CCXXVIII. LIVER GLYCOGEN.

IV. MOLECULAR STRUCTURE OF GLYCOGEN FORMED AFTER INGESTION OF GALACTOSE BY FASTED RABBITS.

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THIS paper describes the application of Haworth's "end-group assay" method [Haworth & Percival, 1932] to the elucidation of the molecular structure of glycogen formed in rabbits' livers subsequent to the oral administration of galactose. The glycogen was methylated and then hydrolysed and the cleavage products were separated and identified by the author's procedure [Bell, 1935]. A yield of 6% of 2:3:4:6-tetramethylglucose was obtained, corresponding with a molecular unit in the methylated glycogen of 18 glucose units.

The following table records the general results of the investigation which was carried out on two groups of rabbits, A 25 animals and B 10 animals. The experimental conditions were purposely different in the two groups.

Table I.

Group	A	B
Fasted for	24 hrs.	48 hrs.
Galactose fed	5 g./kg.	10 g. each
Time allowed for absorption	2 hrs.	4 hrs.
Tetramethylglucose % found after hydrolysis	6.0	6.2
of methylated glycogen		
Number of glucose units in glycogen molecule	18	18

The methoxyl contents (corrected, Bell [1935]) of the methylated glycogens, coupled with the amounts of tetramethylglucose found in the cleavage products, corresponded most closely with an octadecasaccharide having, in A, 54 of the available 56 OH groups methylated, and in B, 53 OH groups methylated. Table II shows detailed results of the separation of the cleavage products.

Table II.

Specimen of methylated glycogen	A	B
OMe %	45.6	44.8
Amount of material hydrolysed (g.)	15.00	12.28
Yield of tetramethylglucose (g.)	0.901	0.768
Yield of trimethylglucose (g.)	12.650	9.592
Yield of dimethylglucose (g.)	1.60	1.760
Recovery of cleavage products %	93.6	91.0

That the assay of the relative amounts of dimethyl- and trimethyl-glucose affords additional aid in estimating the chain-length of the glycogen is shown in Table III.

Table III.

Theoretical.							
No. of mols. of cleavage products							
No. of C ₆ units in chain	No. of free OH groups	OMe %	X	Y	Z	Ratios by weight	
			Tetra- methyl- glucose	Tri- methyl- glucose	Di- methyl- glucose	Y/X	Y/Z
18	1	46.0	1	16	1	14.1	16.0
18	2	45.4	1	15	2	13.1	7.4
18	3	44.9	1	14	3	12.2	4.6
Found A		45.6				14.0	7.9
Found B		44.8				12.5	5.4

DISCUSSION.

Haworth [1935] reported that his research school had found that glycogen could apparently exist in two forms, one composed of 12, the other of 18 glucose units per molecule. Previously, Haworth & Percival [1932] and Bell [1935] had found 12 glucose units as the basic molecular structure of rabbit liver glycogen.

The formation of glycogen as a result of galactose feeding has been studied by a number of investigators, but always with an emphasis on the physiological aspects of the problem. Harding *et al.* [1934] showed that such glycogen in rats was composed solely of glucose units. This fact was confirmed in the present instance by Dr Stephenson, using the technique of Stephenson & Yudkin [1936]. Deuel *et al.* [1933] obtained some evidence that galactose feeding in rats led to the formation of a glycogen which was more slowly broken down or utilized than in normally dieted animals.

In the light of present knowledge, the formation of this new glycogen apparently can be attributed only to the process of galactose feeding. The author's previous investigation of the molecular structure of rabbit liver glycogen consisted in the examination of two samples of material each obtained from 12–20 animals. At the time of publication, the fact that the two groups of animals were supplied with carbohydrate under radically different conditions was not considered to be of significance, particularly as glycogens of identical molecular size were obtained from both groups. Stated briefly, one group received a carbohydrate-rich diet, largely carrots and sucrose, whilst the other animals, after fasting, received glucose by intravenous infusion through the ear-vein by the elegant technique of Bridge & Noltie [1935]. By the second method, rapid formation of large amounts of liver glycogen took place.

The formation of the 18-unit glycogen, therefore, would not seem to be the result of mere rapid storage of carbohydrate in the liver.

A further important fact has emerged from this work. Although investigation of the methylated glycogen showed that it had a molecular structure of 18 glucose units, examination of the glycogen itself revealed no significant differences from the 12-unit polysaccharide. It therefore follows that such properties of glycogen itself as lend themselves to examination afford no clue as to the chemical molecular structure [see Bell & Young, 1934; Bell & Kosterlitz, 1935; Oakley & Young, 1936]. The suggestion by Donhoffer & Macleod [1932] that more than one kind of liver glycogen exists therefore seems to warrant further investigation.

EXPERIMENTAL.

Preparation of the glycogen.

Group A. 25 rabbits, fasted 24 hours, were given galactose, 2.5 g./kg. body weight, by stomach tube. About 2 hours later the animals were killed, the livers excised and ground with ice-cold 5% trichloroacetic acid. After centrifuging, the glycogen was precipitated by the addition of 1 vol. alcohol. Subsequent purification was effected by the procedure of Bell & Young [1934]; yield 20 g.

Group B. 10 large rabbits were fasted 48 hours and to each were then given 10 g. of galactose in concentrated solution by stomach-tube. After 4 hours the animals were deeply anaesthetized with "Numal-Roche" and the livers excised and extracted by boiling water; subsequent purification as for group A; yield 13.5 g. from 700 g. liver tissue.

Properties of glycogens A and B.

Aqueous solutions of both were rather difficult to free from traces of nitrogenous material. This was finally effected by centrifuging at 15,000 r.p.m. The purified solutions were rather more opalescent and had very slightly higher opacities than 12-unit glycogen but otherwise appeared similar and gave the same red-brown colour with iodine. The properties are tabulated below:

Table IV.

Specimen	A	B
$[\alpha]_D$ in H_2O ($c=0.5\%$)	+201°	+200°
Reducing power [Macleod & Robison, 1929]	1.2%	1.1%
Phosphorus, organic	Nil	Nil
Phosphorus, inorganic	Nil	Nil
Ash	Nil	Nil

Biochemical investigation of hydrolysis product of glycogen.

(With M. STEPHENSON.)

The carbohydrate, $[\alpha]_D + 52^\circ$ (in water), formed by aqueous acid hydrolysis of the glycogen "A" was investigated by a fermentation method. A strain of *S. cerevisiae* was used which, when grown on glucose broth, centrifuged and washed, failed to ferment galactose at a measurable rate [Stephenson & Yudkin, 1936].

The CO_2 eliminated per mg. carbohydrate was measured in the Barcroft manometer as described in the above paper. The total CO_2 eliminated from the unknown sugar was compared with that from pure *d*-glucose. As is shown below, the CO_2 /mg. sugar corresponds very closely in the two instances:

Pure glucose	Hydrolysis product
μ l.	μ l.
165.9	162.0

Carrying out a parallel large scale experiment, the yeast-free liquors, after fermentation had ceased, were found to contain less than 0.02% of reducing material (Macleod & Robison).

Acetylation.

This was carried out by the procedure of Haworth and Percival:

Table V.

Specimen	A	B
Amount of starting material (g.)	8.5	13
Yield of acetate (g.)	14	19
Yield %	93	82
$[\alpha]_D$ in $CHCl_3$ ($l=2$, $c=2\%$)	+170°	+170°
$COCH_3\%$	44.0	44.4

Methylation.

A. 26 g. acetate were treated according to Haworth & Percival. After 10 methylations and subsequent purification, 15.8 g. of methylated glycogen were obtained (OMe, 45.6%. $[\alpha]_D^{25} + 210^\circ$: in CHCl_3 : $l=2$, $c=6\%$). The product possessed the appearance and properties described by Bell [1935].

B. 19 g. acetate gave, after 8 treatments, 12.50 g. (93%) of methylated glycogen. (OMe, 44.8%. $[\alpha]_D^{25} + 207^\circ$: in CHCl_3 : $l=2$, $c=2.1\%$.)

Hydrolysis by aqueous acid and separation of cleavage products.

The procedure followed was exactly as described by Bell [1935] except that isolation of the tetramethylglucose in a state of purity, owing to the different relative proportions of tri- and tetra-methylglucoses, required that a 5% solution in chloroform of the crude product, obtained after the chloroform water partitions, should be washed four times with one-third of its volume of water.

A. 15.00 g. were hydrolysed giving:

2:3:4:6-Tetramethylglucose, 0.901 g.; n_D could not be measured owing to crystallization in refractometer. $[\alpha]_D^{20}$ in H_2O , $+82.1^\circ$; M.P. $85-88^\circ$; mixed M.P. showed no depression; OMe, 52.5%.

2:3:6-Trimethylglucose, 12.650 g.; n_D^{20} , 1.4758; $[\alpha]_D^{20}$ in H_2O , $+70^\circ$; M.P. $113-117^\circ$; mixed M.P. showed no depression; OMe, 41%.

Dimethylglucose, 1.60 g; n_D^{20} 1.4860; OMe, 30.0%.

B. 12.40 g. were hydrolysed. A small residue of partly hydrolysed material, 0.120 g., was isolated [see Bell, 1935]. This was deducted from the amount of starting material, making 12.28 g. The following fractions were obtained:

2:3:4:6-Tetramethylglucose, 0.768 g.; n_D^{20} 1.4570; $[\alpha]_D^{20}$ in $\text{H}_2\text{O} + 82^\circ$; OMe, 52.1%; M.P. $85-88^\circ$; mixed M.P., no depression.

2:3:6-Trimethylglucose, 9.005 g.; n_D^{20} 1.4740; $[\alpha]_D^{20}$ in H_2O , $+70.7^\circ$; OMe, 41.5%; M.P. $111-116^\circ$; mixed M.P., no depression.

"Dimethylglucose", 2.347 g.; n_D^{20} 1.4820; OMe, 32.2%. The refractive index and methoxyl content indicate that the dimethyl-fraction here contains about 25% of trimethylglucose. Correcting for this, the amounts of trimethylglucose and dimethylglucose become respectively, 9.592 and 1.760 g.

No trace of isomeric trimethylglucoses could be detected.

SUMMARY.

Glycogen formed in rabbit livers after ingestion of galactose has been shown to have a chemical molecular magnitude corresponding to 18 glucose units, the normally formed glycogen so far investigated being built up of 12. The new type of glycogen was obtained from two groups of animals, 35 in all.

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