and plasmalogen compounds and sphingomyelin was computed from the analysis of the fractions.

3. Choline plasmalogen was present in similar proportions in the whole muscle and myofibrils of rabbit muscle, but was virtually absent from trout muscle.

4. The constituent fatty acids and aldehydes from the separated phospholipids and some triglyceride fractions were identified and estimated by gas chromatography.

5. In general the results showed a preponderance of C_{18} saturated acids or aldehydes, and of polyenoic acids, in the kephalins, and of the C_{18} saturated compounds in phosphatidylcholine and choline plasmalogen, with the exception of liver and trout-muscle lecithins, which contained large amounts of C_{22} acids.

6. The fatty aldehydes fall in a narrower range (C_{14} – C_{18}) than the fatty acids (C_{14} – C_{22}), and included both unsaturated and branched-chain aldehydes, which varied considerably in proportion in different species.

7. The different fatty acid composition of lecithin, kephalin and triglyceride components of various tissues should be considered in possible routes of biosynthesis.

The support of this work by the British Empire Cancer Campaign is gratefully acknowledged. Mr M. J. Shaw assisted in the work on rabbit muscle.

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Biochem. J. (1961) **81**, 488

Alternative Pathways of Carbohydrate Metabolism in Foetal and Adult Tissues

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(Received 3 March 1961)

The recognition of pathways of carbohydrate metabolism other than the classical Embden-Meyerhof glycolytic sequence has stimulated efforts to clarify the reaction mechanisms involved and to evaluate the relative importance of these alternative pathways in different tissues. Evidence

about the fractions of glucose metabolized via the glycolytic sequence and via the hexose monophosphate pathway has been obtained from experiments in which the differential utilization of specifically labelled glucose or other carbohydrate intermediates has been measured. There is as yet

no complete agreement as to the most valid method of calculating, from such experiments, the relative amount of carbohydrate metabolized by the two pathways. A widely used method compares the rates at which C-1 and C-6 of glucose are converted into carbon dioxide. C-6 of glucose is converted into carbon dioxide by glycolysis and the tricarboxylic acid cycle. C-1 of glucose is converted into carbon dioxide both by this pathway and by the hexose monophosphate pathway. C-1 of glucose is removed as carbon dioxide in the oxidative decarboxylation of 6-phosphogluconate to give ribulose 5-phosphate and carbon dioxide, the second enzymic reaction of the pathway. The ratio of the rates at which C-6 and C-1 are metabolized to carbon dioxide gives an estimate of the importance of the two pathways. If all the glucose is metabolized via glycolysis and the tricarboxylic acid cycle the ratio will be unity. If a large fraction of glucose is metabolized via the hexose monophosphate pathway the ratio will be less than unity and will approach zero. Some of the difficulties involved in interpreting such results have been discussed in detail by de Duve & Hers (1957) and Katz & Wood (1960).

Both the dehydrogenation of glucose 6-phosphate to 6-phosphogluconate and the dehydrogenation of 6-phosphogluconate to ribulose 5-phosphate require triphosphopyridine nucleotide as hydrogen acceptor, whereas the dehydrogenations in glycolysis are diphosphopyridine nucleotide-linked. The finding that reduced triphosphopyridine nucleotide is specifically required for certain steps in the synthesis of fatty acids, steroids, purines, amino acids and certain other metabolically important compounds has led to the suggestion that the major role of the hexose monophosphate pathway is to provide reduced triphosphopyridine nucleotide for these synthetic reactions (Siperstein, 1958). We decided therefore to compare the relative rates of glycolysis and hexose monophosphate pathway in foetal and adult tissues, and particularly in those tissues such as the adrenal and ovary in which the synthesis of steroids may be occurring at a high rate. Evidence of high activity of the hexose monophosphate pathway in human foetal adrenal has been presented by Vilee, Engel & Vilee (1959).

MATERIALS AND METHODS

Samples of liver, brain, heart, lung, kidney, adrenal and skeletal muscle were obtained from foetuses within a few minutes of delivery. Human foetuses were removed from the uterus either by miniature caesarean section or by vaginal hysterotomy. Foetal tissues were obtained from rat and rabbit foetuses by dissecting open the uterus and removing the foetuses. Tissues were obtained from adult dogs and human beings under anaesthesia in the course of

operations performed for a variety of purposes. All tissues were placed in ice-cold 0.9% sodium chloride for transfer to the laboratory. Tissue slices, 0.3 mm. thick, were cut with a Stadie-Riggs microtome and a portion was taken for analysis of glycogen content. Tissue slices (about 200 mg.) were weighed on a Roller-Smith torsion balance and placed in Warburg vessels in a medium containing/ml.: 40 μ g.atoms of K^+ , 80 μ g.atoms of Na^+ , 10 μ g.atoms of Mg^{2+} , 100 μ g.atoms of Cl^- and 40 μ moles of phosphate, with an initial pH of 6.9. [^{14}C]Glucose or [^{14}C]glucose was placed in the side arm of the Warburg vessel and was tipped in at zero time, after the vessels had been gassed with oxygen or nitrogen for 7 min. The final concentration of the glucose in the incubation medium was 11.1 mM. The slices were incubated for 1 hr. unless otherwise stated at 37° either in 3.0 ml. of medium in a Warburg flask of about 15 ml. capacity or in 1.5 ml. of incubation medium in a Warburg flask of about 7 ml. capacity.

After the incubation period the tissue slices were removed, blotted dry on filter paper, weighed, and homogenized in chloroform-methanol (1:1, v/v) for extraction of lipids by the method of Folch, Lees & Sloane-Stanley (1957). The total lipid extract was evaporated to dryness and weighed, and a portion was transferred to a stainless-steel planchet for ^{14}C analysis. Another portion of the tissue slice after incubation was analysed for glycogen content by the method of Walaas & Walaas (1950).

Portions of the incubation medium were analysed for glucose (Nelson, 1944) and lactate (Barker & Summerson, 1941). To other portions of the incubation medium carrier glucose was added and glucose phenylsazones were prepared and purified for ^{14}C analyses (Vilee, 1953). The respiratory carbon dioxide was trapped in alkali placed in the centre of the Warburg flask (0.2 ml. of 5% sodium hydroxide, carbon dioxide-free). After the incubation this solution was washed into centrifuge tubes containing water, and the carbon dioxide was precipitated as barium carbonate by the addition of barium chloride at pH 10.5. The barium carbonate was washed with water and ethanol and quantitatively transferred to stainless-steel planchets for ^{14}C analysis. ^{14}C analyses were made with a window-less proportional-flow counter and were corrected for self-absorption. Each sample was counted long enough to reduce the probable error of counting to less than 3%.

RESULTS

The results of experiments with slices from human foetal tissues are summarized in Table 1. The tissues were from foetuses ranging in weight from 35 to 1385 g. and in crown-rump length from 6.5 to 25.5 cm. There was no significant trend in the relative rates of metabolism of glucose by these two pathways with age in the tissues tested, and all the observations for a given tissue were averaged. From the ratios of conversion of glucose carbon to carbon dioxide (column 4) it would appear that the activity of the hexose monophosphate pathway is very small or absent in cerebral cortex but present in medullary tissue from the brain. Other tissues have various degrees of phosphogluconate activity as judged by their

Table 1. *Metabolism of [1-¹⁴C]- and [6-¹⁴C]-glucose to carbon dioxide and to lipid by human foetal tissues incubated under aerobic conditions*

The values are mean $\pm$ S.E. expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Tissue	No. of expts.	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
		C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
Liver	17	0.61 $\pm$ 0.066	0.15 $\pm$ 0.022	0.26 $\pm$ 0.042	0.14 $\pm$ 0.033	0.21 $\pm$ 0.043	1.50 $\pm$ 0.25
Lung	5	0.087 $\pm$ 0.0071	0.029 $\pm$ 0.0033	0.43 $\pm$ 0.047	0.037 $\pm$ 0.0046	0.14 $\pm$ 0.031	3.9 $\pm$ 0.52
Heart	8	0.18 $\pm$ 0.024	0.11 $\pm$ 0.017	0.59 $\pm$ 0.067	0.020 $\pm$ 0.0030	0.020 $\pm$ 0.0021	1.00 $\pm$ 0.13
Brain, cerebral cortex	8	0.48 $\pm$ 0.051	0.44 $\pm$ 0.049	0.92 $\pm$ 0.088	0.088 $\pm$ 0.0092	0.089 $\pm$ 0.0074	0.79 $\pm$ 0.081
Brain, medulla	7	0.22 $\pm$ 0.031	0.096 $\pm$ 0.011	0.44 $\pm$ 0.08	0.11 $\pm$ 0.014	0.099 $\pm$ 0.013	0.91 $\pm$ 0.088
Kidney	8	0.26 $\pm$ 0.030	0.044 $\pm$ 0.008	0.17 $\pm$ 0.014	0.068 $\pm$ 0.010	0.10 $\pm$ 0.014	1.48 $\pm$ 0.061
Diaphragm	4	0.11 $\pm$ 0.016	0.036 $\pm$ 0.006	0.33 $\pm$ 0.048	0.017 $\pm$ 0.0040	0.018 $\pm$ 0.0031	1.07 $\pm$ 0.14
Adrenal	9	1.00 $\pm$ 0.12	0.055 $\pm$ 0.010	0.055 $\pm$ 0.006	0.11 $\pm$ 0.011	0.14 $\pm$ 0.015	1.36 $\pm$ 0.18
Adrenal tumour	2	0.55 $\pm$ 0.061	0.048 $\pm$ 0.008	0.087 $\pm$ 0.02	0.14 $\pm$ 0.031	0.16 $\pm$ 0.026	1.12 $\pm$ 0.12

characteristic ratios of glucose C-6 to glucose C-1 metabolized to carbon dioxide. The lowest ratio of C-6/C-1 conversion into carbon dioxide was found in the foetal adrenal gland. A similarly low ratio was found in an adrenal tumour from a 6-year-old boy. Other metabolic properties of the foetal adrenal and of this tumour will be presented elsewhere (Villee, Engel, Loring & Villee, 1961). In certain of these foetal tissues the absolute rate of conversion of glucose C-6 into lipid exceeds the rate of its conversion into carbon dioxide. Foetal liver, lung, kidney and adrenal have a very high rate of lipogenesis (Villee, Hagerman, Holmberg, Lind & Villee, 1958). At the earliest age tested, about 10–12 weeks gestational age, each of these tissues has established a characteristic pattern of carbohydrate metabolism which yields reproducible ratios for the conversion of C-6/C-1 into carbon dioxide and of C-6/C-1 into lipid. The ratios do not change as the foetus develops to term.

The effects of anoxia on the relative rates of metabolism of C-1 and C-6 of glucose to carbon dioxide and to lipid were measured by incubating tissue slices in vessels that were flushed with nitrogen instead of oxygen. The fact that there was no pressure change during the period of incubation was taken as evidence of complete anaerobiosis, i.e. that there was no oxygen in the vessel to be utilized. With each tissue, the ratio of glucose C-6 to C-1 metabolized to carbon dioxide was lower in nitrogen than in oxygen (Table 2), indicating that under anaerobic conditions the fraction of glucose

metabolized via the hexose monophosphate pathway is larger than the fraction metabolized by that pathway aerobically. Under anaerobic conditions the hexose monophosphate pathway would be limited by the concentration of oxidized TPN (required as cofactor for glucose 6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase) and by the mechanisms available for reoxidizing reduced TPN. Even under aerobic conditions tissues contain relatively little oxidized TPN; most of the TPN is present in the reduced state (Glock & McLean, 1955), thus the second factor is probably more important than the first. Although the hexose monophosphate pathway has been termed the 'direct oxidative pathway' of glucose metabolism, it is depressed by anaerobiosis less than are the enzymes of the Krebs cycle, which are involved in converting C-6 of glucose into carbon dioxide. The synthesis of lipid from both C-1 and C-6 of glucose is depressed by anaerobiosis but to a lesser extent than is the conversion of glucose carbon into carbon dioxide. When liver slices were incubated with air as the gas phase instead of oxygen, the metabolism of both C-1 and C-6 to CO₂ and to lipid was lowered.

The effects of ascorbic acid on glucose metabolism by foetal tissues are summarized in Table 3. The addition of ascorbic acid (30 μ moles/vessel) had no effect on the metabolism of glucose C-1 or C-6 to lipid or on the rate of metabolism of glucose C-6 to carbon dioxide. It did, however, have a marked effect on the rate of conversion of glucose C-1 into

carbon dioxide. The increases ranged from 40% in liver to fivefold in lung, and was evident in all tissues tested. It seemed possible that the ascorbic acid might be acting by oxidizing TPNH₂ and that the increases observed were actually the results of an increased concentration of TPN. The direct addition of 2 μ moles of TPN/vessel produced a slight increase in the conversion of glucose C-1 into CO₂, but the increase was not as great as that ob-

served with ascorbic acid (Table 4). In contrast, the addition of 2 μ moles of DPN decreased the conversion of both glucose C-1 and C-6 into carbon dioxide and into lipid. Since the pyridine nucleotides added *in vitro* might have difficulty in entering the cell this small effect with added TPN may not rule out the possibility that ascorbic acid re-oxidizes TPNH₂ and makes oxidized TPN available for metabolism of glucose 6-phosphate via

Table 2. *Metabolism of [1-¹⁴C]- and [6-¹⁴C]-glucose to carbon dioxide and to lipid by human foetal tissues incubated under anaerobic conditions*

The values are mean $\pm$ s.e. expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Tissue	No. of expts.	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
		C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
Liver	9	0.051	0.0096	0.19	0.022	0.024	1.07
		± 0.009	± 0.0023	± 0.031	± 0.0060	± 0.0065	± 0.16
Heart	3	0.062	0.0069	0.11	0.021	0.022	1.0
		± 0.033	± 0.0031	± 0.044	± 0.0024	± 0.0045	± 0.17
Brain, cerebral cortex	3	0.048	0.035	0.73	0.024	0.012	0.5
		± 0.0088	± 0.0074	± 0.081	± 0.0018	± 0.0011	± 0.03

Table 3. *Effects of the addition of 10 mM-ascorbic acid on the metabolism of glucose to carbon dioxide and lipid by human foetal tissues*

The values are mean $\pm$ s.e. of four experiments and are expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Tissue	Ascorbic acid	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
		C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
Liver	-	0.87	0.18	0.20	0.072	0.17	2.34
	+	± 0.091	± 0.022	± 0.045	± 0.015	± 0.033	± 0.46
Lung	-	1.40	0.24	0.17	0.082	0.16	1.99
	+	± 0.11	± 0.029	± 0.037	± 0.018	± 0.030	± 0.26
Brain, medulla	-	0.067	0.029	0.43	0.035	0.085	2.43
	+	± 0.0071	± 0.0033	± 0.047	± 0.0044	± 0.0092	± 0.48
Kidney	-	0.36	0.016	0.045	0.039	0.080	2.05
	+	± 0.039	± 0.0023	± 0.020	± 0.0047	± 0.010	± 0.41
Liver	-	0.33	0.11	0.33	0.092	0.098	1.06
	+	± 0.045	± 0.014	± 0.052	± 0.013	± 0.013	± 0.09
Lung	-	1.01	0.078	0.07	0.082	0.097	1.19
	+	± 0.11	± 0.0097	± 0.028	± 0.012	± 0.012	± 0.11
Brain, medulla	-	0.22	0.040	0.18	0.063	0.090	1.43
	+	± 0.028	± 0.0082	± 0.016	± 0.009	± 0.011	± 0.13
Kidney	-	0.56	0.030	0.054	0.074	0.084	1.13
	+	± 0.074	± 0.0066	± 0.022	± 0.009	± 0.011	± 0.11

Table 4. *Effects of the addition of cofactors on the metabolism of glucose to carbon dioxide and lipid by human foetal liver*

The values are mean $\pm$ s.e. of four experiments and are expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Cofactor added	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
	C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
None	0.56	0.10	0.19	0.11	0.13	1.12
	± 0.072	± 0.016	± 0.039	± 0.024	± 0.025	± 0.13
TPN, 2 μ moles	0.64	0.11	0.17	0.11	0.13	1.14
	± 0.075	± 0.016	± 0.038	± 0.023	± 0.025	± 0.13
DPN, 2 μ moles	0.48	0.059	0.12	0.077	0.093	1.20
	± 0.055	± 0.008	± 0.022	± 0.014	± 0.017	± 0.15

glucose 6-phosphate dehydrogenase. When liver homogenates are used instead of liver slices, the addition of TPN does increase the rate of the hexose monophosphate pathway and the addition of DPN increases the rate of glycolysis (Siperstein, 1958).

The effects of the addition of unlabelled potassium pyruvate (10 μ moles/ml.) and of D-2-deoxyglucose (6.1 μ moles/ml.) to the system are summarized in Table 5. The pyruvate added to the incubation medium would dilute the intracellular pool of labelled pyruvate derived largely from hexose and then decrease the rate of conversion of labelled glucose into acetyl-coenzyme A and thence into fatty acids and, via the Krebs cycle, into carbon dioxide. The extent to which this occurs will depend on the rate of entrance of pyruvate into the cell and the rate at which it is equilibrated with the endogenously formed pyruvate. The added pyruvate should not depress the metabolism of [1-¹⁴C]-glucose to carbon dioxide via the hexose monophosphate pathway but might increase the rate of

this reaction if the added pyruvate, by product inhibition, decreased one or more of the steps in glycolysis.

D-2-Deoxyglucose is phosphorylated to D-2-deoxyglucose 6-phosphate and this inhibits the enzyme which converts glucose 6-phosphate into fructose 6-phosphate (Wick, Drury, Nakader & Wolfe, 1957). It apparently does not inhibit glucose 6-phosphate dehydrogenase, for the conversion of [1-¹⁴C]glucose into carbon dioxide was not depressed but was increased. This may be attributed to the partial inhibition of glycolysis. The conversion of both C-1 and C-6 of glucose into lipid was greatly depressed by D-2-deoxyglucose as one would expect. The net effect of added pyruvate and D-2-deoxyglucose was to increase the fraction of glucose metabolized by the hexose monophosphate pathway and to decrease the fraction metabolized by glycolysis.

Table 6 summarizes the results of observations on tissues from several species of animals. These emphasize the considerable differences that may

Table 5. *Effects of addition of pyruvate and deoxyglucose on glucose metabolism by human foetal liver*

The values are mean $\pm$ S.E. expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Incubation medium	No. of expts.	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
		C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
Glucose (11.1 mm)	9	0.68 ± 0.070	0.17 ± 0.018	0.25 ± 0.030	0.17 ± 0.016	0.26 ± 0.024	1.50 ± 0.16
Glucose + pyruvate (10 mm)	8	0.64 ± 0.066	0.12 ± 0.014	0.19 ± 0.028	0.094 ± 0.0090	0.22 ± 0.021	2.34 ± 0.21
Glucose + pyruvate + deoxyglucose (6.1 mm)	4	0.93 ± 0.082	0.14 ± 0.014	0.15 ± 0.023	0.063 ± 0.0082	0.18 ± 0.019	2.82 ± 0.20

Table 6. *Metabolism of [1-¹⁴C]- and [6-¹⁴C]-glucose to carbon dioxide and lipid by adult and foetal tissues*

The values are mean $\pm$ S.E. expressed as μ moles of glucose converted into product after 1 hr./g. fresh wt. of tissue.

Tissue	No. of expts.	Glucose carbon $\rightarrow$ CO ₂			Glucose carbon $\rightarrow$ lipid		
		C-1	C-6	C-6/C-1	C-1	C-6	C-6/C-1
Liver, rabbit, 25-day foetus	2	0.55	0.10	0.18	0.098	0.102	1.04
Liver, rabbit, newborn	2	0.89	0.15	0.17	—	—	—
Thyroid, dog, adult	11	0.50 ± 0.040	0.22 ± 0.024	0.43 ± 0.048	0.049 ± 0.009	0.058 ± 0.010	1.18 ± 0.28
Thyroid, human, foetal	4	0.24 ± 0.031	0.059 ± 0.0081	0.246 ± 0.028	—	—	—
Thymus, human, foetal	4	0.16 ± 0.027	0.017 ± 0.0024	0.103 ± 0.015	—	—	—
Ovary, rat	5	0.54 ± 0.056	0.46 ± 0.051	0.85 ± 0.12	—	—	—
Adrenal, dog, adult	12	0.26 ± 0.033	0.078 ± 0.0091	0.30 ± 0.036	0.019 ± 0.0021	0.029 ± 0.0032	1.53 ± 0.29
Liver, rat, control	12	1.13 ± 0.090	0.091 ± 0.014	0.081 ± 0.012	0.028 ± 0.007	0.050 ± 0.009	1.78 ± 0.18
Liver, rat, pantothenic acid deficient	8	2.00 ± 0.25	0.21 ± 0.035	0.106 ± 0.014	0.044 ± 0.0060	0.140 ± 0.033	3.2 ± 0.23

exist in the relative importance of the two pathways in tissues from different species of animals. Similar differences were reported recently (Field, Pastan, Herring & Johnson, 1960) in ovary and testis. The ratio of glucose C-6/C-1 metabolized to carbon dioxide reported by Field *et al.*, 0.20 for human ovary and 0.43 for calf ovary, can be compared with the value of 0.85 for rat ovary obtained in these studies. The values were 0.019, 0.17 and 0.45 for the testes of calf, man and rabbit respectively. Not only were the relative rates of conversion of glucose C-1 and C-6 into carbon dioxide different in the tissues of different species, the absolute rate of metabolism per gram of tissue was tenfold greater in calf ovary than in human ovary and fivefold greater in calf testis than in human testis. Although part of the difference might be ascribed to the ages of the donors of the human tissues (the ovaries were those of a woman aged 41 and the testes were those of a man aged 75), it is clear that marked interspecific differences do exist. These marked interspecific differences in the relative rates of glucose metabolized via glycolysis and the hexose monophosphate pathway in a particular kind of tissue suggest that it would be unwise to make extensive generalizations about the possible physiological roles of the phosphogluconate pathway in the intermediary metabolism of a given tissue.

Livers from a series of adult rats maintained on pantothenic acid-deficient diets since their weaning (a total of 12 weeks) were compared with those of litter mates kept on a control diet (Table 6). Although the pantothenic acid-deficient animals were quite ill their livers metabolized glucose at nearly twice the rate of control livers. The conversion of C-1 and C-6 of glucose into both carbon dioxide and lipid was increased but there was no significant difference in the relative rates of metabolism of C-1 and C-6 of glucose. We had supposed that, since both fatty acid metabolism and the tricarboxylic acid cycle require coenzyme A whereas the hexose monophosphate pathway does not, the pantothenic acid-deficient rat might metabolize more of its glucose via the latter pathway. This expectation was not realized and it would appear that rats with all the signs of severe pantothenic acid deficiency still have enough coenzyme A in their tissues for these enzymic processes.

DISCUSSION

In all of the tissues examined there was no demonstrable change in the relative rates of metabolism via these two alternative pathways of carbohydrate metabolism over the period of foetal development measured, roughly 8-25 weeks' gestational age. Each tissue has developed, some-

time before the eighth week, a pattern of carbohydrate metabolism which is characteristic for it. There is no evidence as to when the hexose monophosphate pathway may first appear in these foetal tissues. In sea-urchin eggs undergoing cleavage and gastrulation, the hexose monophosphate pathway appears to be the major one of glucose oxidation, whereas some 24 hr. later glycolysis and the Krebs cycle have become predominant. This was found by Krahle (1956) and our own experiments with sea-urchin embryos (Villem, Loring & Wellington, 1957) have confirmed and extended his observations. It is possible that an analogous situation exists in the human egg during the early blastocyst stage.

The observation that these pathways of carbohydrate metabolism are relatively unchanging throughout foetal life and resemble the adult condition is in some contrast with a number of the reactions of amino acid metabolism and of glucuronic acid metabolism. As Kretschmer (1959) has shown, there are marked changes in the rates of metabolism of tyrosine, tryptophan and certain other amino acids in the late foetal and early newborn periods. Lathe (1959) and Brown, Zuelzer & Bollet (1958) have demonstrated that the enzymes involved in the synthesis of uridine diphosphoglucuronic acid undergo development late in foetal life.

The effects on carbohydrate metabolism observed when pyruvate and 2-deoxyglucose were added to the medium were to be expected on the basis of our present knowledge of intermediary metabolism. They lend confidence to this method of measuring and interpreting alternative pathways of carbohydrate metabolism in tissue slices.

SUMMARY

1. Tissue slices were incubated with [1-¹⁴C]-glucose and [6-¹⁴C]glucose to evaluate the relative rates of glycolysis and the hexose monophosphate oxidative pathway in a variety of foetal and adult tissues.

2. The effects of anaerobiosis and of the addition of unlabelled pyruvate, D-2-deoxyglucose, ascorbate, and di- and tri-phosphopyridine nucleotide were tested.

3. There was no significant change in the relative rates of metabolism via these two alternative pathways of carbohydrate metabolism in any of the tissues over the period of foetal development from 8 to 25 weeks' gestational age.

4. The activity of the hexose monophosphate pathway in different tissues ranged from the foetal cerebral cortex, in which it is essentially zero, to the adrenal cortex, in which it accounts for most of the glucose metabolized. Comparative studies,

however, revealed wide differences in the relative activities of the two pathways in comparable tissues of different species.

We wish to thank Professor John Lind, Department of Pediatrics, Karolinska Hospital, and Professor Carl Schmitterlow, Department of Pharmacology, Kungl. Veterinärhögskolan, for their generosity in providing the foetal specimens and the laboratory facilities for the experiments in Stockholm. This work was aided by grants from the Charles and Marjorie King Fund, from the Association for the Aid of Crippled Children, and Grant C-2400 from the National Institutes of Health.

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Biochem. J. (1961) **81**, 494

A Study of the Citric Acid Cycle in Certain Tumour Tissues

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(Received 13 February 1961)

At present there are two schools of thought about the respiration of cancer cells. Whereas both agree that tumour tissues exhibit high anaerobic and aerobic glycolysis with an excessive production of lactic acid (Cori & Cori, 1925; Warburg, 1926, 1956), they differ as to the extent of the operation of the citric acid cycle in these tissues (Weinhouse, 1955; Weinhouse, Warburg & Burk, 1956; Warburg, 1956). In these studies the activity of certain segments of the cycle has been determined by oxygen consumption and carbon dioxide production procedures (Warburg, 1926; Busch & Potter, 1952; Busch & Baltrush, 1954; Busch, 1955; Weinhouse, 1955; Warburg, 1956; Ibsen, Coe & McKee, 1958), by measuring the glycolytic and oxidative enzymes (Sibley & Lehninger, 1949; LePage, 1950; Meister, 1950; Potter, 1951; Weinhouse, 1951; Wenner, Spirtes & Weinhouse, 1952; Beck, 1958), and the cofactors of the electron-transport system (Dubois & Potter, 1942; Wenner & Weinhouse, 1953; Greenstein, Werne, Eschenbrenner & Leuthardt,

1944), or by following the utilization of the various intermediates of the Embden-Meyerhof scheme and the tricarboxylic acid cycle (Pardee & Potter, 1949; Elliott & Kalnitsky, 1950; Olson, 1951; Wenner & Weinhouse, 1952; Heidelberger, 1953; Busch & Baltrush, 1954). However, none of these investigators has utilized the simultaneous determination of the amounts and radioactivities of all the members of the citric acid cycle *in vitro* in the presence and absence of certain added labelled substrates and blocking agents. Such a procedure could reflect the activity of the cycle if an excess of substrate is present despite the fact that the individual cycle acids allegedly serve a 'catalytic' function. There are examples of variations in the amounts of the individual cycle acids under differing experimental conditions, for example, after the addition of specific blocking agents. The present study was designed to employ such a direct approach. The tumour and normal tissue preparations were incubated with [^{14}C]acetate,