·4–45·5%, values which were not raised by repeated treatment with dimethyl sulphate, although, as will be seen, the substances were not completely etherified. It was found impossible to apply the liquid ammonia method of methylation owing to insolubility of the materials in that medium. The properties of the two products were identical (see below) and both gave, in aqueous solution, a deep reddish brown coloration with iodine, indistinguishable from that of pure rabbit liver glycogen.

After hydrolysis, separation of the cleavage products yielded, in both cases, analytically pure tetramethylglucopyranose, in amount over 9% of the starting-material, indicating on the basis of Haworth and Percival's formula, a molecular magnitude approximating to 12 glucose units. The bulk of the cleavage products was 2:3:6-trimethylglucose, uncontaminated by isomerides; and in addition about 15% of dimethylglucose. That this arose from the presence of unetherified hydroxyl groups present in the methylated glycogen, and not from the elimination of side-chains by hydrolysis, was shown by treating methylated glycogen (a) with nitrogen pentoxide in dry chloroform and (b) with dichloroacetyl chloride in dry pyridine at -10° [Bell, 1935] whereby nitrate was introduced by (a) and dichloroacetyl by (b).

Table I records the general results of the investigation—rabbit glycogen being investigated with respect to two separate preparations.

DISCUSSION.

The methods employed in this work lead to excellent recovery of cleavage products. The amounts of dimethylglucoses obtained assist considerably in assessing the molecular magnitude of these glycogens, since change in chain-length and degree of methylation alters the ratios, trimethylglucose/tetramethylglucose and trimethylglucose/dimethylglucose, indicated by examples in Table II.

Table I.

The values in parentheses indicate theoretical yields calculated on the basis that methylated glycogen gives, on cleavage, 1 mol. tetra-, 9 mols. tri- and 2 mols. di-methylglucose.

	Rabbit glycogen		Fish glycogen III
	I	II	
$[\alpha]_D$ in CHCl_3	+208.5°	+208.7°	+208.5°
$[\alpha]_D$ in H_2O	—	+208.0°	+207.7°
Amount of material hydrolysed (g.)	19.34	20.43	27.052
Yield of tetramethylglucose (g.)	1.806 (1.851)	1.852 (1.955)	2.480 (2.590)
„ trimethylglucose (g.)	15.425 (15.680)	15.856 (16.550)	20.832 (21.940)
„ dimethylglucose (g.)	3.155 (3.360)	3.000 (3.445)	4.094 (4.565)
% recovery of cleavage products	97	94	93
% of starting material as tetramethylglucose	9.3	9.0	9.2
No. of C_6 chains in molecular unit of glycogen	12	12	12

Table II.

				No. of molecules of cleavage products			Ratios, by weight	
				A Tetra- methyl- glucose	B Tri- methyl- glucose	C Di- methyl- glucose	B/A	B/C
No. of C_6 units in chain	No. of free OH groups	OMe %						
1	12	1	46.3	1	10	1	9.4	10.6
2	12	2	45.3	1	9	2	8.5	4.8
3	12	3	44.2	1	8	3	7.8	2.9
4	13	2	45.2	1	10	2	9.4	5.3
5	13	3	44.4	1	9	3	8.5	3.2
6	11	1	45.2	1	9	1	8.5	9.6
7	11	2	44.1	1	8	2	7.8	4.3
Found:	I	OMe %	45.4	Ratio B/A	8.5	Ratio B/C	4.4	
	II	„	45.5	„	8.6	„	5.3	
	III	„	45.5	„	8.4	„	5.0	

The only values which correspond closely to those found are afforded by the cleavage products of a dodecasaccharide in which 36 of the 38 OH groups are etherified (including the reducing group), the free OH groups existing each in a different C_6 unit (line 2, Table II). The glucose units are united in an unbranched chain, since the OH groups were proved to exist as such in methylated glycogen, by the introduction of nitrate and of dichloroacetyl.

These results, besides fully substantiating the conclusions of Haworth and Percival regarding rabbit liver glycogen, further disclose the biologically interesting fact of the chemical identity of rabbit and fish liver glycogens.

EXPERIMENTAL.

Methoxyl estimations were made by an apparatus and reagents carefully calibrated by means of pure substances of known composition, when a straight-line relationship was shown to exist between the found and true values, by which those stated in this paper are corrected.

Methylation of the glycogens. This was effected by Haworth and Percival's modification for glycogen of the process of simultaneous deacetylation and methylation originally used by Irvine and Robertson [1926]. The acetates here

employed have already been described by Bell and Kosterlitz [1935]. Yields were excellent and the derivatives were obtained as white solids after precipitating several times from acetone solution by light petroleum (B.P. 60–80°) and evaporating a centrifuged acetone solution of the precipitate to dryness under reduced pressure. The methoxyl contents attained maximum values after 8 to 10 methylations and in each instance 8 further methylations proved ineffective in increasing them.

The three preparations (rabbit, I and II, fish, III) were all soluble in acetone, chloroform, benzene, pyridine, glacial acetic acid and cold water. In this last solvent all gave with iodine a red-brown coloration indistinguishable from that of rabbit glycogen.

By dissolving in cold water and raising the temperature to 80–90° the methylated glycogens were completely precipitated and could in this way be easily freed from any appreciable amount of ash. The rotatory powers are detailed in Table I. (OMe found: I, 45.4; II, 45.5; III, 45.5 %.)

Hydrolysis by aqueous acid and separation of the cleavage products. An initial difficulty was provided by the insolubility of methylated glycogen in hot water; hydrolysis, however, was easily effected by adding 5 parts of glacial acetic acid to 1 part of methylated glycogen and reducing the pressure inside the flask so as to permeate the solid with solvent, then adding 10 parts 5% HCl and heating on the water-bath; the solid rapidly dissolved to give a yellow solution. Heating was continued until a constant polarimetric reading was obtained. Barium carbonate, 10% in excess of the HCl used, was then added and the whole evaporated to complete dryness under reduced pressure below 50°, water being added from time to time to facilitate removal of acetic acid. The residue was finally dried by alcohol-benzene, and worked up as described diagrammatically below.

It should be noted that Macdonald [1935] has recently substantiated the applicability of chloroform-water extraction to the quantitative separation of tetramethyl- from trimethyl-glucoses, and that the author has long been accustomed to effect quantitative separations in this manner. He has been

aware that trimethyl-sugars, if present in sufficient concentration, are extracted by chloroform, but also that washing with water removes them from the chloroform layer. Accordingly, the procedure adopted to separate approximately 10% of the tetramethylglucose from a mixture of lower homologues is included in the above scheme. In this manner the cleavage products from glycogens I, II and III, yielded analytically pure, largely crystalline 2:3:4:6-tetramethyl- and 2:3:6-trimethyl-glucose, and syrupy dimethyl-glucoses as indicated in Table III.

Table III.

Methylated glycogen	I	II	III
Amount hydrolysed, after deducting residue A	19.340 g.	20.430 g.	27.052 g.
2:3:4:6-Tetramethylglucose:			
Amount found	1.806 g.	1.852 g.	2.480 g.
n_D^{18} of fused substance	1.4590	1.4585	1.4580
$[\alpha]_D^{18}$ in water	+82.6°	+83.1°	+84.0°
$[\alpha]_D^{18}$ in water of authentic material	+83°	+83°	+83°
OMe (%)	52.5	52.3	52.5
Theoretical OMe (%)	52.5	52.5	52.5
m.p. after draining on tile	86–88°	87°	87°
Mixed m.p.	No dep.	No dep.	No dep.
2:3:6-Trimethylglucose:			
Amount found	15.425 g.	15.856 g.	20.832 g.
n_D^{18} of fused substance	1.4750	1.4755	1.4750
$[\alpha]_D^{18}$ in water	+69.8°	+70.0°	+69.8°
$[\alpha]_D^{18}$ in water of authentic material	+70°	+70°	+70°
OMe (%)	41.8	41.9	41.9
Theoretical OMe (%)	41.9	41.9	41.9
m.p. after draining on tile	117°	115–118°	110–113°
Mixed m.p.	No dep.	No dep.	No dep.
Dimethylglucose:			
Amount found	3.155 g.	3.000 g.	4.094 g.
n_D^{18}	1.4850	1.4860	1.4850
OMe (%)	30.0	29.7	30.0
Theoretical OMe (%)	29.8	29.8	29.8

The dimethylglucose was proved to be homogeneous by converting it into the glucoside, which distilled in one fraction leaving no residue. Acetylation of the barium residues yielded no appreciable amounts of material, showing that monomethylglucose and glucose itself were absent from the cleavage products.

Residues A and B. Residue B was always too small in amount to be investigated, but residue A from glycogens I and III was examined in respect of $[\alpha]_D$ and OMe. The substances were obtained in small amount, 19.925 g. of I giving 0.585 g. ($[\alpha]_D$ (in CHCl_3) +160°; OMe, 35%) and 27.936 g. of III giving 0.884 g. ($[\alpha]_D$ (in CHCl_3) +158°; OMe, 34%).

Detection of free hydroxyl groups in fully methylated glycogen. Treatment at 0° of a dry chloroform solution of methylated glycogen with a 30% solution of nitrogen pentoxide in chloroform for 2 min., introduced nitrate groups, as shown by a strongly positive diphenylamine reaction. In addition, approximately two dichloroacetyl groups could be introduced by the action of dichloroacetyl chloride in pyridine at –10°. (Found: Cl, 4.6; $\text{C}_{72}\text{H}_{82}\text{O}_{62} \cdot (\text{CH}_3)_{36} \cdot (\text{C}_2\text{HOCl}_2)_2$ requires Cl, 5.2%.) The solubilities and outward appearance of these derivatives were unchanged from the parent materials.

SUMMARY.

Fish and rabbit liver glycogens, investigated by the methylation method, were shown to have the same chemical structure. Hydrolysis of the fully methylated glycogens (OMe, 45.5%) followed by quantitative separation of the cleavage products (obtained in over 93% yield) into analytically pure tetramethylglucopyranose, 2:3:6-trimethylglucose and dimethylglucoses showed that both kinds of glycogen are built up of unbranched chains of 12 glucose units.

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