The Structure and Deposition of Human-Liver Glycogens

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(Received 24 August 1965)

1. Glycogen was extracted from and the amount determined in human foetal livers ranging in age from 13½ to 26 weeks. 2. The detailed structures of human foetal- and child-liver glycogens were examined and shown to be essentially the same. 3. The deposition of glycogen in different mammalian species is discussed.

Most studies on glycogen deposition in the foetal liver have been made on laboratory animals, e.g. rats (Jacquot, 1959), guinea pigs (Ducommun-Lehmann, 1951; Nemeth, 1954), rabbits (Jost & Jacquot, 1955) and lambs and monkeys (Shelley, 1960). In all cases the initial concentrations of the polysaccharide are very low and they tend to rise sharply in the later stages of gestation, until at birth they have reached or surpassed the normal adult level. Such studies as have been made with human foetal material (e.g. Kitamura, 1942; Shapovalov, 1961, 1962) employed histochemical techniques and indicated that a similar pattern of development occurs. Histochemical techniques should, however, be accepted with caution, as they may detect polysaccharides other than glycogens, and there is controversy about the first appearance of glycogen in the liver tissues. No determinations of the detailed structures, e.g. measurements of CL(av.)† and ECL(av.), of normal human foetal glycogens have previously been made. Data on the structures of other mammalian foetal glycogens are also limited. Chain lengths of 11 D-glucose units for a pig-liver glycogen (Manners & Archibald, 1957), 13 units for a sheep-liver glycogen (Bell & Manners, 1952) and 11·5 units for a guinea-pig-liver glycogen (Illingworth, Cori & Lerner, 1952) have been reported, but no indications of the foetal ages were given. The most comprehensive survey is that by Warren & Whittaker (1959), who studied the structure of glycogens from foetal goat livers and obtained CL(av.) and ECL(av.) values in the normal range, i.e. 10–14 and 7–9 D-glucose units respectively.

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† Abbreviations: CL(av.), average chain length; ECL(av.), average exterior chain length; ICL(av.), average interior chain length, i.e. CL(av.) – ECL(av.) – 1.

The present study was undertaken in an attempt to determine the earliest stages at which recognizable glycogens may be isolated from the human foetal liver, and to examine the detailed structures of these polysaccharides and compare them with glycogens from more mature liver tissues. A preliminary account of this work has been given (Bourne, McLean & Pridham, 1964).

METHODS AND MATERIALS

Human liver specimens. These specimens, both foetal and mature, were kindly provided by Dr B. Clayton, The Hospital for Sick Children, Great Ormond Street, London, W.C. 1. These were cooled to –20° as rapidly as possible after removal from the body, and stored at this temperature.

β-Amylase. This was obtained from the Wallerstein Co., Mariners Harbor, Staten Island, N.Y., U.S.A. The units of activity are expressed as mg. of maltose liberated by 1 mg. of enzyme under the conditions specified by Hobson, Whelan & Peat (1950) but at a temperature of 30°.

α-Amylase. This was prepared by the method of Fischer & Stein (1954). The crystallization stage was omitted, and the enzyme freeze-dried in 0·2M-sodium citrate buffer, pH 7·0 (Liddle & Manners, 1957). The unit of activity is the quantity of enzyme that liberates 1 mg. of apparent maltose from 1 ml. of starch solution (1%, w/v) in 3 min. at 35° (Manners & Wright, 1962).

Glycogen extraction. Homogenization with cold aq. 10% (w/v) trichloroacetic acid solution was employed as the general method (Bloom, Lewis, Schumpert & Shen, 1950). The glycogen was then precipitated from the extract with ethanol and centrifuged, and the polysaccharide was washed with ethanol followed by ether and finally dried under reduced pressure in the presence of P₂O₅.

Determination of purity of glycogens. Glycogens were hydrolysed with 2N-H₂SO₄ at 100° for 2 hr. After appropriate dilution with water, portions were assayed for glucose by the method of Somogyi (1952).

Determination of CL(av.). Samples were analysed by incubation with α-amylase as described by Manners & Wright (1962), except that the incubation time was 15 hr.; 4 units of α-amylase/mg. of glycogen were used. Apparent

maltose production was measured by the method of Park & Johnson (1949). Larger samples were assayed by periodate oxidation (Potter & Hassid, 1948) with the modification due to Manners & Archibald (1957).

Determination of ECL(av.). When relatively large amounts of glycogen were available, a 30 mg. specimen was incubated with β -amylase (1800 units) and 0.2M-sodium acetate buffer, pH 4.6 (5 ml.), made up to 25 ml. with water, at 30° for 24 hr. (Bell & Manners, 1952). The maltose liberated was determined by the method of Somogyi (1952).

For the smaller samples the digests were reduced to glycogen (1 mg.), β -amylase (50–70 units) and 0.2M-sodium acetate buffer, pH 4.6 (0.4 ml.), in a total volume of 10 ml. After suitable dilution, the maltose was measured by the method of Park & Johnson (1949).

Measurement of absorption spectra of glycogen-iodine complexes. Glycogen (2.5 mg.) in iodine solution (5 ml. of 0.1% I₂ in 1% KI) was made up to 25 ml. with water. The absorption spectrum was measured in the range 400–700 m μ in a Unicam SP.500 spectrophotometer with 1 cm. glass cells. The reference solution used was 0.02% I₂ in aq. 0.2% KI.

Partial acid hydrolysis. Partial hydrolysis of glycogen was carried out by using the conditions of Wolfrom, Lassetre & O'Neill (1951). The neutralized hydrolysates were examined on paper chromatograms with ethyl acetate-acetic acid-water (9:2:2, by vol.) as solvent, and the sugars located with *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950). The hydrolysates, after reduction with NaBH₄, were also examined by paper electrophoresis with 0.062M-sodium molybdate solution as electrolyte (Bourne, Hutson & Weigel, 1961).

RESULTS AND DISCUSSION

Foetal livers ranging in age from 11½ to 26 weeks were examined. In most cases, liver tissues of the same foetal age were pooled to obtain sufficient material for analysis. Foetal livers aged 11½ and 12 weeks gave only traces of precipitate when extracted with trichloroacetic acid and then treated with ethanol. Partial acid hydrolysis of these

precipitates, followed by chromatography and electrophoresis, revealed no reducing sugars, whereas the treatment of glycogen from a 26-week human foetal liver yielded glucose, maltose and isomaltose, thus revealing the presence of the expected α -(1→4)- and α -(1→6)-glucosidic linkages in the polysaccharide. The nature of the precipitates from the early foetal livers was not established.

Milligram quantities of glycogen were obtained from foetal livers at 13½ weeks (Table 1) and a sharp increase in the amount deposited was observed in 25–26-week livers.

The chemical and enzymic methods employed in this study demonstrated that the detailed structures of these glycogens and those from the livers of children aged from 6 weeks to 11 years (Table 2) were essentially the same. Most of the foetal glycogens (Table 1) were within the normal range of variation (see Manners, 1957). The values for CL(av.) varied from 12.5 to 14.5 D-glucose units (average 13.7) and those for ECL(av.) from 7.7 to 10.2 units (average 8.8). An absorption maximum of 470 m μ for an iodine complex with the 26-week foetal glycogen fell within the normal range 420–490 m μ (Manners, 1957), as did those for the other glycogen samples examined in this way (Table 3). This provided additional evidence that the foetal glycogen had a normal structure.

The CL(av.) values for the child-liver glycogens fell within the limits of 12.6 and 15.2 D-glucose units (average 13.3) and the ECL(av.) values ranged from 7.9 to 9.3 units (average 8.7) (Table 2). A CL(av.) value of 15.2 D-glucose units for the glycogen from 7-week liver, although high, can be accounted for by the fact that there is an intrinsic error of ± 1 D-glucose unit in the method employed.

Kitamura (1942) suggested that glycogen deposition first occurs in human foetal liver at 17 weeks and that the amount begins to increase sharply

Table 1. *Structures of human foetal-liver glycogens*

Foetal age (weeks)	Fresh wt. of liver sample (mg.)	Purity of glycogen (%)	Wt. of glycogen (mg.)	Glycogen recovered from liver (%)	CL(av.) by α -amylolysis	ECL(av.) by β -amylolysis	ICL(av.)
13½	420	51.0	1.65	0.39	14.1	9.2	3.9
16	846	45.2	1.95	0.23	13.0	10.2	1.8
17	929	58.0	2.26	0.24	13.5	8.7	3.8
18½	554	58.0	2.55	0.46	12.5	7.7	3.8
20	870	22.0	2.57	0.30	14.5	—	—
21½	465	27.2	2.23	0.48	13.7	9.1	3.6
21½	1567	68.0	3.13	0.20	13.6	8.0	4.6
23½	1944	60.0	4.68	0.24	14.1	9.0	4.1
25	246	83.5	1.75	0.71	14.0	8.7	4.3
26	180	66.3	3.05	1.70	13.5	8.4	4.1
26	28.33 g.	86.6	320	1.13	13.9*	8.9	4.0

* CL(av.) by periodate oxidation = 13.4 D-glucose units.

Table 2. *Structures of liver glycogens obtained from children*

Age of patient	Fresh wt. of liver sample (mg.)	Purity of glycogen (%)	Wt. of glycogen (mg.)	Glycogen recovered from liver (%)	CL(av.) by α -amylolysis	ECL(av.) by β -amylolysis	ICL(av.)
6 weeks	50	45.0	1.4	2.80	—	*	—
7 weeks	566	62.5	4.5	0.80	15.2	9.2	5.0
8 weeks	44	57.3	2.0	4.55	12.6	8.9	2.7
12 weeks	1090	85.3	19.7	1.81	13.4	7.9	4.5
12 weeks	1298	69.7	15.9	1.23	12.9	8.3	3.6
14 weeks	359	67.6	4.3	1.20	13.9	9.3	3.6
20 months	189	45.0	1.4	0.74	13.1	9.2	2.9
28 months	235	98.6	13.2	5.62	12.7	8.6	3.1
11 years	502	76.6	8.4	1.67	12.9	8.2	3.7

* This sample gave 45% conversion into maltose (the normal range is 40–50% conversion).

Table 3. *Wavelengths of absorption maxima of glycogen–iodine complexes*

Source of liver glycogen	CL(av.) by periodate oxidation	CL(av.) by α -amylolysis	ECL(av.) by β -amylolysis	ICL(av.)	$\lambda_{\max.}$ (m μ)
26-week human foetus	13.4	13.9	8.9	4.0	470
Baboon	13.3	13.3	8.3	4.0	435
Mouse	13.1	13.8	8.6	3.9	455
Rabbit	13.9	12.6	8.1	4.2	470

at 26 weeks. Claims have been made that glycogen is present in the liver as early as 6 weeks (Shapovalov, 1961, 1962), but both conclusions were based on histochemical studies. Mackay, Adams, Hertig & Danziger (1956) have, however, suggested that a 4-week foetal liver is almost ready to assume the function of glycogen storage. At this stage the yolk sac still fulfils many of the liver's functions, such as glycogen storage, but has begun to transfer its enzyme systems to the liver. Thus 5-nucleotidase and non-specific esterase begin to appear in the liver and decrease in activity in the yolk sac, and Mackay *et al.* (1956) suggest on this basis that the function of glycogen storage will soon be taken over by the liver. In contrast, Lelong, Rossier & Laumonier (1951) could not detect glycogen in human foetal liver, by histochemical techniques, until a few weeks before birth.

The methods employed in the present study are, of course, too insensitive to reveal very small amounts of glycogen, and we believe that the polysaccharide, and hence the enzyme system necessary for its synthesis, is present in foetal livers younger than 13½ weeks.

The stage at which glycogen storage begins to increase rapidly appears to bear some relation to the class of mammal. In rats, guinea pigs and rabbits (rodents) it occurs very late in gestation, i.e. after 90% (Jacquot, 1959), 80% (Ducommun-Lehmann, 1951) and 75% (Jost & Jacquot, 1955)

respectively of the gestation period has elapsed. In primates it occurs at a much earlier stage, for example in man after 66% of gestation has elapsed, and in Rhesus monkeys (Shelley, 1960) glycogen deposition is already high at 113 days (67% of gestation). Ungulates vary widely, the rapid increase occurring after 88% of gestation in pigs (Mendel & Leavenworth, 1907; Aron, 1922) and 61% in sheep (Shelley, 1960). Since the pituitary and adrenal hormones and insulin stimulate glycogen synthesis, it appears that the hormonal systems of primates and probably ungulates mature earlier than those of the rodents. Newborn and full-term infants respond, by pituitary discharge of adrenocorticotrophic hormone, to adrenaline (Jailer, Wong & Engle, 1951), whereas rats and mice do not respond until the eighth and eleventh days of life respectively (Jailer, 1950; Thompson & Blount, 1954), but direct comparative evidence of hormonal activities during the foetal stage is lacking.

We thank Dr B. Clayton for advice and encouragement. A. McL. was in receipt of a Medical Research Council Grant.

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