Studies on the Structure and Metabolism of Glycogen in Some Abnormal Human Infants

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1. The liver and muscle tissues of 14 human anencephalic babies were examined for glycogen content and structure, and for the activities of several glycogen-metabolizing enzymes. 2. In both tissues glycogen content increased with gestation age; the muscle glycogens had a slightly but significantly lower degree of branching than the corresponding liver glycogens. 3. All the expected glycogen-metabolizing enzymes were present; acid maltase activities were higher and phosphoglucomutase activities were lower than the results reported for human adult tissues. Glucose 6-phosphatase activity increased significantly with gestation age.

One of the most drastic changes occurring in the life of any individual is the transition from intra-uterine dependence to extra-uterine independence. Before birth, physiological processes in the mother have an important role in regulating metabolism in the foetus, and after separation from the placenta the infant has to rely at least partly on relatively untried processes of absorption and excretion. The maintenance of adequate carbohydrate synthesis and breakdown are of first importance and, although the perinatal changes usually proceed smoothly, this is not always true. Serious hypoglycaemia occurs and can be protracted. It is therefore important to study aspects of glycogen metabolism in this period and to have reliable information on the glycogen content and structure as well as on the relevant enzyme activities in tissues concerned. Although some results have been reported for several mammalian species (Dawkins, 1966; Shelley & Neligan, 1966), corresponding information for human newborn infants is not readily available.

In the present study glycogen structure and metabolism in liver and muscle of 14 anencephalic human foetuses was examined. Absence of cerebral tissue is accompanied by a pituitary anomaly and virtual absence of the foetal adrenal cortex (Boyd, 1961). For this reason it is likely that the values detected represent minimal ones (Jacquot & Kretchmer, 1964). Nevertheless the results may give some indication of the variation in glycogen structure and metabolism in the newborn infant.

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MATERIALS AND METHODS

Source of tissues. All specimens of liver and muscle from anencephalic babies were obtained within 1 h and usually within 30 min of delivery or death, whichever was the later. The gestational age, birth weight and probable death–delivery interval are shown in Table 1. The time of death refers to the time when the foetal heart was last heard by an observer and does not necessarily relate to the actual time of demise. Anencephalic foetuses were chosen because there was a degree of predictability about their birth and because permission for *post mortem* could be obtained from the father before or at delivery. All tissue specimens were kept at -15°C in sealed containers.

Analytical methods. A phenol- H_2SO_4 reagent was used for the determination of glycogen (Dubois, Gilles, Hamilton, Rebers & Smith, 1956); the production of reducing sugars during the α - and β -amylolysis of glycogen was measured by the method of Nelson (1944). A modification of the method of Allen (1940) was used for the determination of P_1 .

Isolation and analysis of glycogen. Glycogen was isolated by heating the frozen tissue (100–600 mg) in 40% (w/v) KOH solution (1–3 ml) for 45 min and was purified by six ethanol precipitations. The extent of degradation by α -amylase and β -amylase was measured as described by Kjolberg & Manners (1962); the results are expressed as percentage conversion into maltose (P_M). The average chain length (CL), expressed as D-glucose residues, was estimated from the extent of α -amylolysis (Manners & Wright, 1962) by using the equation:

$$\frac{100}{\text{CL}} = 23.4 - 0.20 (\text{P}_M)$$

The average chain length was also determined by iodine staining (Archibald *et al.* 1961), in which the wavelength

of maximum absorption ($\lambda_{\max}$) of the glycogen-iodine complex is measured in the presence of half-saturated $(\text{NH}_4)_2\text{SO}_4$ and by using the equation:

$$\text{CL} = 16 + 0.114 (\lambda_{\max} - 500)$$

The average exterior chain length (ECL) and the average interior chain length (ICL) are calculated from the equations:

$$\text{ECL} = \text{CL} \times \beta\text{-amylolysis limit} + 2.0$$

and

$$\text{CL} = \text{ECL} + \text{ICL} + 1$$

Enzyme assays. Glucose 6-phosphatase (EC 3.1.3.9), phosphorylase (EC 2.4.1.1), acid maltase (EC 3.2.1.20) and amylo-1,6-glucosidase (EC 3.2.1.33) activity were

measured by the methods described by Hers (1964). The amylo-1,6-glucosidase assay used involved the incorporation of radioactive glucose into glycogen and the unit of activity is defined as the amount of enzyme incorporating 0.1% of the c.p.m. added as glucose/h. All other activities are expressed as μmol of product formed/min per g of tissue. Phosphoglucomutase (EC 2.7.5.1) activity was assayed as described by Hers (1959).

RESULTS AND DISCUSSION

Liver and muscle glycogen. The structure of the glycogens from the liver and muscle tissues of the anencephalic babies, together with the glycogen contents of these tissues, are shown in Tables 2 and

Table 1. *Details of the anencephalic cases examined*

Case	Gestation age (weeks)	Birthweight (kg)	Time of death
A1	31	0.992	24 h before delivery
A2	32	1.304	24 h before delivery
A3	32	1.191	Not known
A4	33	0.964	Not known
A5	33	0.907	50 min before delivery
A6	33	1.162	1 h before delivery
A7	34	1.701	22 h before delivery
A8	36	1.985	12 h before delivery
A9	36	1.474	1 h before delivery
A10	38	1.616	Not known
A11	39	2.466	30 min after delivery
A12	40	2.495	5 h before delivery
A13	41	2.495	1 h before delivery
A14	42	3.600	1 h before delivery

Table 2. *Liver glycogen content and structure*

Average chain length (CL), average exterior chain length (ECL) and average interior chain length (ICL) are expressed as D-glucose residues. The sources of 'normal adult' values are as follows: glycogen content (Sokal & Gerszi, 1959; Hers, 1964); glycogen structure (Manners, 1962).

Case	Glycogen content (% of wet wt.)	CL by iodine staining	α -Amylolysis limit (%)	CL by α -amylolysis limit	β -Amylolysis limit (%)	ECL	ICL
A1	0.05	—	—	—	—	—	—
A2	1.61	12.5	77	12.6	39	6.9	4.7
A3	1.90	12.0	77	12.6	43	6.8	4.5
A4	1.84	13.0	77	12.6	40	7.1	4.7
A5	1.90	12.5	77	12.6	41	6.9	4.7
A6	2.40	12.0	68	10.3	38	6.2	4.0
A7	1.50	12.0	80	13.7	38	6.9	4.9
A8	0.94	15.0	80	13.7	42	8.1	5.3
A9	1.77	12.5	75	12.0	38	6.7	4.6
A10	2.17	14.0	78	13.0	39	7.3	5.2
A11	0.09	—	—	—	—	—	—
A12	1.96	12.0	66	9.7	38	6.1	3.7
A13	0.04	—	—	—	—	—	—
A14	1.03	12.5	77	12.6	39	6.9	4.6
Average	1.37	12.7	75.6	12.3	39.5	6.9	4.6
Standard deviation	0.81	—	4.5	1.3	1.8	0.5	0.5
Normal adult	2-5	10-14	—	10-14	40-50	5.5-8.5	3.5-4.5

3. The glycogen content of both tissues varied greatly from one case to another, and in contrast with the findings of others (Shelley & Neligan, 1966), we were unable to demonstrate a correlation between glycogen content and the maturity for either tissue. There was also no correlation between

the glycogen contents in liver and muscle. The ranges for both tissues were wide with the mean value for muscle glycogen content being higher than that for liver glycogen content. Low hepatic glycogen contents were frequently associated with moderately high values for muscle glycogen,

Table 3. *Muscle glycogen content and structure*

Average chain length (CL), average exterior chain length (ECL) and average interior chain length (ICL) are expressed as D-glucose residues. The sources of 'normal adult' values are as follows: glycogen content (Sokal & Gerszi, 1950; Hers, 1964); glycogen structure (Manners, 1962).

Case	Glycogen content (% of wet wt.)	CL by iodine staining	α -Amylolysis limit (%)	CL by α -amylolysis limit	β -Amylolysis limit (%)	ECL	ICL
A1	1.40	13.5	77	12.9	50	8.7	3.5
A2	1.24	14.0	80	13.9	49	8.9	4.1
A3	1.50	16.0	84	15.0	50	9.6	4.9
A4	2.08	14.0	79	13.6	49	8.8	4.0
A5	1.50	15.0	80	13.9	49	9.1	4.4
A6	2.60	16.0	80	13.9	49	8.9	5.1
A7	3.00	14.9	78	13.0	49	8.9	4.1
A8	0.22	16.0	84	15.0	50	9.7	4.8
A9	1.97	15.0	84	14.9	49	9.3	4.7
A10	1.75	15.5	84	14.9	51	9.7	4.5
A11	0.70	12.5	75	12.0	47	7.7	3.5
A12	2.50	15.5	84	14.9	46	9.0	5.2
A13	2.15	13.0	77	12.6	47	7.7	4.1
A14	1.51	15.0	84	15.0	51	9.6	4.4
Average	1.72	14.7	80.7	14.0	49	9.0	4.4
Standard deviation	0.74	1.0	3.2	1.0	1.4	0.6	0.5
Normal adult	1	10-14		10-14	40-50	5.5-8.5	3.5-4.5

Table 4. *Anencephalic liver enzyme activities*

The sources of 'normal adult' values are as follows: phosphorylase, glucose 6-phosphatase, acid maltase and amylo-1,6-glucosidase (Hers, 1964) and phosphoglucumutase (Weber & Cantero, 1957).

Case	Phosphorylase (μ mol/min per g of tissue)	Glucose 6-phosphatase (μ mol/min per g of tissue)	Phospho- glucumutase (μ mol/min per g of tissue)	Acid maltase (μ mol/min per g of tissue)	Amylo-1,6- glucosidase (units per g of tissue)
A1	17	0.4	24	1.4	102
A2	7	0.6	43	1.7	97
A3	15	0.75	42	1.7	88
A4	14	2.6	36	1.2	131
A5	15	0.8	57	1.7	100
A6	28	1.0	124	2.5	120
A7	11	2.3	123	2.2	135
A8	15	3.0	95	1.2	140
A9	28	2.6	112	1.6	197
A10	25	3.4	69	1.1	123
A11	18	1.8	64	1.6	111
A12	28	1.0	124	2.5	120
A13	28	7.1	46	1.7	97
A14	8	3.0	86	1.0	194
Average	18.4	2.2	74.6	1.7	125.4
Standard deviation	7.7	1.8	36.9	0.5	33.6
Normal adult	15-20	5	86-254	0.7	150

Table 5. *Anencephalic muscle enzyme activities*

The sources of 'normal adult' values are as follows: phosphorylase, acid maltase and amylo-1,6-glucosidase (Hers, 1964) and phosphoglucomutase (Weber & Cantero, 1957).

Case	Phosphorylase ($\mu\text{mol/min per g}$ of tissue)	Phosphoglucomutase ($\mu\text{mol/min per g}$ of tissue)	Acid maltase ($\mu\text{mol/min per g}$ of tissue)	Amylo-1,6-glucosidase (units per g of tissue)
A1	106	39	0.06	58
A2	95	34	0.13	98
A3	65	61	0.09	21
A4	64	77	0.07	103
A5	65	42	0.09	109
A6	88	41	0.08	60
A7	44	40	0.07	98
A8	91	57	0.04	65
A9	120	28	0.20	71
A10	120	63	0.06	74
A11	89	48	0.04	102
A12	114	67	0.07	68
A13	116	57	0.13	104
A14	117	37	0.06	73
Average	92.4	49.4	0.09	78.9
Standard deviation	24.8	14.4	0.04	24.7
Normal adult	100	86-254	0.05	100

indicating that adequate glycogen deposition had been possible and that these foetuses may have drawn on their liver glycogen reserves before delivery.

The molecular structures of the liver glycogens resembled those of adults, although the β -amyolysis limits were slightly lower. The results are also similar to those obtained by Bourne, McLean & Pridham (1966) who examined a series of human foetal and child liver glycogens. This suggests that, in an anencephalic foetus, the ratio of relative activity of glycogen synthetase to branching enzyme is both normal and fairly constant, since this ratio will control the degree of branching in the glycogen.

The muscle glycogen content was about twice that of the average value for human adults and there was a small but significant difference in structure from the liver glycogen. Muscle glycogen chain-length values averaged 14.0 and 14.7 D-glucose residues respectively, by the enzymic and iodine-staining methods, as against 12.3 and 12.7 D-glucose residues for the corresponding liver glycogen samples. The β -amyolysis limits show a more pronounced difference (about 10%). Both the average chain lengths and the exterior chain lengths were slightly longer than those of the liver glycogen from the same infant. This could indicate that glycogen synthesis in the two tissues is controlled by different mechanisms.

Enzyme activities. The results of the enzyme assays of the anencephalic tissues are shown in Tables 4 and 5. Glucose 6-phosphatase activity

Fig. 1. Changes in the activity of liver glucose 6-phosphatase during gestation.

showed some correlation with gestational age (Fig. 1) and to a smaller extent with birth weight. This has previously been demonstrated in other mammalian species (Dawkins, 1966) although the increases in the specimens examined by us were

not as great as those described in rat livers. Acid maltase activity in both liver and muscle tissues remained approximately constant at a value that was greater than the normal ones of about 0.7 and 0.05 unit respectively reported for human adults (Hers, 1964).

There was considerable variation in the individual muscle phosphorylase and amylo-1,6-glucosidase activities, but in general the values approached those of adult tissues. On the other hand the muscle phosphoglucomutase activities were consistently lower than those in human adults. With the exception of glucose 6-phosphatase, we were unable to demonstrate either a positive or a negative correlation of the enzyme activities with each other, or with glycogen contents, birth weight or gestation. It should be emphasized that the results in Tables 4 and 5 represent minimum values, since the exact time of death *in utero* was difficult to determine and resulted in inevitable delay in isolation of tissues during which loss of activity could have occurred. Moreover, foetal decapitations in rats have been shown to prevent an increase in enzyme activity and glycogen accumulation in the last days before birth (Jacquot & Kretchmer, 1964) and an intact pituitary-adrenal axis is obviously important for normal glycogen synthesis and breakdown. As this is almost certainly disturbed in anencephalic babies this could be an additional reason for low values. Placental transfer of maternal hormones may, however, compensate for this, and it is clear that the development of many of the glycogen-metabolizing enzymes can continue in the partial or complete absence of normal pituitary function.

Shelley (1961) has written: 'for obvious reasons there is no reliable information on the glycogen reserves of newborn human infants'. In the present

work, it is recognized that because of the morphological condition, the tissues cannot be regarded as 'normal'; nevertheless, since the primary defect is not a disturbance of carbohydrate metabolism (Boyd, 1961) the results may give an indication of the probable minimum values for glycogen content and for the activities of those enzymes that are directly concerned with its metabolism in liver and muscle tissue.

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REFERENCES

- Allen, R. J. L. (1940). *J. biol. Chem.* **153**, 375.
Archibald, A. R., Fleming, I. D., Liddle, A. M., Manners, D. J., Mercer, G. A. & Wright, A. (1961). *J. chem. Soc.* p. 1183.
Bourne, E. J., McLean, A. & Pridham, J. B. (1966). *Biochem. J.* **98**, 678.
Boyd, J. D. (1961). In *Ciba Found. Symp.: Somatic Stability in the Newly Born*, p. 282. London: J. and A. Churchill Ltd.
Dawkins, M. J. R. (1966). *Br. med. Bull.* **22**, 27.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Analyt. Chem.* **28**, 350.
Hers, H. G. (1959). *Rev. int. Hepatol.* **9**, 35.
Hers, H. G. (1964). *Adv. metab. Disorders*, **1**, 1.
Jacquot, R. & Kretchmer, N. (1964). *J. biol. Chem.* **239**, 1301.
Kjolberg, O. & Manners, D. J. (1962). *J. chem. Soc.* p. 4596.
Manners, D. J. (1962). *Adv. Carbohydr. Chem.* **17**, 371.
Manners, D. J. & Wright, A. (1962). *J. chem. Soc.* p. 1597.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Shelley, H. J. (1961). *Br. med. Bull.* **17**, 137.
Shelley, H. J. & Neligan, G. A. (1966). *Br. med. Bull.* **22**, 34.
Sokal, J. E. & Gerszi, K. E. (1959). *J. Lab. clin. Med.* **53**, 876.
Weber, G. & Cantero, A. (1957). *Science, N.Y.*, **126**, 977.