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Comparative histochemical studies on carbohydrate, lipid and RNA metabolism in the placenta and foetal membranes

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INTRODUCTION

Carbohydrate (particularly glycogen), lipid and RNA were among the substances earliest to be described in the placenta. The papers of Wislocki and his associates (1946*a*, *b*, *c*, 1948) have been supplemented by studies in several species (Hafez, 1964). Recently the distribution of glycogen has been re-examined using modern specific techniques (Christie, 1966*a*) and some correlation between the distribution of glycogen and certain specific alkaline phosphatases has been made (Christie, 1967*a*).

Many enzymes relevant to the metabolism of glycogen, lipid and RNA can be demonstrated histochemically, and some deductions regarding possible metabolic pathways which these substances may follow in the tissues can be made from the enzymes present therein. The distribution of some of these enzymes has already been described (Christie, 1966*b*, 1967*b*). In this paper the distribution of dehydrogenase enzymes concerned with the metabolism of glycogen, lipid and RNA is described in each of the major placental types, and some deductions regarding possible function of the metabolic pathways thus demonstrated in the tissues are made. In addition the descriptions by previous authors of the distribution of lipid and RNA are extended to allow correlation between their occurrence and certain of the enzymes demonstrated (for details of glycogen distribution see Christie (1967*a*)).

MATERIALS AND METHODS

The material used in this study is summarized in Table 1.

All material was processed either for paraffin sections—fixation in Lillie's (1954) acetic acid–alcohol–formalin fixative for nucleic acids followed by staining in chrome alum–gallocyanin at pH 1·64 (Pearse, 1960) with ribonuclease (Sigma) controls—or for fresh frozen sections following freezing on Drikold (I.C.I.). Frozen material was sectioned at 14 μ in a cryostat maintained at -30 °C, sections picked up on cover-slips, briefly air-dried at room temperature, and stained for lipid (Sudan III and Sudan Black B in propylene glycol), or processed for dehydrogenases acting on the following substrates: α-glycerophosphate (α-GPDH), β-hydroxy-butyrate (β-OHDH), glucose-6-phosphate (G-6-PDH), 6-phospho-gluconate (6-PGDH), lactate (LDH), isocitrate (IDH), succinate (SDH) (no NAD added as the enzyme is FAD-dependent), malate (MDH), glutamate (GDH), alcohol (ethanol) (ADH), furfuryl alcohol (FDH), sorbitol (SorbDH).

Incubations were carried out in a medium containing substrate, 0·1 M phosphate or

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Tris–HCl buffer pH 7·4, NAD or NADP, NitroBT, KCN, and polyvinyl-pyrrollidone (7·5 %) to minimize enzyme diffusion.

As the majority of the enzymes studied depend for their demonstration on the presence in the tissues of diaphorases acting on NADH or NADPH the distribution of these was also examined with NADH or NADPH as substrate and omitting the NAD or NADP from the incubation medium described above. Only sites in this material which did not contain sufficient of both diaphorases to give a blue reaction will be described.

Table 1. *List of material used, and specimens obtained*

(F = foetal placenta, M = maternal placenta, YS = yolk-sac.)

Placental type	Animal	Foetal age (d) or length (cm)	Specimen obtained
Epithelio-chorial	Horse	340 d	F
Syndesmo-chorial	Sheep	2·5 cm (B.R.)*	F + M (cotyledon and inter-cotyledonary area)
		15 cm (B.R.)	
		25 cm (B.R.)	
		40 cm (B.R.)	
		(Term) 55 cm (B.R.)	
Endothelio-chorial	Cat	7 cm (C.R.)	F + M
		12·5 cm (C.R.)	F + M
	Dog	24 d	F + M
		32 d	F + M
		45 d	F + M
		(Term) 60 d	F
	Ferret	22 d	F + M
Haemochorial	Human	1·5 cm (C.R.)	F + YS
		1·8 cm (C.R.)	F
		5·5 cm (C.R.)	F
		7·5 cm (C.R.)	F + M (decidua + muscle)
		15 cm (C.R.)	F
		Term	F + M (decidua)
	Rat	10½ d	F + M
		14½ d	F + M
		17½ d	F + M
		18½ d	F + M
		20½ d	F + M
	Rabbit	13 d	F + M
		17 d	F + M
		20 d	F + M
		30 d	F + M
	Guinea-pig	20 d	F + M
		63 d	F + M
Yolk-sac	Chick	3 d	F (YS)
		5 d	F (YS)
		10 d	F (YS + chorioallantoic membrane)
	Limia maculata	Mid-late pregnancy	F (YS) + M

* B.R. = boss–rump length.

RESULTS

As this paper is primarily concerned with histochemical findings the morphology of the main placental types will not be described here. The reader is referred to reviews by Mossman (1937) and Amoroso (1952) for details.

Lipid

Epithelio-chorial placenta. Lipid is observed in the foetal placenta of the horse in the trophoblast, particularly that of the arcades, with little in the villi.

Syndesmo-chorial placenta. In the sheep placenta some lipid is observed in the diplokaryocytes early in gestation, and in the extra-cotyledonary trophoblast. The uterine glands show some lipid throughout gestation.

Endothelio-chorial placenta. Only the cat shows any lipid staining in the labyrinthine trophoblast, although some is present in all three species in the basal cytotrophoblast related to the histiotrophe. The latter, particularly in the cat and ferret, contains marked quantities of lipid. The trophoblast of the haematoma region shows some staining in all three species, and some is present in the epithelium of the uterine glands.

Haemo-chorial placenta. In the rat the main concentration of lipid is seen in the visceral layer of the yolk-sac endoderm. Some is also observed in the giant cells, the decidua basalis, and traces in the metrial gland cells.

The guinea-pig endoderm also shows some lipid in the yolk-sac and placental layers, but more is observed in the degenerating tissue of the junctional zone.

In the rabbit a similar zone of degenerating tissue—the uterine symplasma, later the separation zone—is present, and here again lipid is observed. Some is also seen in the visceral endoderm.

The human syncytiotrophoblast shows some lipid staining, as does the epithelium lining the uterine glands earlier in gestation.

RNA

Epithelio-chorial placenta. The horse placenta shows only traces of RNA in the trophoblast and allantoic endoderm.

Syndesmo-chorial placenta. Early in gestation the cytotrophoblast and syncytiotrophoblast of the sheep placenta show moderate quantities of RNA, the diplokaryocytes being less positive. While syncytial staining decreases towards term cytotrophoblastic staining persists.

Traces of RNA are seen in the maternal and foetal stromal cells, in the chorionic epithelium outside the cotyledon, in the maternal epithelium, and increasing during gestation in the gland epithelium.

Endothelio-chorial placenta. In both cat and dog the trophoblastic staining for RNA, which is quite marked in earlier pregnancy, decreases later. In the cat and ferret the basal cytotrophoblast shows more staining than the syncytial layer while in the dog the reverse is the case. Both exhibit trace amounts in the trophoblast of the haematoma region.

In all three species small amounts of RNA are seen in the epithelium of the

maternal glands of the spongy zone and haematoma region, and in the yolk-sac endoderm.

The thickened maternal endothelium of the ferret placenta shows marked RNA content.

Haemo-chorial placenta. In both rat and guinea-pig the spongy zone trophoblast shows considerable RNA staining, while later in pregnancy that of the labyrinth appears less marked. In the rabbit also, RNA concentration in the trophoblast appears to decrease during pregnancy. However, this lesser degree of staining may well be due to thinning of the trophoblast as pregnancy advances. In the rat and rabbit the trophoblast giant cells contain RNA in contrast to those of the guinea-pig.

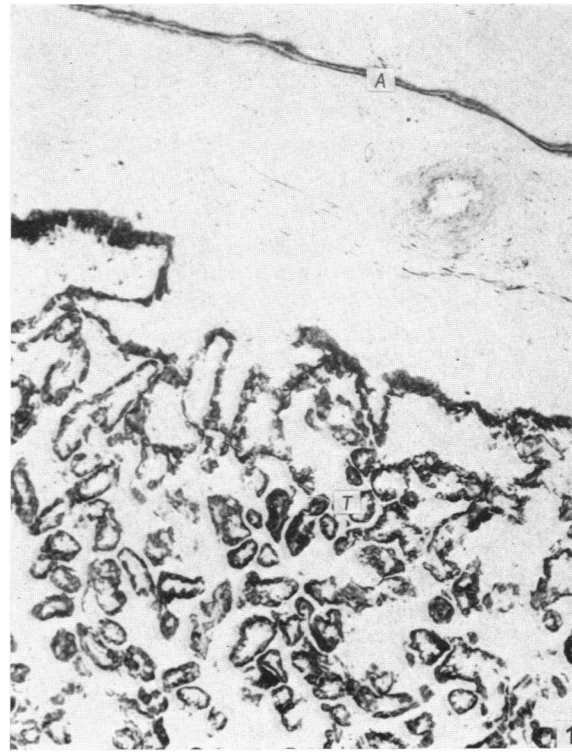
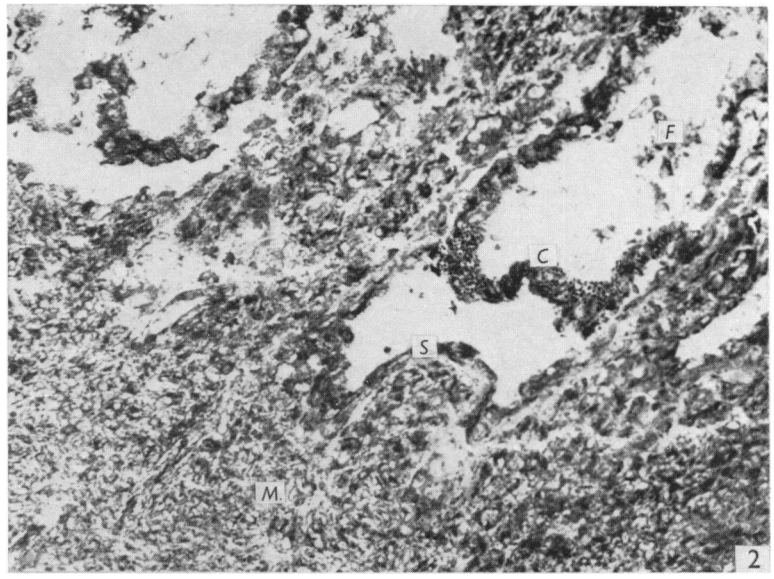


Fig 1. G-6-PDH in the trophoblast and allantoic endoderm of the term horse placenta.

Throughout these figures the maternal side of the placenta lies at the bottom. The following abbreviations are used:

T	trophoblast (general)	*Me*	mesoderm
A	allantoic endoderm	*L*	labyrinth
C	cytotrophoblast	*G*	giant cells
S	syncytiotrophoblast	*Z*	spongy zone
F	foetal stroma	*V*	visceral layer of yolk sac endoderm
M	maternal stroma	*P*	parietal (or placental) layer of yolk-sac endoderm
E	maternal endothelium	*E.S.*	endodermal sinus
D	decidua	*Y*	yolk sac endoderm
Ec	ectoderm		

All three species show RNA in the visceral yolk-sac endoderm, the decidua, and the uterine epithelium. In the rat, RNA is heavily stained in the endovascular plasmodium at $17\frac{1}{2}$ and $18\frac{1}{2}$ d but less so earlier; in the rabbit, in the layer of decidual cells immediately adjacent to the vessel wall; and in the guinea-pig, in the subplacenta.



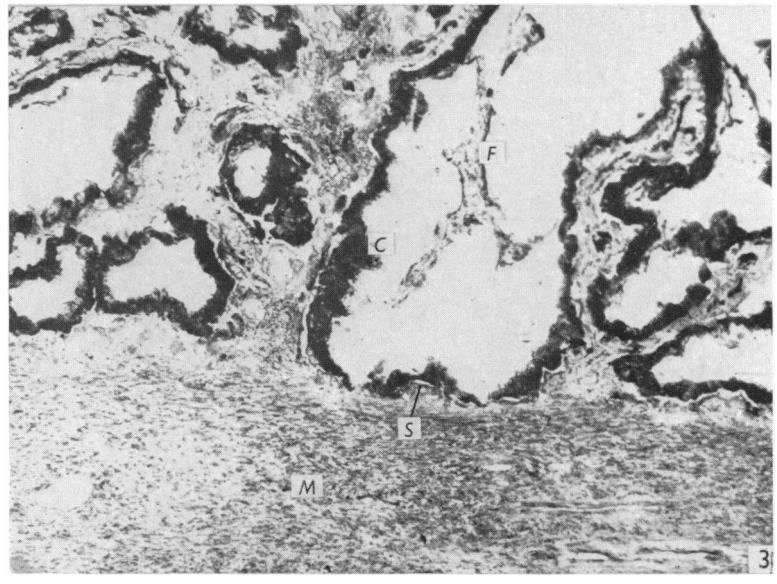
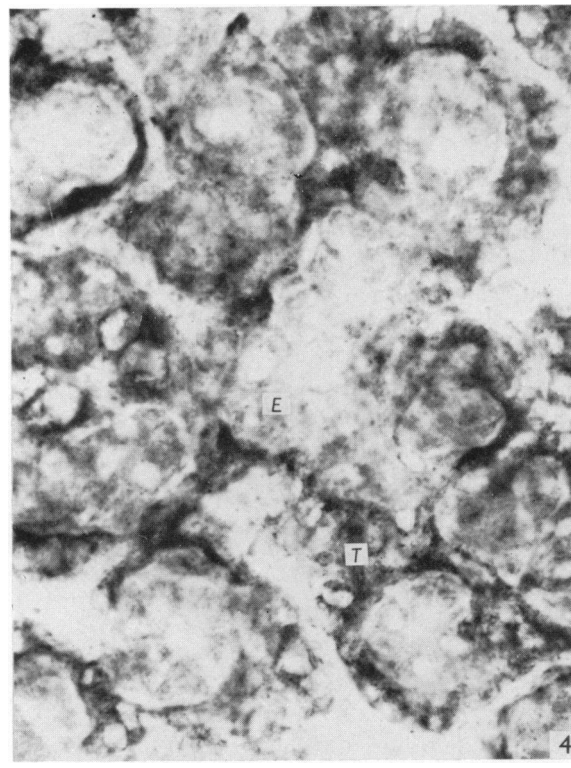


Fig. 2. MDH in the cytotrophoblast, syncytiotrophoblast, maternal and foetal stroma of the 2·5 cm sheep placenta.

Fig. 3. G-6-PDH in the cytotrophoblast, maternal and foetal stroma, with little activity in the syncytiotrophoblast, of the 15 cm sheep placenta.



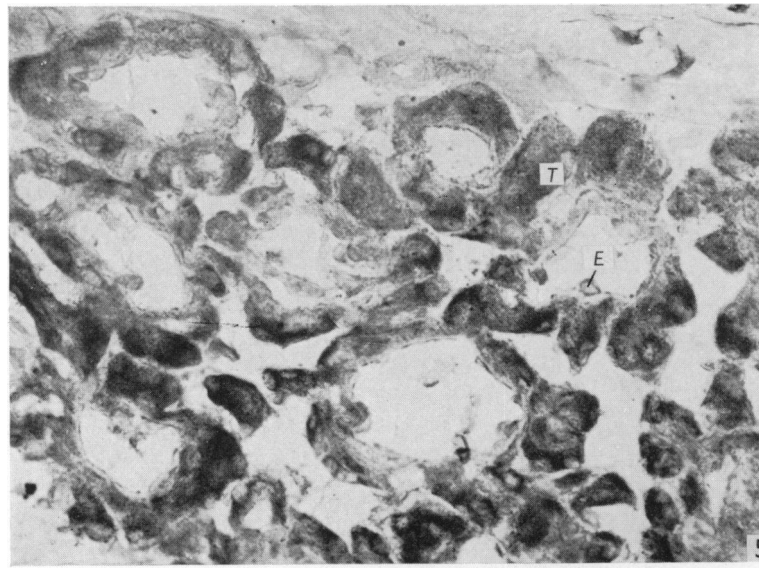


Fig. 4. MDH in the labyrinthine trophoblast and maternal endothelium of the ferret placenta.

Fig. 5. G-6-PDH in the trophoblast and maternal and maternal endothelium of the term dog placenta.

In the human placenta RNA is found in both layers of trophoblast, but particularly the syncytiotrophoblast and the decidua, and less in the foetal stroma. Some staining is also seen in the yolk-sac endoderm.

Carbohydrate dehydrogenases

In this study the carbohydrate dehydrogenases examined fell into three groups within which the distribution of enzymes was similar (with occasional exceptions which will be noted), and a fourth group containing four enzymes whose distribution was very variable. The enzymes included in the groups were as follows.

Group 1: α-glycerophosphate dehydrogenase and β-hydroxy-butyrate dehydrogenase.

Group 2: lactate, malate, succinate, isocitrate dehydrogenases.

Group 3: glucose-6-phosphate and 6-phospho-gluconate dehydrogenases.

Group 4: alcohol, furfuryl alcohol, glutamate, sorbitol dehydrogenases.

To facilitate comparison between species results for these enzymes will not be presented under species headings, but under tissues common to different species. These headings will also be used in the discussion.

Trophoblast-placental

Group 1. These enzymes are chiefly seen in the trophoblast of the chorionic plate of the horse, and in the extra-cotyledonary trophoblast and the cytotrophoblast within the cotyledon in the sheep up to 15 cm. The cat labyrinthine trophoblast is moderately positive, but trace activity is seen in that of the dog and ferret. In the rodents α-GPDH is only seen in the rat labyrinth, and that of the rabbit later in pregnancy, but β-OHDH is present in the trophoblast of rat, rabbit, and term guinea-pig. Both enzymes increase their activity during gestation in the syncytiotrophoblast of the human placenta.

Group 2. Intense activity of all enzymes of this group is seen in the trophoblast of the horse (villous and chorionic plate), and sheep (cytotrophoblast and extra-cotyledonary trophoblast). Activity in the sheep syncytiotrophoblast increases during gestation. In the carnivores LDH and MDH are intensely active (IDH and SHD less so) in the trophoblast of the dog and ferret, and in the deeper parts of the labyrinth of the cat, where the more superficial parts are less active. In the rat group 2 enzymes increase their activity (to very strongly positive at term) during gestation; in the guinea-pig their activity remains consistently high; and in the rabbit it decreases from strongly positive up to 20 d to considerably less active at term. A similar, but lesser, decrease from strongly positive levels is seen in the trophoblast of the human placenta near term.

Group 3. G-6-PDH and 6-PGDH are highly active in the trophoblast of the horse placenta, and the cytotrophoblast of the sheep placenta where peak activity at 15 cm to 25 cm is followed by a slight fall. The syncytiotrophoblast of the sheep, however, exhibits only trace activity. Whereas the cat labyrinthine trophoblast is highly active with these enzymes, again particularly in the deeper parts, that of the dog is only moderately active, and that of the ferret completely negative. In the rat enzyme activity increases to moderate at term, but both rabbit and guinea-pig show a steady

Table 2. *Distribution of group 4 enzymes (ADH, FDH, GDH, SorbDH) in the trophoblast, decidua, and yolk-sac endoderm (only species with positive results are included)*

Species	Tissue	ADH	FDH	GDH	SorbDH
Horse	Trophoblast	+	+	+	Tr
Sheep	Cytotrophoblast	Tr	+ (25 cm)* → Tr (term)	+ → Tr	+ → Tr
	Extra-cotyledonary trophoblast	+ → Tr	+ (25 cm) → Tr (term)	+ → Tr	+ (15 cm) → → Tr
	Syncytiotrophoblast	−	Tr	−	−
Cat	Labyrinthine trophoblast	+	+	Tr	Tr
	Brown-border trophoblast	+	+	++	+
Dog	Labyrinthine trophoblast	Tr	+	−	Tr
	Green-border trophoblast	++ → −	++ → −	−	+ → −
Ferret	Labyrinthine trophoblast	−	+	−	+
	Basal cytotrophoblast	+	+	+	+
Rat	Labyrinthine trophoblast	+ → −	+ → −	+	−
Human	Syncytiotrophoblast	−	Tr	+	−
	Trophoblast of chorion laeve	Tr	Tr	Tr	−
Cat	Decidual giant cells	+	+	Tr	Tr
Rat	Decidua basalis	+	+	++ → Tr	+
Rabbit	Multinucleate decidual cells	−	+	−	−
Guinea-pig	Decidua basalis	++	−	−	−
Cat	Yolk-sac endoderm	−	−	+	+
Ferret	Yolk-sac endoderm	−	+	−	Tr
Rat	Yolk-sac endoderm Visceral	+ → Tr	+ → Tr	+ → Tr	+ → Tr
	Parietal	−	+	−	+
Rabbit	Yolk-sac endoderm	−	++ (13 d) → + (term)	+	++
Guinea-pig	Yolk-sac endoderm Visceral	+ → Tr	+ → Tr	+++ → +	++ → +
	Parietal	−	−	−	Tr

* Enzyme activity ↗ increases, → remains steady, ↘ decreases, throughout gestation or till stage indicated in brackets.

decrease. Increase in activity during gestation is seen in the syncytiotrophoblast of the human placenta, and the early cytotrophoblast is moderately active.

Group 4. The distribution of the enzymes of this group is summarized in Table 2.

Decidua

In this section will be included the maternal cotyledonary stroma of the sheep, the decidual giant cells of the cat, the slightly enlarged decidual cells of the dog, and the decidua basalis of the rat, rabbit, guinea-pig (decidua capsularis also) and human. The decidua underlying the chorion laeve of the latter is also included.

Group 1. Both enzymes of this group are seen in trace activity in the sheep, cat, rat, and human at term. The endothelium lining the decidual blood vessels in the rabbit shows α-GPDH activity earlier in gestation and both enzymes later.

Group 2. The reaction for these enzymes in decidual tissues is very variable. It is slight in the rat, rabbit uninucleate decidual cells, and dog; moderate in the sheep, guinea-pig decidua capsularis, and human with MDH and SDH; and strong in the cat, rabbit multinucleate decidual cells, guinea-pig decidua basalis, and human with LDH and IDH. Transformation of stromal cells to decidual cells in the cat, and of uninucleate to multinucleate decidual cells in the rabbit is accompanied by transient increase in staining for these enzymes to very strong levels.

Group 3. Trace activity of G-6-PDH and 6-PGDH is seen in the sheep and dog, and a very strong reaction in the cat decidual giant cells (decreasing towards term) and the human subplacental decidua. The decidua capsularis of the latter shows less activity, however, as does the decidua basalis of the rat and guinea-pig, in both of which activity decreases towards term. No activity is seen in the decidua of the rabbit, but slight staining occurs in the endothelium lining the blood vessels.

Group 4. The results are summarized in Table 2.

Giant cells

These include the sheep diplokaryocytes, whose staining reactions did not differ from the cytotrophoblast in which they lay (see above), the antimesometrial giant cells in the rat and rabbit, the mesometrial giant cells in the rat and guinea-pig, and what is probably the mesometrial homologue in the rabbit—the multinucleate bodies.

Group 1. Trace activity is seen with both enzymes in rodent giant cells except the rabbit multinucleate bodies.

Groups 2 *and* 3. Moderate activity of both groups of enzymes is seen in all rodent giant cells except the rat mesometrially, where activity decreases from very strong up until 17½ d to moderate at term.

Group 4. Slight FDH activity is seen in the mesometrial cells in the rat (GDH and SorbDH also) and guinea-pig, and increasing in the antimesometrial cells in the rabbit. All other enzymes of this group are negative.

Yolk-sac endoderm

Included in this section are the absorptive yolk-sacs of the chick, oviviviparous fish (*Limia maculata*), human, rodents, and early pregnant dog, and the secretory (Amoroso 1952) yolk-sac of the carnivores later in pregnancy.

Group 1. Trace activity is seen in the yolk-sac endoderm of the cat, and of the

dog prior to separation of the chorio-vitelline placenta, but not thereafter in that species. Slight activity is present in the rat yolk-sac and, increasing with gestation, in that of the rabbit, and in both yolk-sac and placental layers of endoderm in the guinea-pig. Slight activity is also seen in the yolk-sac endoderm of *Limia maculata*, and increases markedly between 4 and 10 d in that of the chick. Insufficient material was available to incubate human yolk-sac for these enzymes.

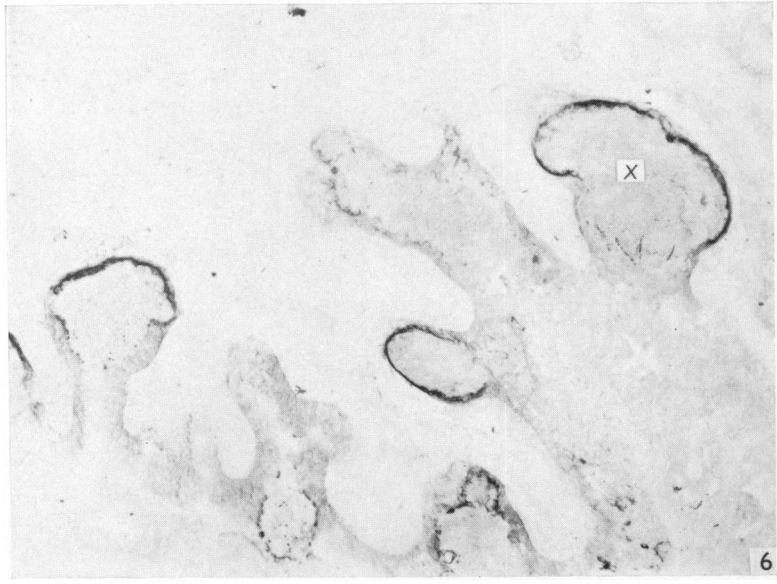


Fig. 6. GDH in areas of brown border associated with absorption of degenerating material (*X*).

Group 2. The carnivore yolk-sac exhibits strong LDH and MDH, moderate IDH and less SDH activity. All three rodents show decrease in activity in the yolk-sac endoderm towards term, this being preceded in the rat by an increase up to 17½ d. Initially the chick yolk-sac shows only LDH activity, but this increases as gestation proceeds, and MDH, IDH and SDH appear. *Limia maculata* shows strong LDH and less MDH activity but is negative for IDH and SDH, and the human yolk-sac is similar but less reactive.

Group 3. G-6-PDH and 6-PGDH in the cat, rabbit, and guinea-pig yolk-sacs decrease their activity from strong levels of staining to moderate at term. In the rat, however, increase to moderate levels at term is observed. Both human and ovivi-parous fish yolk-sacs show moderate G-6-PDH activity.

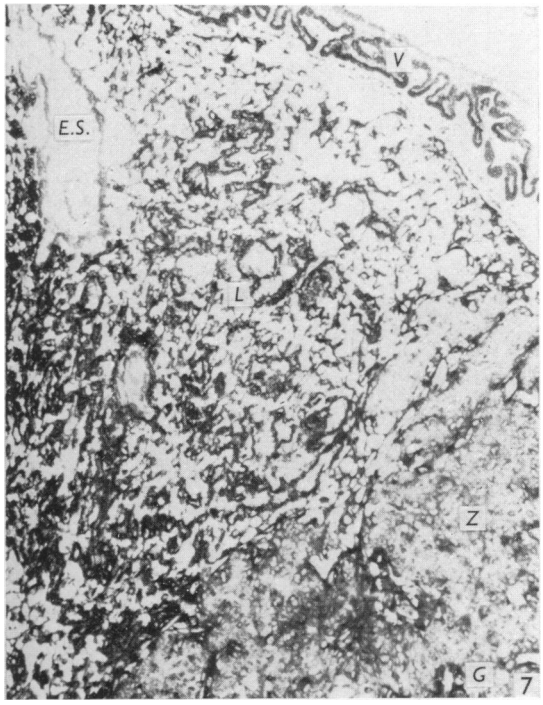
Group 4. Results are indicated in Table 2.

Non-placental chorion and maternal epithelium

For comparative purposes the trophoblast of the basal chorionic plate in the horse, and of the intercotyledonary area of the sheep and the yolk-sac endoderm in the rodents after degeneration of the parietal layer, can be included in this section. Their

content of dehydrogenase enzymes has already been considered (see 'Trophoblast' and 'Yolk-sac endoderm').

All areas of carnivore non-placental trophoblast show moderate to strong group 2, and (except in the ferret) group 3 enzymes, the content of the latter in the dog decreasing to mid-pregnancy and thereafter increasing again. Traces of group 1 enzymes are seen in the dog alone, and group 4 enzymes do not appear.



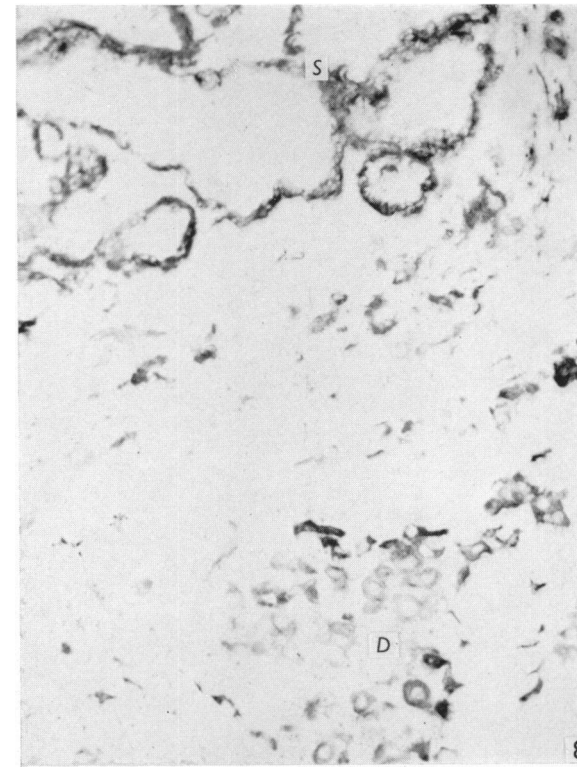


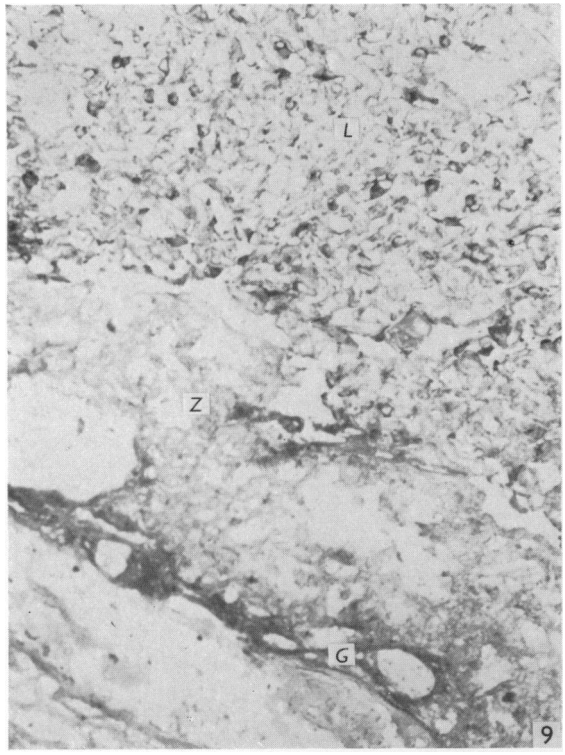
Fig. 7. MDH in the labyrinth, giant cells, spongy zone trophoblast, visceral endoderm, and endodermal sinus of the 17½ d rat placenta.

Fig. 8. IDH in the syncytiotrophoblast and decidua of the term human placenta.

The uterine epithelium in the sheep, carnivores and rodents is moderately to strongly positive for group 2 enzymes. Activity in the sheep increases to 15 cm and thereafter remains constant. Trace to slight activity of group 1 enzymes is seen in the same species. Group 3 enzymes appear in moderate amounts in the sheep (increasing to 15 cm and thereafter steady), dog (decreasing towards term), ferret and rabbit (increasing from 13 d); and trace to slight activity in the cat and guinea-pig, other species being negative. Group 4 enzymes are rather variable from one substrate to another, but are generally at trace to slight levels in the sheep, dog and cat. Rather more activity is seen in the guinea-pig paraplacental epithelium and GDH alone appears in the regenerating epithelium lateral to the term rat placenta. Other species are negative for this group.

Allantoic endoderm

This tissue in the horse, sheep, cat, dog and ferret exhibits moderate to strong group 2 enzyme activity, and similar levels of activity of group 3 enzymes in the horse, but slight to trace activity only in the sheep and carnivores (ferret negative). A slight level of α-GPDH, GDH, and SorbDH activity is seen in the horse allantoic endoderm; and of FDH activity in that of the cat and dog; the latter showing similar levels of SorbDH.



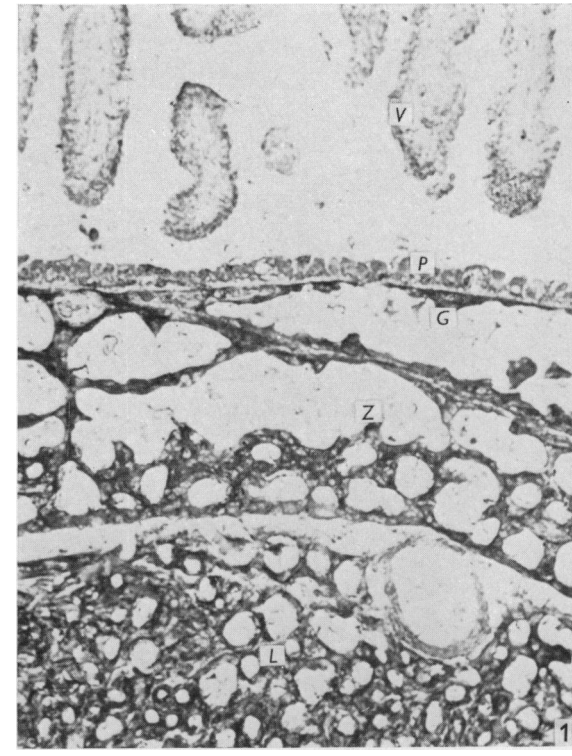


Fig. 9. G-6-PDH in the labyrinth, spongy zone and giant cells of the term rat placenta.

Fig. 10. G-6-PDH in the labyrinth, spongy zone, giant cells, placental and visceral layers of endoderm in the term guinea-pig placenta.

Haematoma region

The maternal epithelium, and foetal trophoblast of the dog 'green border', cat 'brown border' and ferret 'haematoma' exhibit certain differences from those of the non-placental region. In the maternal epithelium GDH activity is increased, particularly in the cat and dog. The trophoblast exhibits PAS-positive diastase-fast droplets (Christie, 1966*a*) and increased activity of groups 3 and 4 enzymes.

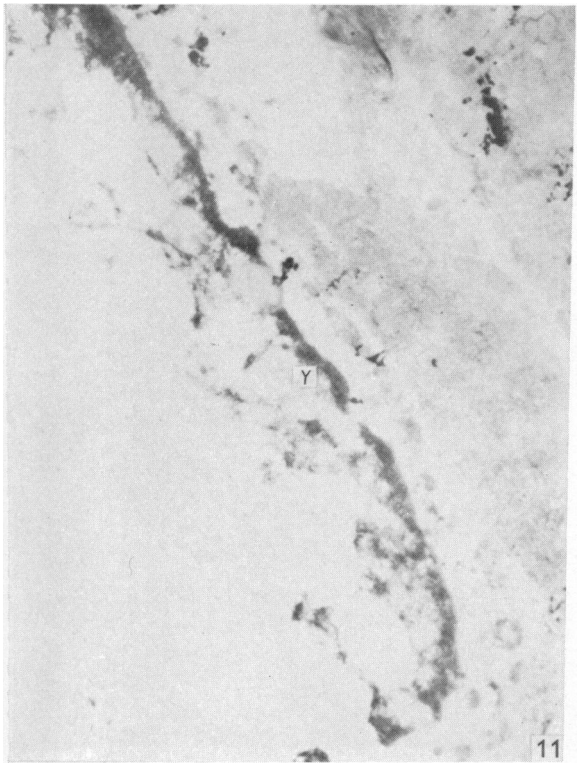
Carnivore spongy zone

The results for this region are summarized in Table 3.

Rodent spongy zone

The trophoblast of the spongy zone in the rat and guinea-pig differs histochemically from that of the labyrinth.

In the rat it exhibits lesser activity of group 2, 3 and 4 enzymes, which appear in the spongy zone with moderate, slight and negative activities respectively. In the guinea-pig, however, the spongy zone trophoblast, while less active than that of the labyrinth with group 3 enzymes (moderate activity), is more active with group 2 enzymes (very strong levels) and with ADH and FDH of group 4 (slight activity).



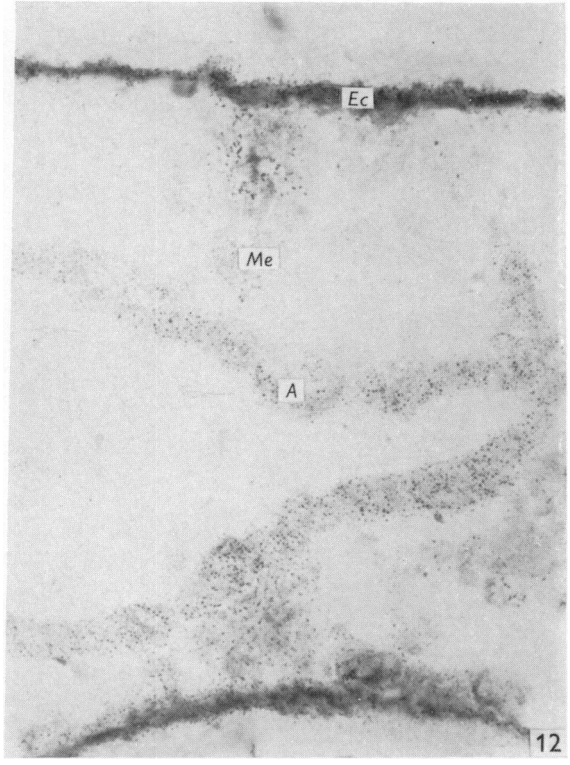


Fig. 11. MDH in the yolk-sac endoderm of the embryo oviviviparous fish *Limia maculata*.

Fig. 12. NADPH diaphorase in the chorio-allantoic membrane of the 10 d chick embryo.

The vacuolated 'glycogen cells' of the rat spongy zone exhibit increasing activity of groups 2 and 3 enzymes to moderate to strong levels at 17½ d, followed by a decrease towards term.

'*Fibrinoid capsule*' (*Bulmer & Dickson*, 1961)

This area of the rat uterine tissues exhibits group 2 enzymes only, the activity of which increases to moderate levels at 17½ d and thereafter decreases.

Endovascular plasmodium

This tissue, the origin of which is disputed, shows strong activity of groups 2 and 3 enzymes, the former increasing to 17½ d and thereafter decreasing, the latter increasing to term. Slight α-GPDH, GDH, FDH and SorbDH activity is also seen.

Table 3. *Distribution of enzyme activity in the tissues of the carnivore spongy zone*

(A = ADH, F = FDH, G = GDH, S = SorbDH.)

Species	Tissue	Group 1	Group 2	Group 3	Group 4
Cat	Maternal epithelium	–	+++	++	A, F, G, S Tr
	Basal cytotrophoblast	–	+++	++++	F +
	Histiotrophe	+++	–	–	A, F ++; G, S +
Dog	Maternal epithelium	–	C* +++	C +++ ↘ + (45 d)	D G, S +
			D + ↘ Tr (term)	D +++ ↘ ↗ + (45 d)	++ (term)
	Basal cytotrophoblast	–	+++	++	–
Ferret	Maternal epithelium	–	+++	+++	F, S +
	Basal cytotrophoblast	+ (α-GPDH)	+++	++	A, F, G +
	Histiotrophe	Tr	–	–	A, F +

* C = contracted portion of glands, D = dilated portion of glands. For arrows see Table 2.

Metrial gland cells

These exhibit slight activity of groups 1 and 4 enzymes, the latter decreasing towards term, and strong groups 2 and 3 enzyme activity at 18½ d which decreases abruptly.

Rabbit separation zone, and guinea-pig junctional zone

Both of these areas contain large quantities of degenerating material. This contains slight to moderate quantities of groups 1 and 4 enzymes in the guinea-pig, and similar activities are seen in cells gathered round the glandular symplasma (which is itself unreactive) in the rabbit separation zone. No activity of groups 2 or 3 enzymes is seen.

Guinea-pig subplacenta

This shows, at 20 d of gestation, strong group 2 enzyme activity, and moderate activity of group 3, and ADH and FDH of group 4.

Chorio-allantoic membrane—chick

The allantoic endoderm at 10 d of incubation exhibits small quantities of the following enzymes—FDH, LDH, MDH, IDH, G-6-PDH and α-GPDH, in that

descending order. LDH, MDH, and diaphorases only are found in the mesoderm. The ectodermal layer applied to the inside of the shell membrane shows moderate LDH, MDH and FDH activities, strong NADH diaphorase activity, but very strong NADPH diaphorase activity despite the lack of histochemically demonstrable NADP-linked enzymes.

DISCUSSION

Enzyme specificity

Recently Kalina, Gahan & Jones (1965) have suggested that all histochemically demonstrated dehydrogenase reactions show only the localization of one or other pyridine nucleotide diaphorase. However, in this study, totally different pictures were observed with different groups of enzymes, the grouping not being dependent on the pyridine nucleotide used. In some sites, too, separate enzymes, both NAD-linked, stained single, but opposite, members of an adjacent pair of tissues, suggesting that the diffusion shown by Kalina, Gahan & Jones in their work had not occurred. This may have been due to the addition to the incubation medium used of large quantities of polyvinyl-pyrrolidone.

Physiological importance of enzymes and other substances studied

(1) *Glycogen.* This is thought to exist in cells chiefly as a readily available source of energy, and is normally bound to protein, (Stetten & Stetten, 1960).

(2) *RNA.* The association of this with protein synthesis, cell growth, and differentiation is well known.

(3) *Carbohydrate dehydrogenases.* Glycogen can be broken down either to pyruvate via the Embden–Myerhof–Cori pathway, or to ribulose-5-phosphate via the Pentose Shunt (G-6-PDH, 6-PGDH). If degraded to pyruvate it then proceeds either to lactate (LDH) in anaerobic conditions, or to carbon dioxide and water via the Krebs tricarboxylic acid cycle (IDH, SDH, MDH) in aerobic conditions, the function subserved in either case being the production of energy. If degraded to ribulose-5-phosphate this may either be passed back into the glycolytic pathway, via xylulose-5-phosphate, or converted to ribose-5-phosphate which may then be used for RNA synthesis.

Of the remaining dehydrogenases studied, the function of α-GPDH and β-OHDH is the introduction of α-glycero-phosphate and β-hydroxy-butyrate respectively (both derived from lipid breakdown) into the glycolytic pathway; that of GDH is the introduction of glutamate derived from protein breakdown into the Krebs cycle, or the divergence of α-keto-glutarate from the cycle into protein synthesis; that of ADH is the degradation of alcohols to the corresponding aldehydes, and that of FDH is the degradation of furfuryl alcohol, which may be derived from RNA catabolism to furfural which is excreted in the urine.

Histochemical considerations

Trophoblast—placental. In general the histochemical findings reported here confirm those of other workers where relevant.

The degree of decrease in activity of the Krebs-cycle enzymes towards term,

indicative of placental ageing, is very variable. Despite the decrease in permeability (possibly due to decrease in active energy-requiring transport) known to occur from nine-tenths of the period of gestation onwards in all placental types except the epithelio-chorial (Flexner & Gellhorn, 1942) only the sheep, rabbit, and human show decrease in LDH, MDH, IDH and SDH activities towards term.

Glycogen in the placenta has been correlated with both high (Szendi, 1934) and low (Dempsey & Wislocki, 1945) rates of metabolism. In this study all cells containing glycogen also contain high activity of dehydrogenases, a result which would tend to confirm, as did Huggett (1961), Szendi's interpretation. A high level of metabolism would be expected in placental trophoblast where considerable activity with respect to transport, degradation and synthesis of materials is taking place.

Total placental glycogen (i.e. glycogen in both maternal and foetal tissues) as opposed to trophoblastic, shows in this material the classification of Huggett (1961) into the glycogen-poor sheep and horse placenta, and the glycogen-rich remainder. Huggett (1961) and others observed that the glycogen-poor placentae are associated with fructose as the foetal blood sugar, while the glycogen-rich placentae are associated with glucose. It was hoped that the staining of sorbitol dehydrogenase in this material might throw some light on the route of formation of fructose in ungulate placentae, in view of the observation of Andrews, Britton & Nixon (1959) that sheep placentae perfused through the umbilical artery yielded sorbitol, which is converted by sorbitol dehydrogenase to fructose, and the ability of sheep placenta to form fructose when perfused with glucose (Huggett, 1961). However, the concentration of sorbitol dehydrogenase, as judged histochemically, is greater in the placentae of the species in the foetal blood of which only small quantities of fructose are found, and the route of metabolism must thus remain uncertain.

RNA is present in lesser quantity in the horse placenta than in that of any of the other species studied. Its significance in the placenta is not certain, but it has been suggested that placental structures containing RNA are probably concerned with protein hormone production (Weber, 1964). In this respect it is thought that the placenta in man produces chorionic gonadotrophin, and the rat is thought to produce luteotrophic hormone (Bourdel & Jacquot, 1956). Thus the moderately high levels of RNA seen in the trophoblast of these species would be anticipated. In the horse, on the other hand, gonadotrophin is produced not from the trophoblast, but from specialized endometrial 'cups' in the first half of gestation. Therefore the lower levels of trophoblastic RNA would not be unexpected.

The possibility exists therefore that other species exhibiting marked RNA concentration in the trophoblast may be secreting protein hormones. Unfortunately comparative evidence on this subject is lacking.

α-GPDH and β-OHDH are chiefly seen in areas which also exhibit lipid—for example, the cat labyrinthine trophoblast, and that of the chorionic plate in the horse—and are presumably concerned with lipid degradation. The enzymes are also found in the extra-cotyledonary trophoblast of the sheep, and here, as in the horse, may be associated with the further metabolism of absorbed uterine milk which is known to contain 1·2 g % of fatty matter in the ewe, and which could be split to α-glycerophosphate and fatty acids by the hydrolase enzymes (acid phosphatase and non-specific esterase) known to be present in these sites (Christie, 1967*b*).

High *LDH*, *MDH*, *IDH* and *SDH* activities in the placenta indicate the high metabolic activity of this organ, and show the production of energy for a variety of processes. The increased activity in the basal parts of the cat labyrinth correlates with the findings as regards steroid production in this animal (Ferguson & Christie, 1967) and suggests some degree of trophoblastic ageing in this animal as has already been noted for the rabbit and human. In the rat, human (up until near term), and sheep the increase in activity of these enzymes noted as gestation proceeds may indicate increase in placental transport of nutriment to the foetus, whose increase in weight in these species is very marked in the later part of pregnancy.

G-6-PDH and *6-PGDH* activity in trophoblast may indicate either RNA synthesis or the generation of reduced NADP which is required for steroid hydroxylation (Deane *et al.* 1962). In this material trophoblast of the rat, guinea-pig and rabbit which does not exhibit steroid synthesis (Ferguson & Christie, 1967) but contains considerable activity of G-6-PDH and 6-PGDH also shows quite marked RNA concentration. On the other hand the horse, dog, cat, human and sheep cytotrophoblast, all of which are concerned in steroid metabolism, show low or decreasing quantities of RNA but contain marked enzyme activity. The sheep syncytiotrophoblast is in an intermediate position, in that both RNA and steroid metabolic enzymes decrease throughout pregnancy, and here only traces of enzyme staining are observed.

ADH, *FDH*, *GDH* and *SorbDH* activities are never very high in trophoblast. FDH tends to be present in areas of decreasing RNA concentration, further confirming its function in that process. ADH appears in the same sites, and in approximately the same concentrations as α-GPDH, suggesting that some non-specific degradation of α-glycerophosphate may be taking place through this enzyme. The only trophoblast in which GDH appears in any quantity is that of the horse and sheep, suggesting that the enzyme may be concerned with the degradation of the protein content of absorbed uterine milk. The function of SorbDH in relation to fructose formation has already been discussed.

Decidua. The function of the decidua is uncertain, and it has variously been ascribed a protective or nutritive role. That it is a very active tissue in all species is evident from the degree of staining of energy-producing enzymes. The significance of the increase in G-6-PDH and 6-PGDH in that of the cat, rodents, and human is uncertain. The possibility exists, however, that they may be concerned with the production either of RNA, which is found in the decidua of the latter two species, or of reduced NADP, which could be utilized to stimulate protein synthesis in the decidua prior to passage to the foetus for nutrition. Certainly NADPH is known to be stimulatory to that process.

Giant cells. Histochemically the antimesometrial giant cells in the rat and rabbit appear to be homologous, those of the rabbit being more active with all enzymes. The function of the carbohydrate dehydrogenases in these cells is uncertain.

The marked enzyme activity in the mesometrial giant cells in the rat, however, would accord with their likely function in steroid biosynthesis (Deane *et al.* 1962, Botte, Materazzi & Chieffi, 1966; Ferguson & Christie, 1967). The mesometrial homologue of these cells in the rabbit, the multinucleate bodies, were described by Sansom (1927) as 'inactive degenerate structures'. That this is not so is demonstrated by their content of dehydrogenase enzymes.

Trophoblastic giant cells are also found in the sheep—the diplokaryocytes. Wimsatt (1951) suggested that these cells form the syncytium lining the maternal crypts, and that they may possibly secrete some material into the lumen. Their dehydrogenase content, indicating marked energy production, would accord with these suggestions and with the possibility that they are concerned with transport across the placental barrier as suggested previously (Christie, 1967*a*, *b*).

Yolk-sac endoderm. The simple yolk-sac, as found in the fish, chick, and human shows moderate Krebs-cycle enzyme activity, and lower activity of the other carbohydrate dehydrogenases. α-GPDH and β-OHDH show interesting changes in the chick yolk-sac endoderm, where their appearance and increase after 5 d corresponds to the change-over from carbohydrate to lipid as the primary energy source in the developing embryo described by Mahler, Wittenberger & Brand (1958).

The main difference observed between the simple absorptive yolk-sac and the secretory yolk-sac of the carnivores (Amoroso, 1952) is the increased activity of the Krebs-cycle enzymes, the degree of staining of which correlates, except in the dog, with the glycogen content. Correlation with the degree of secretion as judged histochemically is also present. As might be expected in sites of protein synthesis, RNA is present in these cells.

The function of the rodent yolk-sac is mainly absorptive, and the high Krebs-cycle enzyme activity reflects energy production for that process. The high activity of the pentose shunt enzymes, unassociated with increase in RNA content, suggests the possibility of further energy production via transhydrogenation from NADPH to NADH, a hypothesis which is further supported by the presence in these cells of 3α- and 17β-hydroxy-steroid dehydrogenases (Ferguson & Christie, 1967), both of which are known to utilize both co-factors. In the guinea-pig GDH activity (and β-glucuronidase) increase markedly towards term, and activity of these enzymes is much greater in the yolk-sac of this species than in that of any others studied.

The parietal endoderm of all rodent placentae exhibits activity of FDH, correlating with tissue, and more particularly RNA breakdown.

Insufficient material was available to compare the endoderm of the chorio-vitelline placenta with that of the free yolk-sac in carnivores. Results on two early dog specimens, however, suggest that the only difference is the presence of α-GPDH and β-OHDH in the former, which would correlate with the presence in the endoderm of lipid (Amoroso, 1952).

Non-placental chorion, maternal epithelium, and uterine secretion

In the horse the presence of α-GPDH and β-OHDH, particularly in the chorion between the bases of the primary villi, and the possible correlation with the absorption of uterine milk has already been noted. A similar possibility of foetal nutrition via the uterine secretion is suggested by the enzyme activity of the non-placental chorion in other species. In the sheep uterine glands the changes in Krebs-cycle and pentose-shunt enzymes suggest that their activity becomes maximal at about 15 cm and thereafter remains steady, and activity here, as in other species, correlates with the degree of uterine secretion as judged histochemically.

The presence of intact maternal epithelium from 2·5 cm (35 d) on in the sheep

non-placental region is of interest, in view of the generally held opinion that the epithelium is destroyed in this site early in pregnancy and not regenerated until the fourth month (foetal length, 38 cm).

Allantois. The carbohydrate dehydrogenase activity observed in the allantoic endoderm would accord with its presumable function in the absorption of fluid from the allantoic cavity.

Haematoma region. The maternal epithelium and trophoblast of the dog 'green border', cat 'brown border', and ferret 'haematoma' exhibit enzyme activities similar to those observed in the yolk-sac endoderm of rodents, which is thought to play a significant part in iron absorption in these species (Lambson, 1966). Iron transport across the gut is regulated by ferritin (Granick, 1946), and it has recently been demonstrated in the visceral endoderm of the rat yolk-sac (Lambson, 1966), where it is observed in apical vacuoles. G-6-PDH and 6-PGDH, and GDH present in the maternal epithelium, may be involved in conjugation of iron with a protein, the material in the lumen as judged histochemically being in conjugated form. Subsequent de-conjugation in the trophoblast would release the iron for use. Further evidence in favour of this suggestion has already been reported (Christie, 1967*b*).

Carnivore spongy zone. In this area the presence of RNA, pentose-shunt enzymes, FDH and GDH suggests protein synthesis in the basal cytotrophoblast, possibly associated with cellular proliferation and trophoblastic growth. Active Krebs-cycle enzyme activity is also present for energy production. In the maternal epithelium energy for secretory processes in the cells to contribute to the histiotrophe can be derived, as judged histochemically, from the glycogen content via the Krebs cycle.

Rodent spongy zone. The increase in degradative dehydrogenases (ADH, FDH) in the trophoblast of this region when compared to that of the labyrinth may accord with its rapid erosion as the latter expands. The difference in the enzyme activity of this zone in the guinea-pig compared to the rat is not unsurprising in view of the differences in morphology. In the spongy zone of the rat the glycogen content of the vacuolated cells increases to a maximum at 17 d and thereafter remains steady. Here the fall-off in LDH, IDH, SDH and MDH activity from $17\frac{1}{2}$ d corresponds to the period of unaltered glycogen content and may be expressive of decreased utilization. The wave of activity seen with G-6-PDH and 6-PGDH could be concerned (via RNA) with synthesis of the protein to which glycogen is bound in cells (Stetten & Stetten, 1960).

The origin of the *endovascular plasmodium* has been questioned in the rat (Mossman, 1937; Bridgman, 1948). Histochemically its cells are almost identical with the labyrinthine trophoblast, and it would appear likely that it is of foetal trophoblastic origin.

In both the rabbit and guinea-pig the area of degenerating tissue—in the former the *separation zone*, in the latter the *junctional zone*—exhibits fairly intense activity of ADH, FDH, GDH, SorbDH, α-GPDH and β-OHDH, suggesting that these enzymes can be considered to be at least partly concerned with tissue breakdown.

The considerable quantities of RNA, of LDH, IDH, MDH and SDH and slightly less of G-6-PDH and 6-PGDH seen in the guinea-pig *subplacenta* confirm and supplement the observations of Wislocki, Deane & Dempsey (1946) and Davies, Dempsey

& Amoroso (1961) and suggest that the subplacenta is concerned in protein secretion, possibly gonadotrophin as suggested by Davies *et al.* (1961).

It is of interest to compare the chorio-allantoic placentae of mammals, concerned in the transport of many substances and synthesis or degradation of several, with the simple chorio-allantoic 'placenta' of the chick concerned purely with gaseous exchange and possibly (in the allantoic endoderm) with reabsorption of water from the allantoic sac. Here the ectoderm applied to the inner aspect of the shell membrane, and presumably most concerned with oxygen and carbon-dioxide exchange, shows intense NADPH diaphorase activity, unaccompanied by activity of any NADP-linked enzymes, and much less activity with NADH diaphorase. This is the reverse of the situation in the analogous trophoblast of all species examined except the horse, where NADPH diaphorase is again higher. The significance of this finding, however, is unclear. The wide range of enzyme activities seen in the chick allantoic endoderm would accord with its function in fluid resorption, and also possibly energy production for its own extension, and is similar to the range seen in the allantoic endoderm of other species.

SUMMARY

The distribution of lipid, RNA and dehydrogenases acting on α-glycerophosphate, β-hydroxy-butyrate, lactate, isocitrate, succinate, malate, glucose-6-phosphate, 6-phospho-gluconate, ethanol, furfuryl alcohol, glutamate and sorbitol, and of NADH and NADPH diaphorases, is described in the placentae and foetal membranes of the horse, sheep, cat, dog, ferret, rat, rabbit, guinea-pig and human, and in the yolk sac of the chick (chorio-allantoic placenta also) and an oviviviparous fish *Limia maculata*. Correlation between the presence of these enzymes and that of glycogen, lipid and RNA is found.

Deductions concerning the physiological significance of these substances and enzymes in the placentae and foetal membranes are made, and possible pathways of their metabolism in the tissues are sought.

The histochemistry of placental structures is discussed on a comparative basis, under the headings trophoblast, decidua, giant cells, yolk-sac endoderm, non-placental chorion and maternal epithelium, allantois, haematoma region, carnivore and rodent spongy zones, fibrinoid capsule, endovascular plasmodium, metrial gland cells, rabbit separation zone and guinea-pig junctional zone, guinea-pig subplacenta, and chick chorio-allantoic placenta.

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