Glucuronide Synthesis in Foetal Liver and Other Tissues

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Glucuronides are synthesized in adult liver by enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPglucuronic acid) to an acceptor (Dutton & Storey, 1954; Storey & Dutton, 1955), which may be from a wide range of substances (Isselbacher & Axelrod, 1955; Dutton, 1956; Axelrod, Inscoe & Tomkins, 1957); other adult tissues, notably kidney and gastrointestinal tract, also form glucuronides and the mechanism there is possibly the same (Dutton, 1958*a*).

In liver slices from young mice and foetal rabbits synthesis of *o*-aminophenyl glucuronide appears to be low or absent (Karunairatnam, Kerr & Levvy, 1949; Hartiala & Pulkkinen, 1955). Investigation of this deficiency seemed important, especially since identification of the 'direct' bilirubin in the van den Bergh reaction as bilirubin diglucuronide (Schmid, 1956; Talafant, 1956; Billing, Cole & Lathe, 1957), and the implication of UDPglucuronic acid and the microsomal enzyme [variously termed glucuronyl or glucuronosyl transferase (Levvy, 1956; Isselbacher, 1956) or uridine diphosphate-transglucuronylase (Dixon & Webb, 1958)] in the formation of this conjugate (Lathe & Walker, 1957; Schmid, Hammaker & Axelrod, 1957; Grodsky & Carbone, 1957).

It was necessary to show whether foetal liver had lower levels than adult liver of enzyme, nucleotide or both, or whether these values were at adult level and apparent deficiency arose from inhibition of the enzyme or hydrolysis of the conjugate. Synthesis in liver slices reflects the formation of both conjugate and UDPglucuronic acid; in liver homogenates fortified with the nucleotide only the final glucuronyl transference occurs. It therefore seemed of value to follow synthesis of *o*-aminophenyl glucuronide in both tissue preparations through foetal into adult life, and to supplement by more direct measurement of levels of liver UDPglucuronic acid. Other foetal tissues would also need to be examined for glucuronide synthesis.

The present work indicates that the relative deficiency in foetal liver is due to low levels of both enzyme and nucleotide, and illustrates their gradual increase up to and after birth. Conjugation below adult level is also shown in foetal kidney, but apparently not in foetal stomach or intestine, under the conditions employed. Evidence was obtained from guinea-pig, mouse and human tissues. Preliminary accounts of this work have already appeared (Dutton & Greig, 1957; Dutton, 1958*a*, *b*).

EXPERIMENTAL

Materials

Acceptor substrates. These were resublimed *o*-aminophenol (OAP), recrystallized (−)-menthol and recrystallized *p*-aminobenzoic acid.

Uridine diphosphate glucuronic acid. Boiled liver extract or barium–ethanol fractionated nucleotide was used with, for confirmation, either chromatographically pure preparations (Storey & Dutton, 1955) or 'uridine diphosphoglucuronic acid' (98–100% quoted purity, ammonium salt) from Sigma Chemical Co., St Louis, Mo., U.S.A. In all cases the amount added was checked to ensure that it was not a limiting factor for conjugation during the incubation period of 30 min.

Other additions. β-Glucuronidase was prepared by the method of Kerr, Graham & Levvy (1948), with incubation for conjugate hydrolysis at pH 5·1 for 3 hr. at 37°. Saccharo-1:4-lactone was prepared in solution according to Levvy (1952). D-Glucurone and potassium glucuronate dihydrate were gifts from the Corn Products Refining Co., New York, U.S.A. Glucose 1-phosphoric acid (dipotassium salt) was from British Drug Houses Ltd.

Animals

Mice. Mice were from a mixed colony and of either sex; litters were segregated. Adults were killed by cervical dislocation, young mice by decapitation. Required organs were rapidly removed to crushed ice, those from very young litter mates being pooled if necessary.

Guinea pigs. These were from a mixed colony. Adults and young were killed by a sharp blow on the head, and organs or foetuses placed in crushed ice, required foetal tissues being removed at once. Foetal ages were estimated from the weight and length of the excised litter, the data of Draper (1920), Ibsen (1928) and Bell (1941) being used. In the group '9 weeks–birth' are included those litters where maternal behaviour and their own physical development indicated imminent parturition.

Human. The human foetuses, each between 3 and 4 months old, were normal and obtained therapeutically; immediately on excision the foetus was placed in crushed ice and the placenta, liver and kidneys were removed and cooled before being rapidly transported to the Laboratory. Allowance for this additional 15–20 min. in ice was made by suitable controls.

Methods

Synthesis with sliced tissue. Tissues were sliced by the method of Cohen (1957), except gastrointestinal tract, which was cut into suitable strips, care being taken to remove loose fat or muscle and to open out the gut. For synthesis of *o*-aminophenyl glucuronide the method of Storey (1950) was used, the slices being shaken for 1 hr. at 37° in sulphate-free Ringer bicarbonate soln. (Krebs & Henseleit, 1932), with $CO_2 + O_2$ (1:19) as gas phase; 0·23 mM-OAP and mM-ascorbic acid were present. Triplicate determinations were made where possible. Tissues were dried at 110° for 2 hr. before weighing. Glucose, glucurone and potassium glucuronate were added in some experiments, to a final concn. of 20 mM. Where menthyl glucuronide was to be measured the procedure, after removal of slices, was as for the corresponding homogenate preparation (Dutton & Storey, 1954).

Synthesis with homogenized tissue. The methods were described previously, for OAP and (−)-menthol (Dutton & Storey, 1954; Storey & Dutton, 1955) and for *p*-aminobenzoic acid (Dutton, 1956). With OAP as substrate, each flask contained 33 mM-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·5, 10 mM-$MgCl_2$, UDPglucuronic acid preparation (see under Materials; when pure, a concn. of 0·1 mM was present), 0·14 mM-OAP, 0·76 mM-ascorbic acid and 0·5 ml. of 10% (w/v) tissue homogenate, in a total volume of 3 ml. Incubation was for 30 min. in air at 37°.

Levels of uridine diphosphate glucuronic acid. The method used for estimation gave a reasonably accurate comparison of the amounts of UDPglucuronic acid present in foetal and adult tissue. Immediately on death the liver was excised and cooled for 1 min. in ice; 1 g. was rapidly weighed, and dropped into 4 ml. of boiling water and boiled for 2 min.; the mixture was cooled, the tissue was disrupted by the homogenizer and the suspension centrifuged at 2000 *g* for 5 min. Flasks had been set up which contained homogenate-reaction medium complete but for UDPglucuronic acid, and 0·5 ml. of the supernatant was then added to them as a source of the nucleotide. Corresponding flasks were run without this addition, and the increase in synthesis of *o*-aminophenyl glucuronide due to the added extract could be determined. With very small livers, less than 1 g. was used and the volume of water was reduced accordingly. A check was always made to ensure that homogenate concentration was not a limiting factor.

Controls. As well as those mentioned above, controls were run in each experiment as a check on the continued suitability of technique and media; for example, foetal preparations were always accompanied by those from an adult. When the choice arose, foetal tissues were exposed least to the chance of accidental loss of activity; and, if necessary, adult tissue was manipulated in amounts small enough to correspond with the foetal samples.

RESULTS

Synthesis of o-aminophenyl glucuronide in young mouse liver

Slices. For sliced tissue, the results of Karunairatnam *et al.* (1949) were confirmed. Formation of *o*-aminophenyl glucuronide by livers of 1- to 5-day-old mice was negligible when compared with that in corresponding adult preparations; about 50 % of the adult level was reached in 16–20 days, and adult values were attained by 4 weeks of age (Fig. 1).

Homogenates. Although negligible conjugation occurred also in UDPglucuronic acid-fortified homogenates of liver from day-old mice, the subsequent rise in activity seemed more rapid than with slices, often reaching 50 % of the adult value by 7 days. Considerable variation between, but not within, litters was noticed; the degree of glucuronide formation appeared to correspond with body weight rather than the age, and since smaller litters (five to six) were heavier for their age than larger ones (12–14) the typical familial difference shown in Fig. 1 is explicable.

Levels of UDPglucuronic acid. Tentative experiments suggested that liver UDPglucuronic acid was at a lower level in young mice than in adults, but the nucleotide is very labile in tissues and results obtained with such small and easily damaged livers required further confirmation.

Synthesis of o-aminophenyl glucuronide in foetal and young guinea-pig liver

Because of its more convenient foetal size, the guinea pig was used in subsequent studies. Since the young are born in an advanced state, investigation was begun on the early foetus.

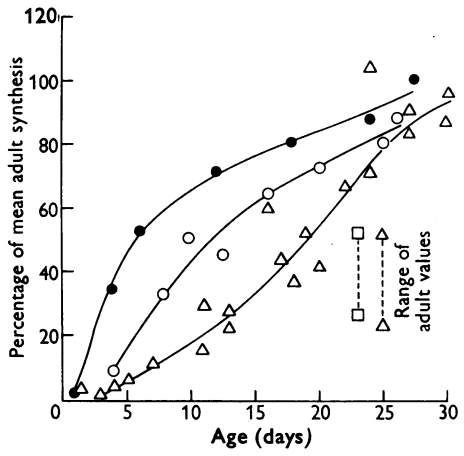

Fig. 1. Glucuronide synthesis by mouse-liver preparations at various ages, expressed as percentage weight of *o*-aminophenyl glucuronide formed, the mean adult value from 25 animals (for slices) or from 30 animals (for homogenates) being taken as 100. Sliced tissue: △, shaken for 1 hr. at 37°, gas phase $CO_2 + O_2$ (1:19), with 0·23 mM-*o*-aminophenol and mM-ascorbic acid. Homogenates: ●, from a small litter; ○, from a large litter; □, from adults. Homogenate (0·5 ml.) was present in a total volume of 3 ml. containing 33 mM-tris buffer, pH 7·5, 10 mM-$MgCl_2$, UDPglucuronic acid (see text), 0·14 mM-*o*-aminophenol and 0·76 mM-ascorbic acid, incubated in air at 37° for 30 min. All values are means of duplicate determinations.

Slices. Formation of *o*-aminophenyl glucuronide could not be shown in liver slices from foetuses estimated at 5–6 weeks' gestation, and the amount of conjugation in later specimens increased slowly. Glucose, glucose 1-phosphate, glucurone or glucuronate (all 20 mM) did not increase the extent of synthesis. Just before birth foetal slices had, on the dry-weight basis employed, about 10% of the adult conjugation. After birth the increase was more rapid, reaching 30–40% at 4 days, as shown in Table 1. It should be noted that in this and subsequent tables the division of foetal ages into groups is one of convenience; since increase in extent of conjugation is progressive with age, the range quoted in each foetal group reflects this and not necessarily experimental error or random individual variation.

Homogenates. In UDPglucuronic acid-fortified homogenates from early foetal guinea-pig liver, formation of *o*-aminophenyl glucuronide was very low, suggesting a deficiency of UDP-transglucuronylase activity, compared with the adult. Just before birth the level had risen to about 40% of the mean adult figure, and after 1–2 days to about 60%, adult values being reached by 5 days of age (Table 2).

A few experiments with microsomal preparations (see Dutton, 1956) confirmed that foetal liver conjugated less *o*-aminophenol than did the adult tissue, but the lengthy procedure was unsuitable for this work. In agreement with the observations made on young mice, fortified homogenates of foetal guinea-pig liver showed a level of glucuronide formation nearer to that of the adult than did

Table 1. *Glucuronide synthesis by guinea-pig tissue slices at various ages*

Incubation was for 1 hr. at 37° in sulphate-free Ringer bicarbonate soln. containing 0·23 mM-*o*-aminophenol (OAP) and mM-ascorbic acid; 20 mM-glucose was present where indicated; gas phase, $CO_2 + O_2$ (1:19). Individual results are the means of duplicate or triplicate determinations. In this and subsequent tables, where results are not individually reported, means are given with ranges in parentheses and the number of animals in superior figures; – means not determined.

		OAP conjugated (μg./50 mg. dry wt. of tissue/hr.)		
Age	Glucose	Liver	Kidney	Stomach
Foetus, 5–7 weeks	–	0, 0, 0·5	0·6, 0·8	2·2, 1·9
	+	0, 0·4, 0·5	0·8, 1·2, 1·3	6·5, 4·7, 3·1
Foetus, 7–8 weeks	–	0·6, 1·2, 1·5	1·0, 1·5	—
	+	0·6, 1·8, 2·0	1·8, 3·2	10·6, 8·5
Foetus, 8–9 weeks	–	1·5, 2·8, 5·0	2·6, 3·1	—
	+	1·8, 2·3	3·2, 3·4, 3·8	6·0, 5·5, 2·3, 1·7
Foetus 9 weeks–birth	–	1·6, 2·8, 3·0	3·3, 4·5	—
	+	1·5, 3·5, 3·8	4·1, 5·6, 6·7	—
Young, 1 day	–	4·0	—	—
	+	5·0	5·5	5·0
Young, 2 days	–	11·0, 14·0	—	—
	+	—	12·8	—
Young, 4 days	–	17·0, 24·0	—	—
	+	—	—	—
Adult	–	35·1 (26·5–42·0)[17]	19·3 (15·1–26·0)[4]	1·2 (0·8–1·5)[8]
	+	26·0 (22·0–30·5)[3]	33·2 (31·5–35·6)[7]	3·5 (1·5–9·2)[7]

Table 2. *Glucuronide synthesis by guinea-pig liver homogenates at various ages*

Each flask contained 3 mM-tris buffer, pH 7·5, 10 mM-$MgCl_2$, UDPglucuronic acid preparation (see text), aglycone and 0·5 ml. of 10% (w/v) liver homogenate in a total of 3 ml. Aglycone for (*a*) was 0·14 mM-*o*-aminophenol (with 0·76 mM-ascorbic acid); for (*b*), 6·4 mM-(–)-menthol; for (*c*) 0·14 mM-*p*-aminobenzoic acid. Incubation was in air for 30 min. at 37°. Individual figures quoted are the means of duplicate determinations.

	Aglycone conjugated (μg./50 mg. wet wt. of liver/30 min.)		
Age	(*a*)	(*b*)	(*c*)
Foetus, 5–7 weeks	0·5, 0·6, 0·8, 1·2, 1·8	1·0	0
Foetus, 7–8 weeks	2·1 (1·6–2·5)[10]	—	—
Foetus, 8–9 weeks	3·1 (2·2–3·9)[6]	6·3	0·8
Foetus, 9 weeks–birth	4·2, 4·8, 5·3, 6·7	10·0	—
Young, 1 day	3·9, 7·2, 8·5	—	—
Young, 2 days	5·6, 9·5	—	—
Young, 5 days	12·6	—	—
Adult	12·2 (7·9–14·5)[30]	18·1, 16·2, 19·1	5·8, 8·1

slices of a corresponding age or, more strikingly, than did slices from the same foetal animal.

Levels of uridine diphosphate glucuronic acid. The above phenomenon suggested a low level, or poor utilization, of UDPglucuronic acid in intact foetal liver. The amount of this nucleotide in foetal, as compared with that in identically prepared adult, boiled liver extracts was measured by the increase in conjugation each afforded when added to an unfortified homogenate of adult guinea-pig liver (Table 3). In spite of the inexactitude of this method the differences were outstanding. It was obvious that guinea-pig liver did not gain its high content of UDPglucuronic acid until birth, and it was thus reasonable to suppose that this lack superimposed on low activity of UDP-transglucuronylase accounted for the poorer synthesis in slices.

Weight of foetal guinea-pig liver

It was noticed that livers of guinea-pig foetuses of some 8 weeks onwards became enlarged, pale and fatty. According to Imrie & Graham (1920), such livers contain some 15% by weight of fatty acids, these disappearing a few days after birth. The fat would be included in both the wet- and dry-weight methods used in this work, and would interfere significantly during the last stages of gestation.

Total body and total liver wet weights were determined, together with the final weights of 1 g. of wet liver dried at 110° for 5 hr. Total wet and total dry liver weights were then separately expressed as a percentage of the body weight; for six adults of both sexes these values were in the range 3·3–5·3% and 0·9–1·2% respectively. From the data of Bogdanovitch & Man (1938) the mean figure for the former value was 3·1%, for thirty animals of both sexes, 1–35 months old. Six foetuses from separate litters at 9 weeks–full term, and one animal at a day old, were examined similarly. The wet-liver weight ranged from 5·0 to 7·4 (mean 5·7)% of body weight and the dry-liver weight from 1·9 to 2·5% (mean 2·1, one not determined). Two earlier foetuses (6–8 weeks), as expected, yielded results about the adult level, as did an animal 5 days old.

In the late foetus therefore both the ratios (wet-liver weight:body weight) and (dry-liver weight:body weight) are higher than in the adult, up to roughly two and three times respectively, this estimate being based on extreme examples of results so far obtained. This would have the effect of diminishing that discrepancy noted above between synthesis in slices and in homogenates of the same foetal liver. Five days after birth the livers were visibly similar to those of the adult; the extra fat, according to Imrie & Graham (1920), has disappeared and this is noticeable in the fallen liver (dry weight:body weight) ratios.

Further evidence for low levels of uridine diphosphate-transglucuronylase

Although low activity of UDP-transglucuronylase was noted in homogenates of foetal liver, this might have been due, not to a smaller amount of the enzyme itself, but to its inhibition or to destruction of the conjugate by the known high level of β-glucuronidase in young tissues (Karunairatnam *et al.* 1949). To test the first supposition, various amounts of homogenate of foetal liver were added to a similar preparation of adult liver (Table 4). The conjugation observed in such a mixture, where not increased additively, shows an insignificant fall,

Table 3. *Levels of uridine diphosphate glucuronic acid in guinea-pig liver at various ages*

Conditions were as described in Table 2 (*a*), except that UDPglucuronic acid preparation was replaced by 0·5 ml. of extract obtained by boiling 1 g. (wet wt.) of liver sample in 4 ml. of water (see text); adult guinea-pig liver was used as enzyme source. The resulting weight of conjugated OAP was subtracted from that measured in control flasks without any added source of UDPglucuronic acid. Individual results are the means of duplicate determinations.

Age	Increased OAP conjugation due to added extract (μg./50 mg. wet wt. of liver/30 min.)
Foetus, 5–7 weeks	0·1, 0·2
Foetus, 7–8 weeks	0·4, 0·5, 0·8
Foetus, 8–9 weeks	0·5, 0·7, 0·8, 0·8
Foetus, 9 weeks–birth	0·4, 0·9, 0·9, 1·2, 1·3
Young, 1 day	1·7, 1·8
Young, 2 days	2·4
Young, 5 days	5·9
Adult	5·5 (4·2–9·1)[20]

Table 4. *Effect of various additions on glucuronide synthesis by homogenates of foetal or adult guinea-pig liver*

Media and incubation were as described in Table 2, except where indicated. Boiled saccharate soln. was used as source of saccharo-1:4-lactone (see text). Figures are means of duplicate determinations. *A* and *C* were homogenates of livers of foetuses of 5–7 weeks and of 7–8 weeks respectively. *B* and *D* were homogenates of adult livers.

Liver homogenate present	OAP conjugated (μg./30 min.)
A, 0·5 ml.	0·8
B, 0·5 ml.	11·1
A, 1·0 ml.	1·3
B, 1·0 ml.	18·7
A, 0·5 ml. + *B*, 0·5 ml.	10·8
A, 1·0 ml. + *B*, 0·5 ml.	9·0
C, 0·5 ml.	2·5
D, 0·5 ml.	10·5
C, 0·5 ml. + *D*, 0·5 ml.	12·0
C, 0·5 ml. + boiled 0·15 mM-saccharate	2·2
C, 0·5 ml. + boiled 1·5 mM-saccharate	1·8
D, 0·5 ml. + boiled 0·15 mM-saccharate	10·0

Table 5. *Glucuronide synthesis in human foetal tissues*

Conditions were as described in Table 1 for sliced tissue, as in Table 2 (*a*) for homogenates; UDPglucuronic acid levels were estimated by a method similar to that described in Table 3. *A* is a human foetus of 3·5–4 months; *B*, of 3–3·5 months. Tissues from mouse *X* were treated exactly as those from *A* and *B* (see text); those from mouse *Y* were used immediately after death. Figures quoted for adult-human liver were communicated by Dr I. D. E. Storey. Values are means of duplicate determinations.

Tissue	OAP conjugated (μg./50 mg. dry wt. of slices/hr.)	OAP conjugated (μg./50 mg. wet wt. of homogenate/hr.)	UDPglucuronic acid (% of that in equal wt. of fresh adult-mouse liver)
Placenta from *A*	0	0	10
Kidney from *A*	0·6	—	—
Kidney from *B*	0·5*	—	—
Liver from *A*	0·3	0·1	12
Liver from *B*	0·2	0·2	10
Adult human liver	25·0, 29·5, 27·0	—	—
Liver from mouse *X*	24·1	5·0	62
Kidney from mouse *Y*	5·0, 10·2*	—	—
Liver from mouse *Y*	28·2	5·8	100

* Glucose present (20 mM).

and indicates that an inhibitor of UDP-transglucuronylase activity is unlikely in foetal liver. It also suggests that low synthesis is not due to rapid destruction of UDPglucuronic acid, as may happen in kidney and gut homogenates (Dutton & Greig, 1957). Saccharo-1:4-lactone, the specific inhibitor for β-glucuronidases (Marsh & Levvy, 1958), when added to homogenates of foetal liver did not increase their conjugating power. Thus hydrolysis of formed glucuronides is not responsible for their low yield in preparations of foetal liver. UDP-transglucuronylase itself would therefore seem to be at a low level in foetal liver.

Synthesis of other glucuronides in guinea-pig liver

Because of the rapid, sensitive and specific method available for this substrate, the conjugation of *o*-aminophenol only was followed in detail through foetal development. Evidence was obtained that the glucuronides of (−)-menthol and of *p*-aminobenzoic acid were formed to very small extent, if at all, in the early foetal guinea-pig liver, increasing later as with the previous substrate (Table 2). *p*-Aminobenzoyl glucuronide is an ester-type glucuronide, synthesized in liver in the same way as the ether-type glucuronide of *o*-aminophenol (Dutton, 1956).

Synthesis of o-aminophenyl glucuronide in other guinea-pig tissues

Foetal kidney. Since slices of guinea-pig kidney formed *o*-aminophenyl glucuronide at a rate, when glucose was present, comparable with that of liver, this organ was studied in the foetus (Table 1). Conjugation was low, and though increased with glucose to levels above those in corresponding foetal-liver slices, it remained below adult value until after birth. Glucuronide formation in crude homogenate of adult kidney is small (Dutton & Greig, 1957), so that its low level in foetal homogenates was not profitable to investigate under these conditions.

Placenta and uterus. The early foetal guinea-pig being unable to form glucuronides to any extent through its own liver and kidney, the maternal uterus and placenta were examined; neither gave evidence of significant formation of *o*-aminophenyl glucuronide at different foetal ages.

Other foetal tissues. Considerable synthesis of glucuronide occurs in slices of stomach and intestine of various adult animals, provided that glucose is present (Zini, 1952; Shirai & Ohkubo, 1954; Hartiala, 1955; Schachter, Kass & Lannon, 1958; Dutton, 1958*a*), though much variation in activity is encountered. By several methods it has been proved that this tissue forms the glucuronide of *o*-aminophenol in the guinea-pig foetus. On a dry-weight basis, the activity seems to be at or above adult level in foetal stomach from at least the sixth week, and to fall gradually (Table 1). Because of the use of entire stomach-wall strips instead of the active but not easily separable mucosa, this fall may merely reflect the increasing inclusion with growth of non-conjugating tissue. Evidence for the mechanism of glucuronide synthesis at extra-hepatic sites such as stomach and kidney will be presented more fully elsewhere. Stomach is the most active early foetal tissue yet examined, slices of lung, spleen or brain having negligible conjugating power under the conditions employed.

Synthesis of o-aminophenyl glucuronide in human placenta, foetal liver and foetal kidney

Results obtained with two normal human foetuses of 3–4 months and with human placenta of the same period are shown in Table 5, together with results observed in control preparations from

adult-mouse tissues, both fresh and subjected to the same delay as the human specimens. Adult human-liver slices form the glucuronide at roughly the level of those from adult-mouse liver (Dr I. D. E. Storey, personal communication). The loss of activity at 0° consequent on delay did not affect the mouse preparations to a great extent, and the low values recorded for the human examples must therefore have been due to their foetal state. This bears out the results obtained with mouse and guinea pig, by showing negligible formation of *o*-aminophenyl glucuronide in early foetal liver and kidney, or in placenta, traceable in the liver to low levels of UDP-transglucuronylase activity and of UDPglucuronic acid.

DISCUSSION

It follows from previous work (Dutton & Storey, 1954; Storey & Dutton, 1955) that in liver homogenates the step

$$\text{Acceptor} + \text{UDPglucuronic acid} \rightarrow \text{acceptor glucuronide} + \text{UDP}$$

only is being studied, whereas in slices the synthesis (see Strominger, Maxwell, Axelrod & Kalckar, 1957) of the nucleotide from glycogen or α-glucose 1-phosphate also takes place. Since excess of UDPglucuronic acid is supplied to homogenate preparations, their synthetic power may be largely a measure of UDP-transglucuronylase activity, whereas that of slices, being dependent on endogenously formed nucleotide, may reflect more accurately the position in the intact animal. Any striking difference between these synthetic performances is due possibly to lack of availability of UDPglucuronic acid for the transference reaction; actual levels of the nucleotide in liver would be deduced from the behaviour of the boiled extract.

In the early foetal guinea-pig liver it is clear that formation of the glucuronides studied is negligible and this arises from very low levels of both enzyme and nucleotide. This condition is seen also in the human foetus of 3–4 months, and it is probable that the lack of synthesis of *o*-aminophenyl glucuronide observed in early foetal rabbit-liver slices (Hartiala & Pulkkinen, 1955) is from the same cause. Though foetal-mouse liver has not been studied a low level of enzyme may be presumed present, for this is certainly the case with the organ from new-born animals. Glucuronide synthesis below adult level has been shown in guinea-pig and human foetal-kidney slices, and has been reported from similar preparations in the young mouse (Karunairatnam *et al.* 1949).

It would thus seem that negligible amounts of UDP-transglucuronylase and UDPglucuronic acid are typical of the first two-thirds of foetal life in the species studied, and this is associated with low formation of *o*-aminophenyl glucuronide in the kidney. There is considerable glucuronide synthesis in the stomach, and some in the gut, of early foetal guinea pig, but because of the shortcomings in its liver and kidney conjugatory function the foetus of this age is relatively poorly equipped, compared with the adult, for 'detoxication' through glucuronide formation. Since the placenta and uterus seem ineffective in this role, the early foetus must largely rely on 'detoxication' by the maternal liver, kidney and gastrointestinal tract.

This dependence may persist until birth in the mouse, but is to some extent ameliorated by full term in the guinea pig, whose greater development at birth possibly accounts for the species difference. Apparently UDP-transglucuronylase activity increases more rapidly in foetal guinea-pig liver than does UDPglucuronic acid, and this is reflected in the slow development of the glucuronide-synthesizing system in slices. In the full-term guinea pig the enlarged fatty foetal liver must be taken into account. From the maximum increases in liver wet and dry weight: body weight ratios, UDP-transglucuronylase has increased at full term to

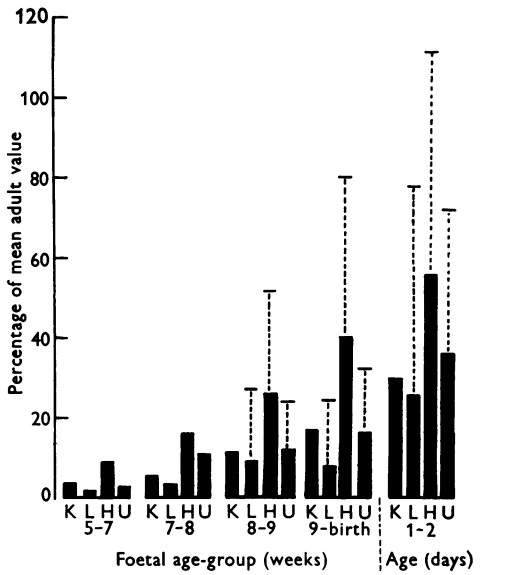

Fig. 2. Synthesis of *o*-aminophenyl glucuronide and levels of UDPglucuronic acid in preparations of guinea-pig tissue at various ages, expressed as percentage of the mean value found in adult tissue. K, Kidney slices; L, liver slices; H, liver homogenate; U, UDPglucuronic acid estimated as in text. Filled-in columns show mean percentages as determined for 50 mg. dry wt. (K, L) or wet wt. (H, U) of tissue; broken-line extensions illustrate the mean percentage attainable in total liver if maximum allowance for its fatty enlargement in the late foetus is assumed (see text).

about 80–100 % of the mean adult value, UDPglucuronic acid to about 60 %, and conjugation in slices to about 30–40 %. In spite of the complexity introduced by fatty infiltration, the pattern in the total liver is still of a rather higher enzyme than nucleotide level, with a poorer synthesis in slices than in fortified homogenates (Fig. 2); however, whereas low formation of the nucleotide probably accounts for the latter discrepancy in foetuses up to some 8 weeks, after this period the greater interference by fat in the dry weights recorded for slices would diminish the observed effect of any increased endogenous supply of UDPglucuronic acid.

Four to five days after birth these late-foetal characteristics have almost disappeared in the guinea pig, and both liver UDPglucuronic acid and glucuronide synthesis in slices are at adult level. Recent work by Schachter *et al.* (1958) would confirm this, inferring that in slices of young guinea-pig liver synthesis of both ether- and ester-type glucuronides of salicylic acid increased from 'minimal' values at birth to 50–80 % of adult figures after 3–4 days.

It is reasonable to suppose that since the mechanism studied above is a general one for glucuronide formation in liver, then its deficiency would affect the synthesis of other examples than those reported here. Particular interest would be attached to bilirubin diglucuronide. It is known that UDPglucuronic acid transfers its glucuronic acid to bilirubin in the livers of the human infant and adult, and of adult rats (Lathe & Walker, 1957; Grodsky & Carbone, 1957; Schmid *et al.* 1957). That the same microsomal enzyme is involved as the one studied here is probable from the reduced synthesis of both phenyl and bilirubin glucuronides in congenitally jaundiced adult humans and rats (Axelrod, Schmid & Hammaker, 1957; Carbone & Grodsky, 1957). The rats had normal UDPglucuronic acid levels and so in their case probably, and in the other possibly, the enzyme was deficient. Lathe & Walker (1957) report a deficiency in enzymic transfer of glucuronic acid to bilirubin in the jaundiced new-born human, and the recent work of Brown, Zuelzer & Burnett (1958), and of Lacson & Waters (1957), cited by Faber *et al.* (1958), indicates that in the guinea pig, and possibly in man, the system synthesizing bilirubin glucuronide develops in foetal and neonatal life similarly to the one forming *o*-aminophenyl glucuronide studied above.

From these results it seems that an enzymic deficiency in glucuronyl transfer, accompanied by low UDPglucuronic acid levels, is a foetal characteristic in the species studied, and hence it may be that certain types of neonatal jaundice are due to persistence of this foetal metabolism, particularly in premature infants. Where lack of UDPglucuronic acid is a limiting factor, it is possible, though unlikely, that its administration, or that of glucose, might prove of use; attempts to induce formation of glucuronide by treatment with glucuronidogenic drugs could lead, in deficiency of UDP-transglucuronylase, to competition with bilirubin for reaction at the few enzyme molecules available.

SUMMARY

1. Low *o*-aminophenyl glucuronide synthesis in foetal liver of guinea pig and man and in neonatal mouse liver has been shown to be due to low levels of uridine diphosphate glucuronic acid and of uridine diphosphate-transglucuronylase (glucuronyl transferase) activity, the enzyme itself probably being present only in small amount.

2. In early foetal guinea-pig liver both these levels are negligible, but increase towards full term. Relatively greater conjugation was observed in homogenates fortified with uridine diphosphate glucuronic acid than in corresponding slices; the bearing of this phenomenon on formation of the nucleotide and on fatty infiltration of foetal liver is discussed. After birth, guinea-pig liver rapidly assumes the glucuronide-synthesizing capacity of the adult. A somewhat similar pattern is described for the infant mouse.

3. Synthesis below adult levels also of (−)-menthyl glucuronide and of the ester-linked *p*-aminobenzoyl glucuronide occurs in homogenates of foetal guinea-pig liver.

4. *o*-Aminophenyl glucuronide synthesis in early foetal kidney is negligible but increases towards full term, being at birth, when fortified with glucose, higher than in corresponding liver slices, though still below adult level.

5. The most active site of glucuronide synthesis so far examined in the early foetus of the guinea pig is the stomach.

6. 'Defective' glucuronide formation in liver and kidney appears to be a foetal characteristic of the species studied, and its relation to bilirubin conjugation in neonatal jaundice is discussed.

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Incorporation of Amino Acids into some Subcellular Fractions of Hepatic Tissues of the Rat

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After the injection of an isotopically labelled amino acid there is a marked difference in the rate at which the protein of the various subcellular constituents of the liver cells becomes labelled. This was first shown by Hultin (1950) with chicks, then by Borsook, Deasy, Haagen-Smit, Keighley & Lowy (1950) for guinea pigs, and later by Keller, Zamecnik & Loftfield (1954) for rats. When slices of rat liver are incubated with a labelled amino acid and subsequently homogenized, the distribution of radioactivity in the different subcellular fractions is very similar to that in the experiment *in vivo* (Campbell, Greengard & Jones, 1957). If homogenates of liver are incubated with a radioactive amino acid and a suitable energy source, the distribution of radioactivity is still similar to that in the intact cell (Siekevitz, 1952). Thus a certain characteristic of the amino acid-incorporating system is maintained in the disrupted cells, suggesting that it might be possible to study the regulation of protein synthesis in subcellular preparations.

In view of the above considerations it was naturally of interest to continue our experiments, designed to elucidate differences between protein synthesis in the normal and malignant liver cell, on cell-free systems. As in our previous studies, liver tumours have been induced by feeding 4-dimethylaminoazobenzene to rats. In order to make comparisons with a rapidly growing normal tissue, preparations from regenerating liver have also been used.

Since the microsomal fraction of the liver cell incorporates amino acids more rapidly than any of the other subcellular fractions so far studied, emphasis, in the present experiments, has been placed on this fraction. Zamecnik & Keller (1954) were the first to demonstrate the incorporation of