J. Anat. (1973), **115**, 3, pp. 327–346
With 29 figures
Printed in Great Britain

The postnatal development of the liver in a marsupial, *Didelphis virginiana*

1. Light microscopy

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(*Accepted* 25 *April* 1973)

INTRODUCTION

The appearance and structure of the adult mammalian liver have been well established as a result of numerous studies (e.g. Elias, 1949, 1955*a*; Elias & Bengelsdorf, 1952; Hampton, 1964; Flaks, 1971) and considerable attention has been directed to the development of the liver in the embryo (e.g. Lewis, 1912; Bloom, 1926; Elias, 1955*b*; Du Bois, 1963; Severn, 1971). Additionally, there have been some reports upon certain specific features of postnatal development and differentiation of the liver, where clearly profound changes in both structure and function occur. Features investigated include deposition of lipid material within hepatic cells (Deane, 1944), size of hepatic cells and width of the lobule (LeBouton & Marchand, 1970), cellular composition within the lobule (Greengard, Federman & Knox, 1972), and the distribution of haematopoietic foci (Sorenson, 1960; Thomas, Russell & Yoffey, 1960). However, there has been a dearth of systematic studies on hepatic development during this period. Recently, in an account of the general features of postnatal development in the rabbit liver, Leeson & Cutts (1972) described hypertrophy and hyperplasia of hepatic cells, presence of lipid material within hepatic cells and its subsequent loss, mitotic activity and the appearance of binucleate cells, organization of cells into plates, and the extent of haematopoietic activity and the nature of its decline during the postnatal period.

The opossum (*Didelphis virginiana*) is born approximately 12½ days after conception and continues its development within the maternal marsupium. It remains firmly attached to the nipple for at least 60 days and thereafter begins to move about freely, both within and without the pouch (Hartman, 1952). The present study details the sequence of changes, as seen with the light microscope, that occurs in liver architecture during this postnatal period.

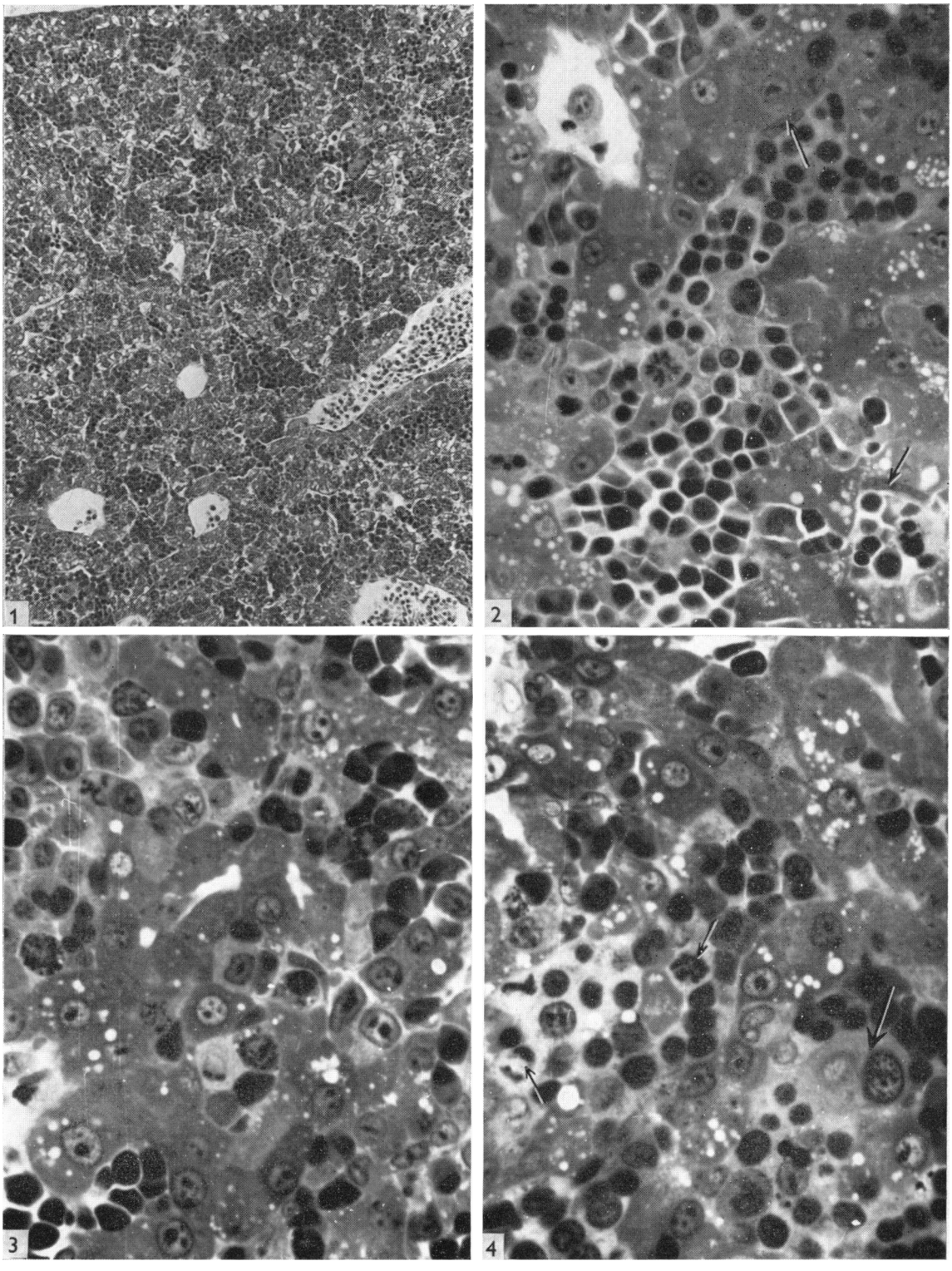
MATERIALS AND METHODS

One hundred and two opossums (*Didelphis virginiana*) were used in the study. Pouch-young opossums were divided into the following ten groups according to their snout:rump lengths. 1·5,† 2·0, 2·5,‡ 3·0, 4·5, 7·5, 10·0, 15·5, and 20 cm. Five

* Recipient of a grant from the American Philosophical Society, No. 6515 Penrose Fund, and of a University Research Council Grant NSF 1074 for general research support.

† Animals in this group (newborn) were known to be less than twenty four hours old.

‡ Animals in this group were known to be nine days old.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adults also were used. Each group had a minimum of six animals and some groups had as many as fifteen. The animals were killed by decapitation, and as quickly as possible blocks of liver were placed in Bouin's solution or in 10 % buffered neutral formalin. Tissues were processed routinely, embedded in paraffin, and stained with haematoxylin and eosin. Additional liver material, fixed in phosphate-buffered 4 % glutaraldehyde and post-fixed in osmium tetroxide, was processed and embedded in Araldite as for electron microscopy. Thick sections of this material were cut at 0·5–3·0 μm and stained with toluidine blue. After clearing in xylene, they were cover-slipped and examined by light microscopy (Leeson & Leeson, 1970).

The quantitative studies were performed exclusively on material embedded in Araldite. Two or three fields from each of three sections were examined for each animal in the different stages of development. The cells of hepatic and haematopoietic origin were counted per high power field, with not less than 12,000 cells enumerated for each group of animals. The haematopoietic cells were classified further according to their type and stage of maturation, and note was made also of the mitoses that occurred among the hepatic and haematopoietic cells.

RESULTS

Qualitative findings

The liver at birth (1·5 cm) appears markedly immature (Fig. 1) when compared with that of the adult opossum (Fig. 26). Within lobules, hepatic cells lack organization into plates. The cells are concentrated around central veins, and peripherally they appear in groups separated by large islands of haematopoietic elements. The small hepatic cells contain pale, vesicular nuclei with distinct nucleoli (Figs. 2–4). The cytoplasm is finely granular and appears vacuolated because of the presence of discrete lipid droplets. Haematopoietic elements occur as large islands scattered between the groups of hepatic cells (Figs. 1, 2). Cells of the erythrocyte and granulocyte series appear in about equal numbers and additionally there are scattered megakaryocytes (Figs. 3, 4). At this stage, the latter cells are immature and most possess only one or two nuclei. The relationship of the haematopoietic cells with respect to sinusoids is difficult to determine. Although no distinct lining cells can be

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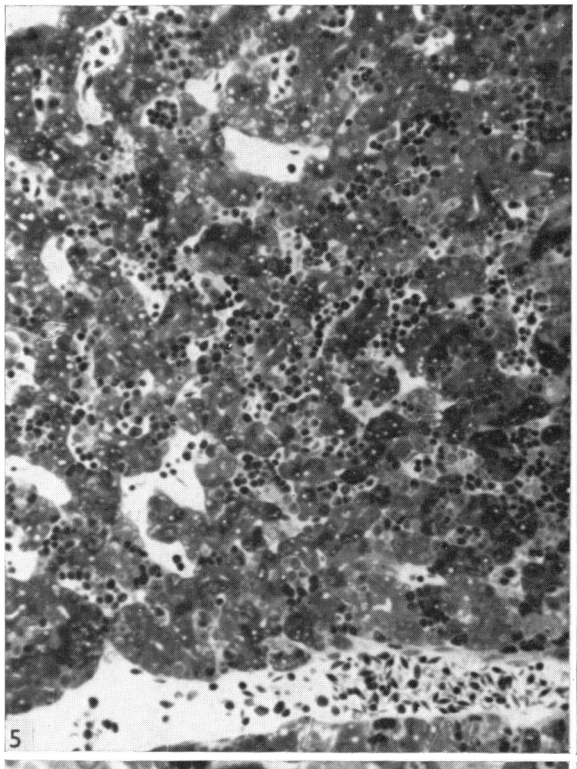
Figs. 1–4 are from the newborn opossum (1·5 cm).

Fig. 1. Hepatic cells appear concentrated around the central veins, and islands of darkly staining haematopoietic cells are scattered between them. Note the nucleated red blood cells within the large sublobular vein (right centre). Paraffin section. Haematoxylin and eosin. × 85.

Fig. 2. Hepatic cells contain pale, vesicular nuclei and the finely granular cytoplasm is vacuolated due to the presence of discrete, lipid droplets. Large islands of haematopoietic elements, one of which contains a mitotic figure (centre), are scattered between hepatic cells. Note the pale elongated nuclei (arrowed) between hepatic and haematopoietic cells. Araldite section. Toluidine blue. × 235.

Fig. 3. Intermediate region of a lobule. Scattered haematopoietic elements, many of the granulocyte series, occur between irregular groups of hepatic cells. Araldite section. Toluidine blue. × 500.

Fig 4. Hepatic cells possess a finely granular cytoplasm and scattered small lipid droplets. Two mitotic figures (small arrows) are present in haematopoietic cells. A megakaryocyte (large arrow) is also present. Araldite section. Toluidine blue. × 625.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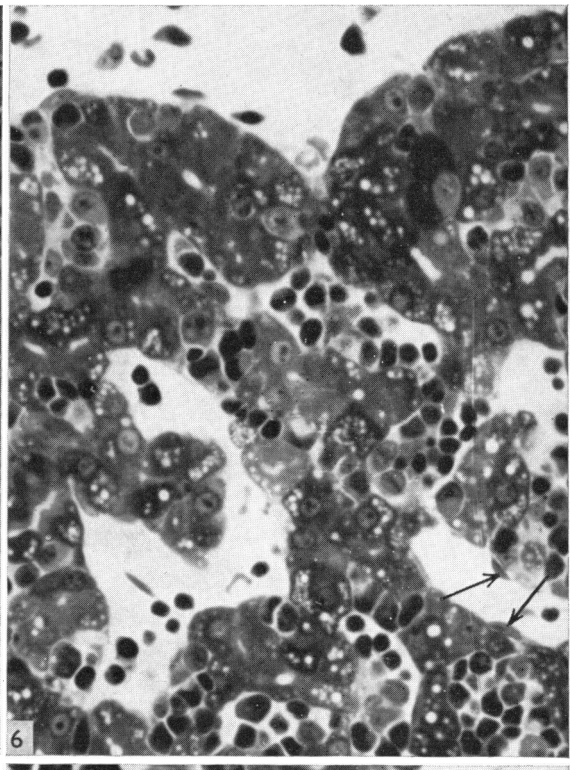
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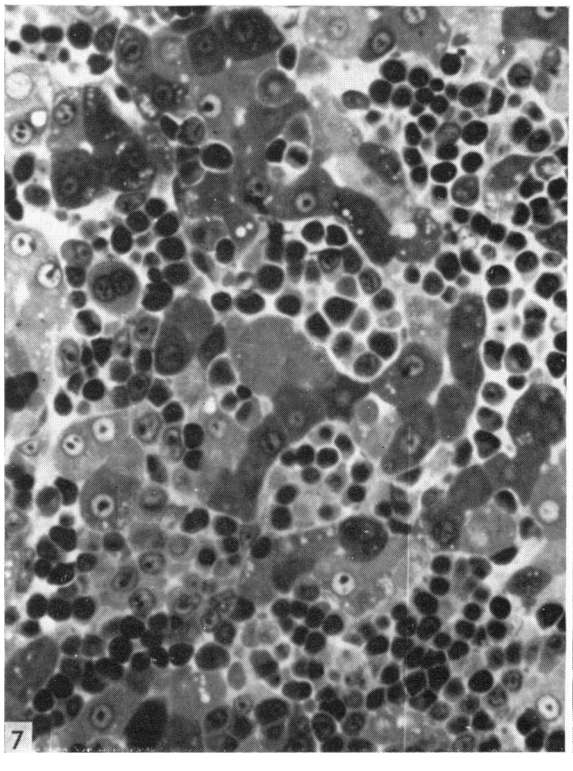
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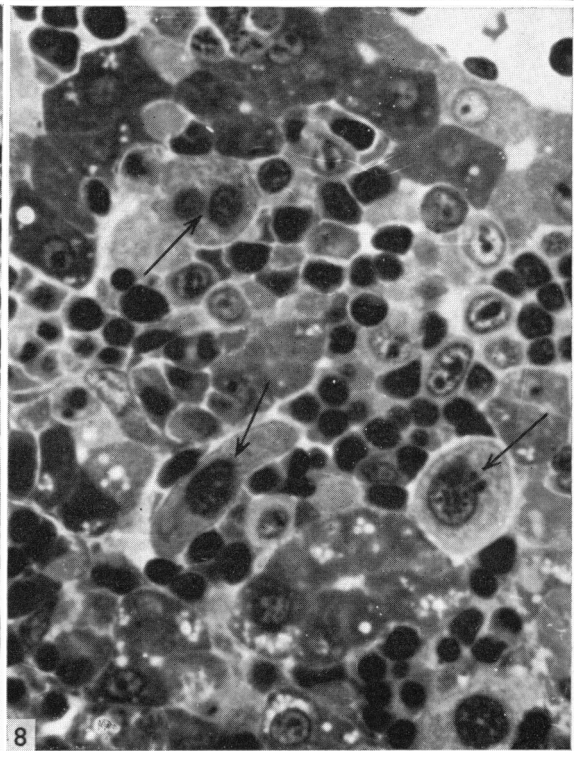
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identified between hepatic and haematopoietic cells, pale, elongated nuclei are occasionally interposed between the two cell types (Fig. 2). Numerous mitoses are present within haematopoietic cells (Figs. 2, 4) and occasional mitotic figures occur in hepatic cells. At this stage, the large sublobular veins contain considerable numbers of nucleated red blood cells (Fig. 1).

By the 6th day of postnatal development* (2·0 cm), lobules generally appear less compact (Figs. 5, 6). Hepatic cells in the interior of lobules are arranged in irregular plates separated by wide spaces containing blood elements. Occasional flattened nuclei, presumed to be those of sinus-lining cells, are interposed between hepatic cells and the spaces (Fig. 6). Nucleated red blood cells are still present within large veins (Fig. 5). Hepatic cells are generally similar in appearance to those of the earlier stage. The cytoplasm is finely granular and the amount of lipid deposition in the form of discrete droplets appears unchanged. However, there is now some variation between cells in the depth of staining of the cytoplasm, and fat droplets are present in larger numbers within the more densely staining cells (Figs. 7, 8). Occasional mitoses occur within hepatic cells. Compared with the earlier stage, there is a definite increase in the number of haematopoietic elements, although they now appear more scattered throughout the lobule and there is less tendency for them to form large islands (Figs. 5, 7). While most elements are of the erythrocyte series, granulocytes also are increased in total number. Although there is a significant increase in the number of progranulocytes at this stage, they constitute only a small proportion of the haematopoietic cells and most granulocytes seen represent the later stages of development. Megakaryocytes also are present in increased numbers and their cytoplasm exhibits an irregular granularity (Fig. 8). While most of these cells still contain only one or two nuclei, a few do exhibit three or four nuclei.

Hepatic cells show a more regular arrangement of plates radiating from the central vein in the 2·5 cm stage (9 days postnatal) (Fig. 9). However, at the periphery of the lobule, they still lack organization and occur as large groups of cells separated by scattered, irregular islands of haematopoietic cells. Hepatic cells appear to be some-

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Figs. 5–8 are from the 2·0 cm opossum (6 days after birth).

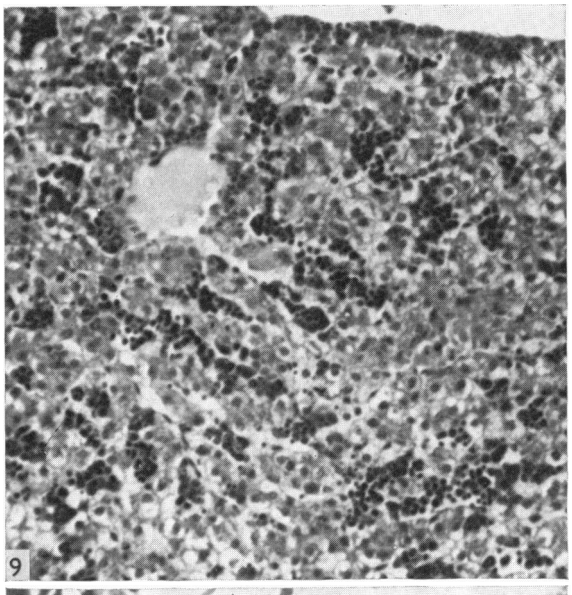
Fig. 5. Irregular plates of hepatic cells are separated by wide vascular spaces. Haematopoietic cells are scattered throughout the lobule. Nucleated red blood cells are present in the sublobular vein (bottom). Araldite section. Toluidine blue. × 100.

Fig. 6. Peripheral region of the lobule, with a portion of a sublobular vein above. Wide spaces between irregular plates of hepatic cells contain circulating blood elements and small groups of haematopoietic cells. Occasionally the wide spaces are separated from hepatic cells by flattened nuclei (arrows). Araldite section. Toluidine blue. × 300.

Fig. 7. Intermediate zone of a lobule. There is some variation in the depth of staining of hepatic cells. The wide spaces between hepatic cells are crowded with haematopoietic elements, principally of the erythrocyte series. Araldite section. Toluidine blue. × 375.

Fig. 8. Central region of a lobule, with a portion of a central vein (upper right). Fat droplets appear more concentrated within the more densely staining hepatic cells. Three megakaryocytes (arrowed), with a granular cytoplasm, a small group of granulocytes (top centre), and numerous cells of the erythrocyte series occur between hepatic cells. Araldite section. Toluidine blue. × 550.

* Approximate age determinations are based on results presented by Moore & Bodian (1940) and Reynolds (1942).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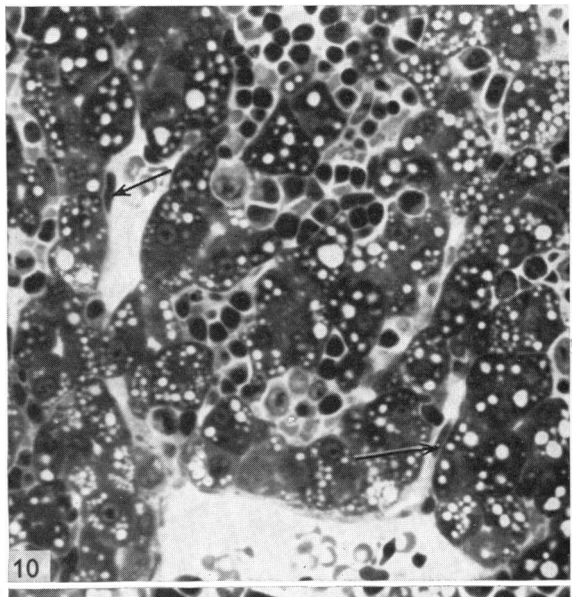
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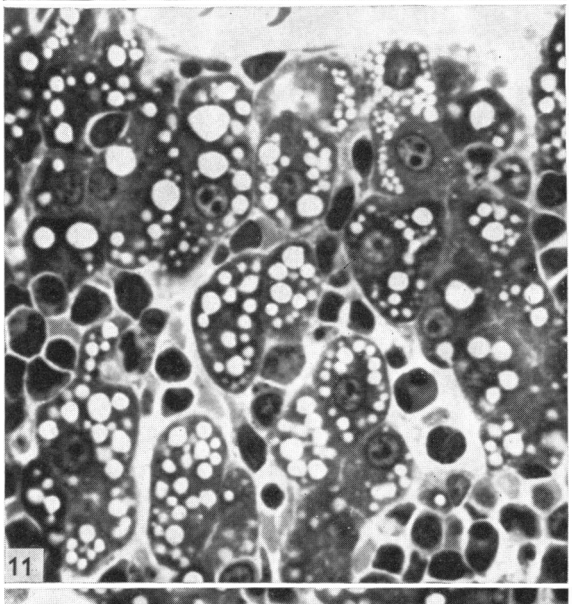
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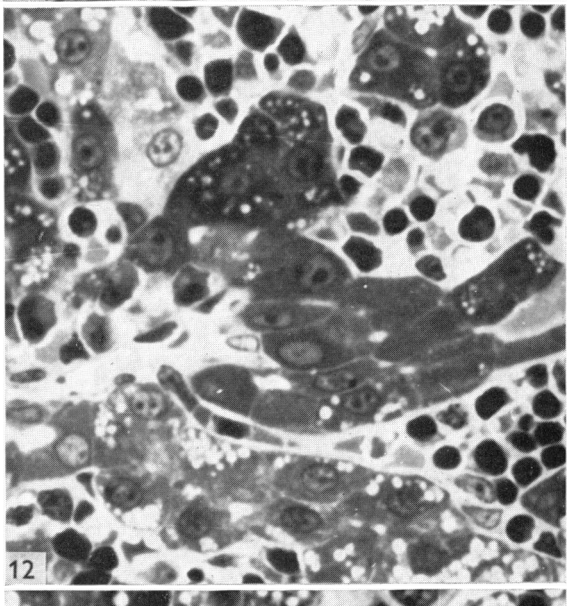
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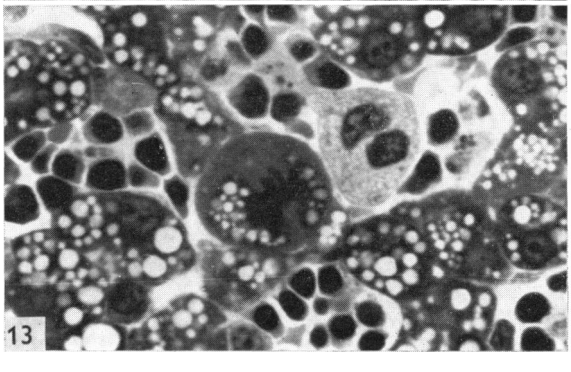
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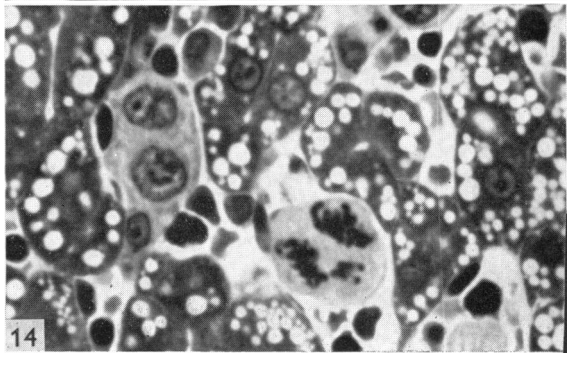
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what larger in size than those of earlier stages, but this is probably due principally to an increase in the amount of lipid deposition. Many cells, particularly those situated centrally within the lobule, contain large numbers of lipid droplets of various sizes (Figs. 10, 11). Nuclei again are small, vesicular, and with distinct nucleoli. At the periphery of the lobule, there commonly are more regular plates of small hepatic cells relatively free of lipid droplets (Fig. 12). These cells appear to be organizing into the limiting plate of the lobule. There is a marked increase in mitotic activity within hepatic cells at this stage (Fig. 13). Although there is some reduction in the total number of haematopoietic elements, the number is still greater than that present at birth. The reduction from the previous stage appears to be due principally to maturation and loss of granular leucocytes. The haematopoietic elements seen are mainly of the erythrocyte series and megakaryocytes (Figs. 12, 14). Some of the latter cells occasionally exhibit mitotic activity (Fig. 14). Nucleated red blood cells are still present in circulating blood at this stage (Fig. 10). Where hepatic cells occur in plates, the sinusoids between are lined by scattered, flattened cells (Fig. 10). However, at the periphery of the lobule, where hepatic cells occur in large groups separated by small clumps of haematopoietic cells, sinus-lining cells are difficult to visualize (Figs. 12, 14).

In the 3 cm stage (15 days postnatal), irregular plates of hepatic cells extend throughout each lobule. They are separated by wide spaces, many of which contain small groups of haematopoietic elements (Fig. 15). Hepatic cells are small, and their densely staining, finely granular cytoplasm still possesses small lipid droplets, although the latter are reduced in number (Fig. 16). Mitotic activity within the cells is decreased to about that of the initial stages. Haematopoietic elements, which occur in small groups, are restricted principally to the erythrocyte series and to megakaryocytes.

By the 4·5 cm stage (20 days postnatal), the lobular arrangement is quite definite. Irregular, radiating plates of hepatic cells extend from a wide central vein to the periphery of the lobule, where there are clearly defined periportal areas that contain small bile ducts, not noted in the earlier stages (Fig. 17). The cytoplasm of hepatic cells is finely granular and the content of lipid droplets is increased somewhat over

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Figs. 9–14 are from the 2·5 cm opossum (9 days after birth).

Fig. 9. Hepatic cells form irregular, radiating plates in relation to the wide central vein (top left). Peripherally within the lobule, hepatic cells lack organization. Darkly staining islands of haematopoietic cells are scattered throughout the lobule. Paraffin section. Haematoxylin and eosin. × 100.

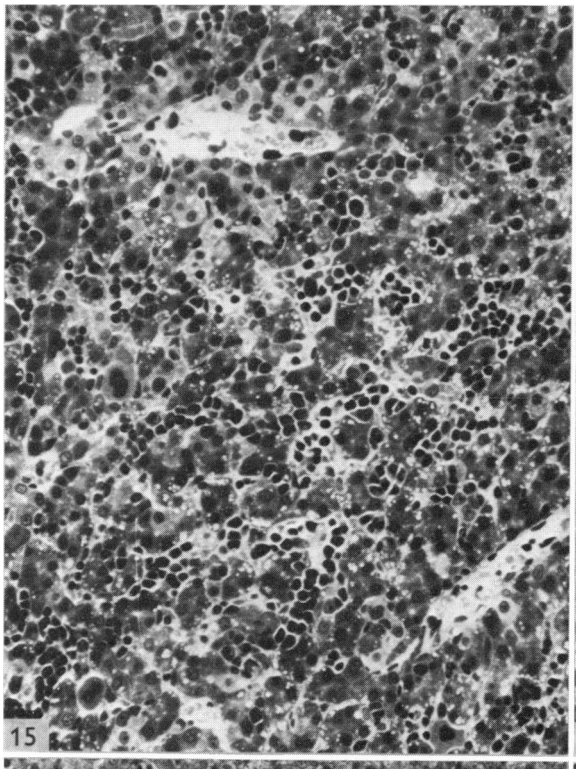
Fig. 10. Central region of a lobule, with a portion of the central vein (below). Hepatic cells contain numerous, discrete lipid droplets. The sinusoids between hepatic plates are lined by flattened cells with densely staining nuclei (arrows). Araldite section. Toluidine blue. × 270.

Fig. 11. Similar region to Fig. 10, with a portion of the central vein (above). Hepatic cells contain large numbers of lipid droplets of various sizes. The haematopoietic elements present include some of the granulocyte series. Araldite section. Toluidine blue. × 500.

Fig. 12. Periphery of a lobule. Small hepatic cells, relatively free of lipid droplets, form regular plates (centre). Araldite section. Toluidine blue. × 375.

Fig. 13. Hepatic cells appear markedly vacuolated due to the presence of numerous lipid droplets. One cell (centre) exhibits mitotic activity. Araldite section. Toluidine blue. × 450.

Fig. 14. Two megakaryocytes, one of which is in mitosis, occur between hepatic cells. Araldite section. Toluidine blue. × 430.



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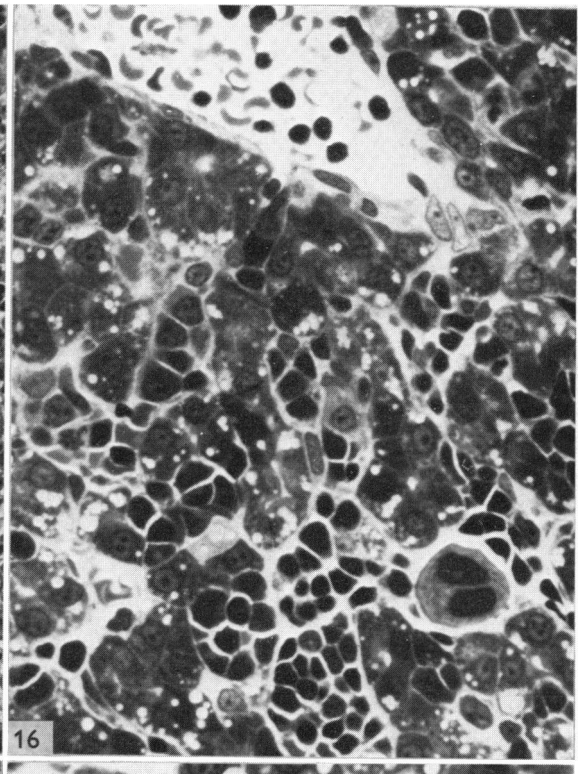

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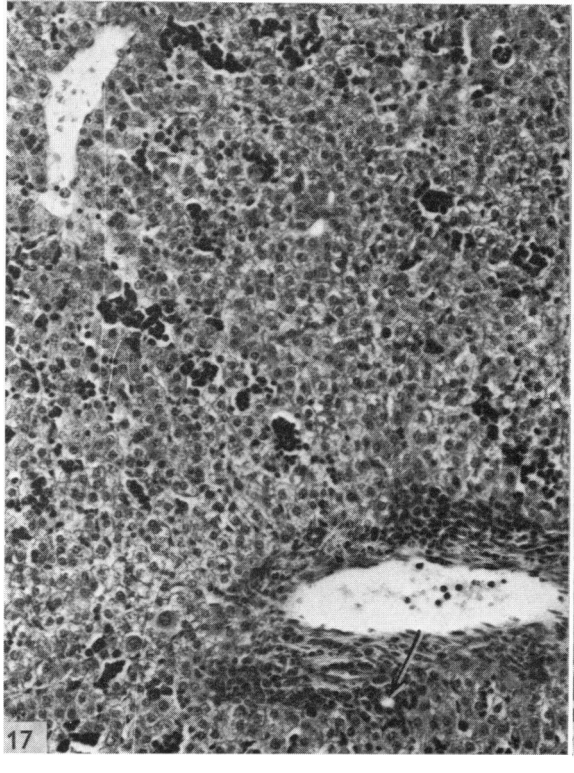

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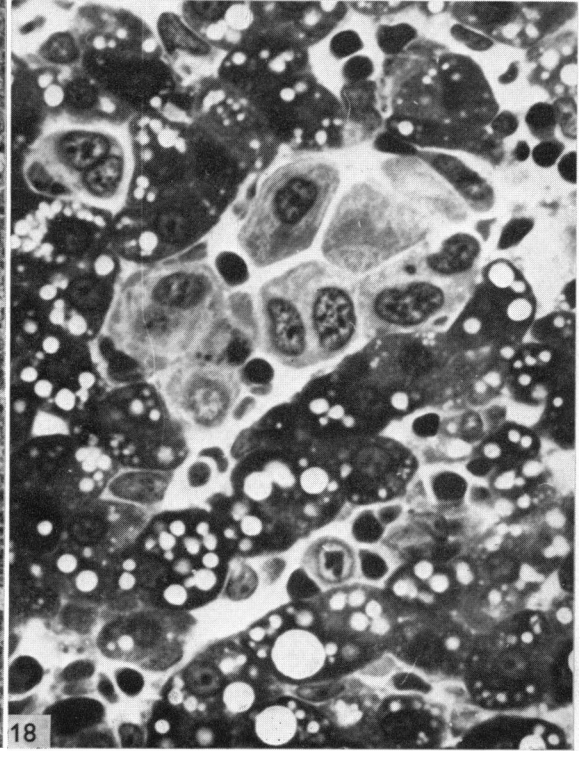

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the previous stage. Additionally, there is increased mitotic activity of the cells. Sinusoids are clearly defined, and many contain small groups of haematopoietic elements, although the latter show a considerable reduction in number from the earlier stages. They are principally of the erythrocyte series and exhibit little mitotic activity. Numerous megakaryocytes, occurring either singly or in large clumps (Fig. 18), are also present.

Changes at 45 days postnatal (7·5 cm) consist of better definition of the individual lobules and of the periportal areas (Fig. 19). Hepatic cells, which appear crowded at the periphery of the lobule, still contain considerable quantities of lipid material in the form of discrete vacuoles (Fig. 20). Some mitotic activity continues within the cells. Haematopoietic elements now occur only as small scattered groups, principally toward the periphery of each lobule (Fig. 19). They consist of megakaryocytes and a few representatives of the erythrocyte series, mainly normoblasts and a few pronormoblasts. No mitotic activity is present within haematopoietic elements at this stage.

Essentially the appearance of the liver in the 10·0 cm stage (60 days) is similar to that of the previous stage. However, even at low magnification, the densely staining nuclei of sinus-lining cells are now clearly apparent (Fig. 21). The cytoplasm of hepatic cells, densely staining and finely granular, contains numerous lipid droplets, many of which are small and give a honeycomb appearance to the cytoplasm (Figs. 22, 23). Haematopoietic activity is similar to that noted in the previous stage, although the erythrocyte series is now represented exclusively by normoblasts. Mitotic activity, no longer a feature of haematopoietic elements, continues in hepatic cells (Fig. 23).

The major change noted 85 days after birth (15·5 cm) relates to the cytoplasm of hepatic cells, which now shows patchy staining (Fig. 24). In the plastic-embedded material, the background cytoplasm is pale and finely granular, and scattered throughout it are densely staining granules and rodlets, thought to be mitochondria. Some cells still contain a few lipid droplets, but many now appear devoid of this material. Between hepatic cells, the densely staining nuclei of sinus-lining cells are seen frequently (Fig. 24). Haematopoietic elements are represented only by a rare late normoblast and scattered megakaryocytes. Hepatic cells still exhibit some mitotic activity.

An almost adult appearance to the liver architecture is achieved by the 20·0 cm stage (juvenile). Liver plates are well-formed and the sinusoids are wide and clearly delineated (Fig. 25). Parenchymal cells are large and nuclear characteristics remain

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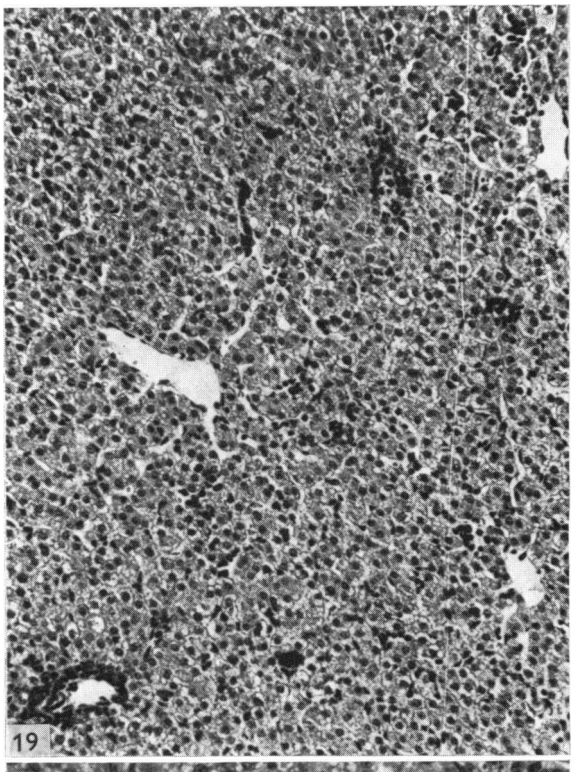
Fig. 15. 3 cm stage (15 days after birth). Irregular plates of hepatic cells, separated by small groups of haematopoietic elements, extend throughout the lobule. Araldite section. Toluidine blue. × 150.

Fig. 16. 3 cm stage. Hepatic cells possess a densely staining, finely granular cytoplasm and few lipid droplets. Haematopoietic elements are principally of the erythrocyte series. A megakaryocyte (lower right) is also present. Araldite section. Toluidine blue. × 300.

Fig. 17. 4·5 cm stage (20 days after birth). The lobular arrangement is quite distinct, and hepatic plates extend from the central vein (upper left) to the periportal area (lower right). The latter contains a small bile duct (arrowed). Small groups of darkly staining haematopoietic cells are scattered throughout the lobule. Paraffin section. Haematoxylin and eosin. × 100.

Fig. 18. 4·5 cm stage. Hepatic cells appear vacuolated due to the content of lipid droplets. Centrally, there is a large group of megakaryocytes. Araldite section. Toluidine blue. × 450.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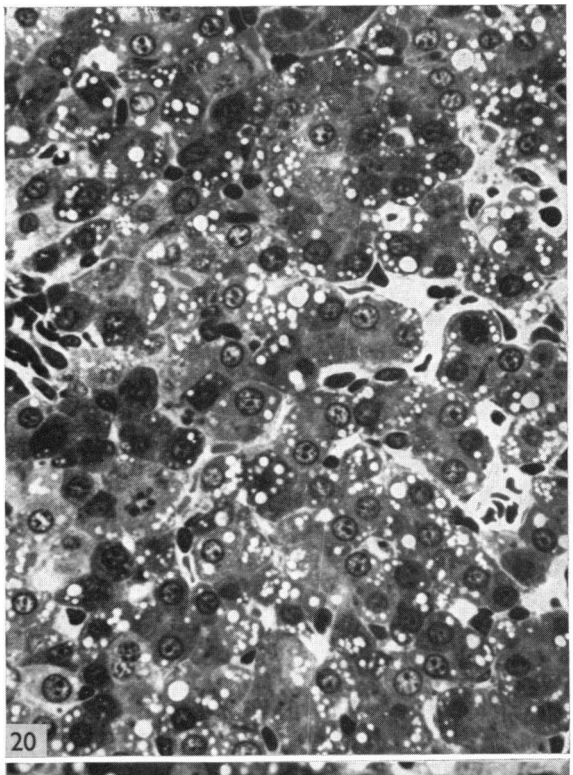
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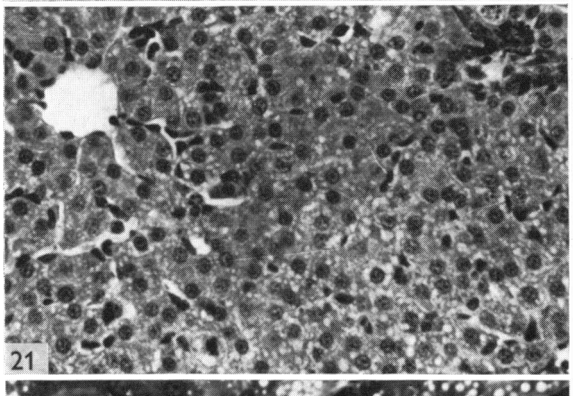
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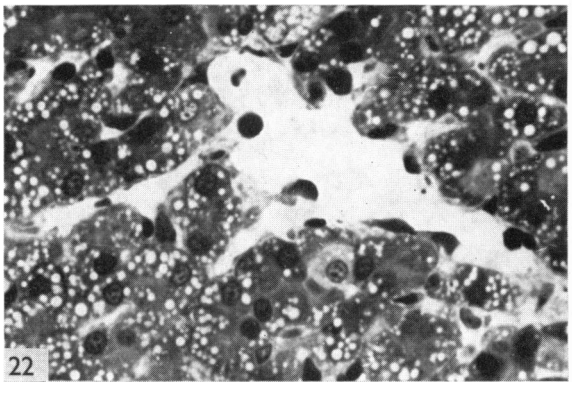
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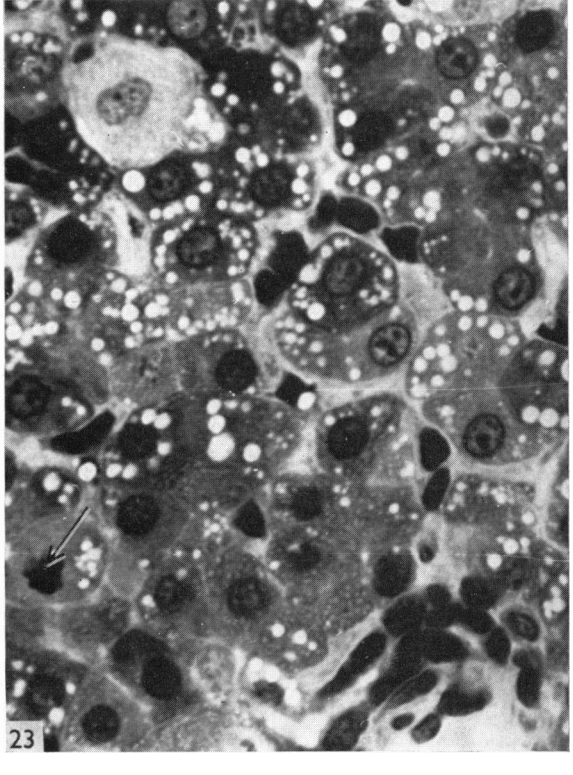
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unchanged. The lipid content is reduced to small droplets in the peripheral cytoplasm. In perinuclear areas, the cytoplasm appears pale. No haematopoietic cells were noted at this stage.

The adult opossum exhibits the typical mammalian pattern of liver architecture. Each lobule, arranged around a central vein, is bounded incompletely by connective tissue (Fig. 26). The periportal areas are generally small and the contained vessels and tributaries of the bile duct are surrounded by sparse amounts of connective tissue. Hepatic cells, arranged in irregular plates, are uniform in size and possess pale, regular nuclei. As in the earlier stages, only a few binucleate cells are present. The cytoplasm appears heterogeneous and contains no obvious lipid droplets (Fig. 27). Irregular, dense nuclei of sinus-lining cells occur between the plates of hepatic cells. Apart from an occasional megakaryocyte, the liver is devoid of haematopoietic elements.

Quantitative findings

The number of hepatic cells per high power field (HPF) at each stage of development is shown in Fig. 28: also presented are the corresponding numbers of mitotic and binucleate cells per 1000 hepatic cells. Two peaks of mitotic activity are noted, one at the 2·5 cm stage and a second at the 4·5 cm stage. Each of these peaks precedes a subsequent increase in the total number of hepatic cells seen per HPF, and they also coincide with a modest increase in the number of binucleate cells. Apart from these two periods, the numbers of hepatic cells in mitosis and of binucleate cells remain fairly constant throughout the period of evaluation. The hepatic cells appear to reach maximum numbers per HPF at the 7·5 cm stage and thereafter maintain a constant level to maturity. The apparent decrease in the number of hepatic cells noted at the 2·0 and 2·5 cm stages coincides with a period of marked haematopoietic activity (Fig. 29).

Haematopoiesis is a prominent feature of the liver, especially up to the 3·0 cm stage (15 days postnatal). This is manifest both in the number of haematopoietic cells per HPF and in the intense mitotic activity. At birth, 79 % of the cells in the liver are of haematopoietic origin, and these cells show a mitotic rate of 40/1000 cells. By nine days postnatal (2·5 cm), haematopoietic elements make up 85 % of the cells seen. The

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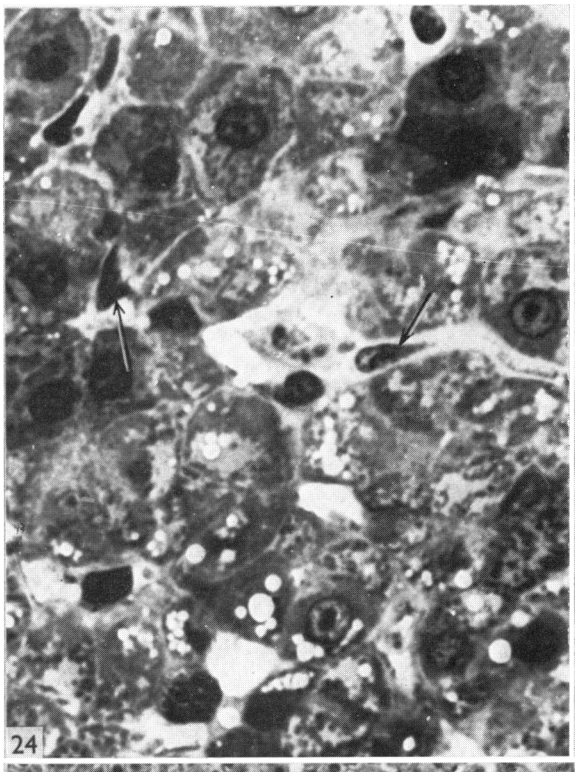
Fig. 19. 7·5 cm stage (45 days after birth). Central veins, radiating plates of hepatic cells, and periportal areas are clearly defined. Only a few scattered foci of haematopoietic elements remain. Paraffin section. Haematoxylin and eosin. × 100.

Fig. 20. 7·5 cm stage. Hepatic cells possess finely granular cytoplasm and still contain numerous lipid droplets. Sinusoids are distinct and contain mature blood elements. Araldite section. Toluidine blue. × 300.

Fig. 21. 10·0 cm stage (60 days after birth). Portion of a lobule, with a periportal area (top right). The small densely staining nuclei of sinus-lining cells can be seen clearly. Hepatic cells appear vacuolated. Paraffin section. Haematoxylin and eosin. × 200.

Fig. 22. 10·0 cm stage. Central region of a lobule. Hepatic cells contain numerous lipid droplets in the peripheral cytoplasm. Araldite section. Toluidine blue. × 230.

Fig. 23. 10·0 cm stage. Hepatic cells contain numerous lipid droplets: many of the latter are now small and give a honeycomb appearance to the cytoplasm. Mitotic activity is still present in hepatic cells (arrow). A small bile duct is also present (lower right). Araldite section. Toluidine blue. × 640.



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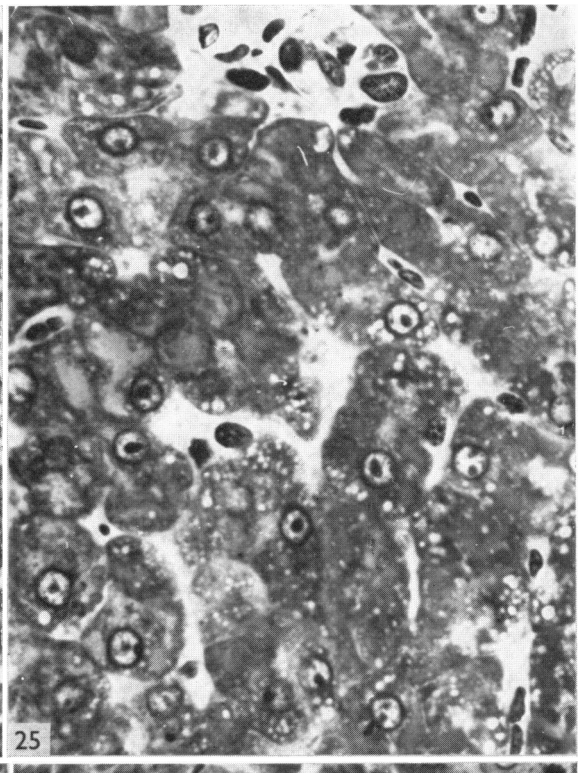

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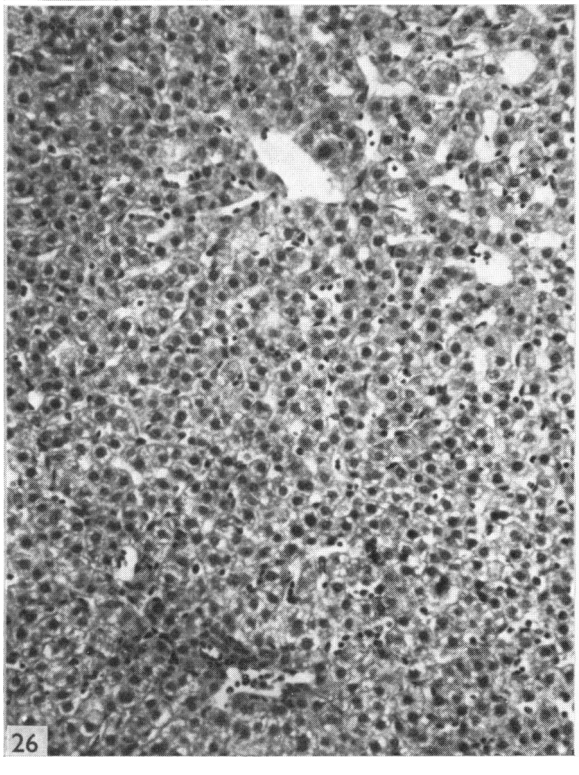

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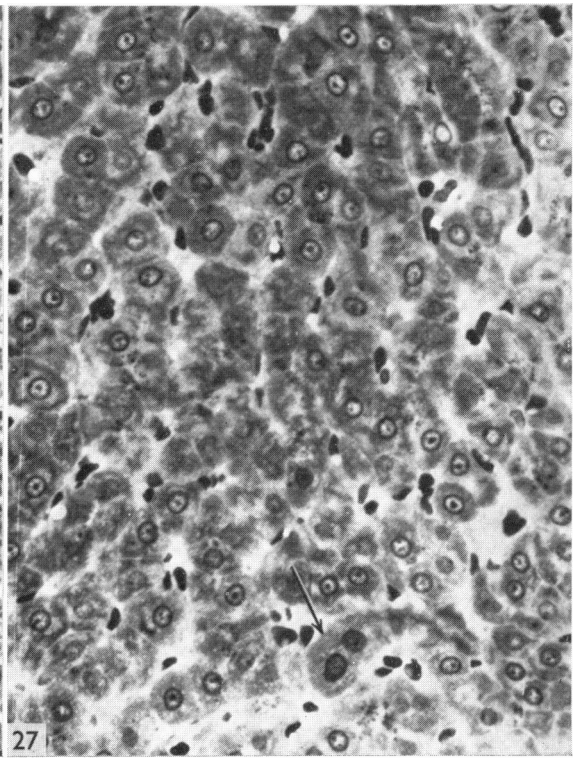

27

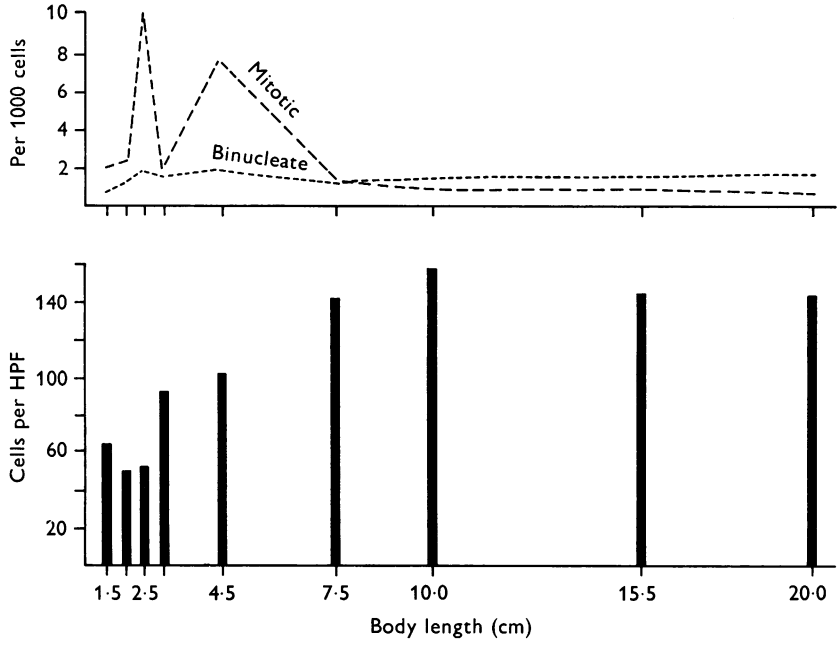

Fig. 28. Quantitative data for hepatic cells. The lower figure shows the number of hepatic cells per HPF at the different stages of development. In the upper figure the corresponding number of mitotic and binucleate hepatic cells are shown per 1000 hepatic cells.

number of haematopoietic cells rapidly declines from these high values so that by 45 days (7·5 cm), haematopoietic cells constitute only 7 % of the cells present in the liver sections. From Table 1 it can be seen that at birth granulocytes and erythrocytes are present in almost equal numbers. The increase in haematopoietic cells at the 2·0 cm stage is due almost entirely to a marked increase in all stages of erythrocyte development. Granulocytes show only a slight increase in total numbers at this stage, but there is a significant increase in the number of progranulocytes and a corresponding decrease in the number of 'blast' cells. The latter are large cells with features similar to those of progranulocytes, but they possess a more vesicular nucleus and are devoid of cytoplasmic granules. Tentatively they are considered to represent primitive

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Fig. 24. 15·5 cm stage (85 days after birth). The cytoplasm of hepatic cells exhibits a patchy staining and contains densely staining granules, thought to be mitochondria. The nuclei of sinus-lining cells (arrowed) appear dark. Araldite section. Toluidine blue. × 625.

Fig. 25. 20·0 cm stage (juvenile). Irregular plates of hepatic cells, which contain a few lipid droplets in the peripheral cytoplasm, are separated by wide sinusoids. No haematopoietic elements are present. Araldite section. Toluidine blue. × 500.

Fig. 26. Adult opossum. Portion of a lobule. Hepatic plates extend from the central vein to the periphery of the lobule, where a small periportal area is present (bottom centre). Paraffin section. Haematoxylin and eosin. × 100.

Fig. 27. Adult opossum. The cytoplasm of hepatic cells appears heterogeneous and contains no obvious lipid droplets. The dense nuclei of sinus-lining cells are apparent between the plates of hepatic cells. One megakaryocyte (arrow) is present. Araldite section. Toluidine blue. × 300.

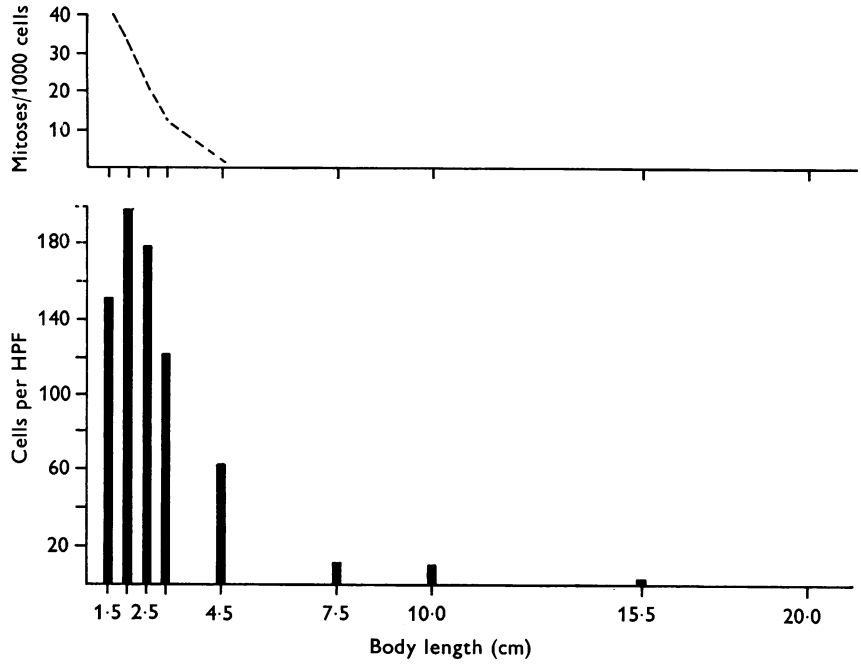

Fig. 29. Quantitative data for haematopoietic cells in the liver of the postnatal opossum. The lower figure shows the total number of haematopoietic cells (erythrocytic, granulocytic and megakaryocytic) per HPF, while the upper figure shows the number of mitotic cells per 1000 haematopoietic cells.

Table 1. *Haematopoietic elements in developing liver*
(No. of cells per HPF.)

	Erythrocytic				Granulocytic				
Body length	Erythro-blasts	Pronormo-blasts	Normo-blasts	Total	'Blast'	Pro-granul-ocytes	Granul-ocytes	Band and PMN*	Total
1·5	3·1	29·2	37·9	70·1	1·2	0·4	25·5	45·9	73·0
2·0	15·4	58·3	51·0	124·7	0·7	7·8	37·9	29·2	75·6
2·5	3·3	36·2	55·2	94·7	—	2·6	15·8	28·5	46·9
3·0	1·5	37·5	29·4	68·4	—	0·5	8·2	14·4	23·1
4·5	1·5	5·7	24·1	31·3	—	0·08	2·1	5·6	6·78
7·5	—	0·8	5·5	6·3	—	—	0·12	4·3	4·42
10·0	—	—	3·7	3·7	—	—	0·02	4·6	4·62
15·5	—	—	0·2	0·2	—	—	—	3·1	3·1
20·0	—	—	—	—	—	—	—	0·2	0·2

* PMN = polymorphonuclear leucocytes.

reticular cells or haemocytoblasts. In subsequent stages the marked diminution in proliferation, accompanied by maturation, results in a rapid decline in both erythrocytic and in granulocytic cells. This decrease occurs earlier among the granulocytic elements.

The number of megakaryocytes per HPF is shown in Table 2. These cells also con-

Table 2. *Megakaryocytes in developing liver*

Body length	Total cells per HPF	Nuclei – percent of cells with				Mean no. nuclei per cell
		1	2	3–4	lobulated	
1·5	0·74	70·2	25·5	4·3	—	1·40
2·0	1·25	55·5	40·2	4·3	—	1·44
2·5	1·20	38·6	48·6	12·8	—	1·58
3·0	0·95	33·4	58·2	16·2	2·2	1·64
4·5	1·10	27·5	46·8	33·5	1·2	1·77
7·5	0·65	21·3	49·6	21·3	7·8	1·83
10·0	0·21	n.d.	n.d.	n.d.	n.d.	—
15·5	Rare	—	—	—	—	—
20·0	—	—	—	—	—	—

n.d. = not determined.

tribute to the total increase in haematopoietic elements seen at the 2·0 cm stage but, unlike the other blood-forming cells, megakaryocytes remain at almost the same level until the 4·5 cm stage. Their decline thereafter is rapid. As judged by the number of nuclei that are present, the cells do undergo a progressive maturation. Only a few of the megakaryocytes show lobulated nuclei and, even in the late stages of development, most of these cells show discrete, rounded or oval nuclei.

DISCUSSION

The present investigation demonstrates that considerable changes occur in the opossum liver during the postnatal period. The reorganization principally involves differentiation of hepatic cells and their rearrangement into plates. At birth, hepatic plates are not evident and sinusoids cannot be defined clearly. In the 20 days after birth, hepatic cells become arranged into irregular plates that extend from the central vein to the periphery of the lobule. At the end of this period, periportal areas are present and the lobular arrangement is quite definite. Coincident with the development of hepatic plates, sinusoids become apparent. This sequence of changes is similar in all respects to that which occurs during postnatal development of the rabbit liver (Leeson & Cutts, 1972), although in the latter species the changes are complete by 15 days after birth.

The process by which sinusoids make their appearance is obscure. Leeson & Cutts (1972), in the rabbit, suggested that sinusoids may be present at birth but that they are masked in the early postnatal period by the concentration of haematopoietic cells. In the present study pale, elongated nuclei, interposed between hepatic cells and the islands of haematopoietic elements, were noted at birth. These may represent sinus-lining cells. It is possible that additional cells of this type may arise from haematopoietic precursors since the quantitative results indicate that haemocytoblasts disappear at a time when sinus-lining cells first become clearly apparent (2·0 cm stage). A previous study on rat liver (Greengard, Federman & Knox, 1972) has indicated that the increase in number of such cells during postnatal development may be extremely modest. In this quantitative study, these authors noted that

Kupffer cells represent less than 3 % of the total liver volume in both fetal and adult rat livers. They also represent 4 % of the total number of cells in fetal liver, but in the adult liver they come to constitute 37 % of the total number of cells, because of the large volume and consequent low frequency of parenchymal cells. Thus the latter figure is somewhat misleading, and the absolute increase in the number of Kupffer cells between fetal and adult livers is only 1·3-fold.

Liver growth during the early postnatal period results principally from hyperplasia. Numerous mitoses are present up until the 7·5 cm stage, with peaks of mitotic activity at the 2·5 cm and 4·5 cm stages. Each of these peaks precedes a subsequent increase in the total number of hepatic cells. This situation differs somewhat from that which occurs in the rabbit, where liver growth is a result both of hyperplasia and of hypertrophy (Leeson & Cutts, 1972). It is of interest that the peaks in mitotic activity in the opossum liver coincide with a modest increase in the number of binucleate cells. A similar sequence of events has been recorded in the rabbit liver (Leeson & Cutts, 1972) and in the rat liver, where McKellar (1949) and Teir & Ravanti (1953) noted a peak of mitosis at 21 days, just prior to the appearance of polyploid cells. Wheatley (1972) observed that binucleate hepatic cells appear in large numbers shortly after weaning in the rat, preceded by a burst of mitotic activity. He also made the interesting observation that liver cell binucleation remains at a very low level if weaning is delayed until 25 days of age, and that thereafter the increase follows a compensatory burst of mitotic activity. This suggests that binucleate cells arise by acytokinetic mitosis. In pig liver, however, where the mitotic index varies from 4·7 in the day old pig to 0·0 in the adult pig, the number of binucleate cells varies from 1·34 % in the newborn to 11·0 % in the adult (White, 1939). In the opossum, the number of binucleate cells never reaches such proportions, and this may be due to the relative absence of hypertrophy as a factor in liver growth, as indicated by the fact that hepatic cells reach maximum numbers per HPF at the 7·5 cm stage and thereafter maintain a constant level. The uniformity of cell size throughout the series also indicates a lack of hypertrophy in the opossum liver, and this is quite unlike the situation in the rat, where the mean volume of individual parenchymal cells doubles between the 12th and 28th postnatal days (Greengard *et al.* 1972), and in the pig, where again there is a twofold increase in cell size after birth (Bischoff, Richter & Stein, 1969).

The presence of numerous fat droplets within hepatic cells is a prominent feature of the opossum liver during the postnatal period. The amount of lipid deposition appears constant during the first postnatal week but is increased at the 2·5 cm stage (9 days postnatal). Thereafter there is a gradual loss of lipid material from the cells and only a few droplets remain by the 20·0 cm stage (juvenile). Although the functional significance of lipid deposition is obscure, it has been reported in numerous species, including the cat (Chavres, 1923), guinea-pig (Imrie & Graham, 1920; Chavres, 1923), mouse (Deane, 1944), and rabbit (Leeson & Cutts, 1972). In all these species, lipid stores are high at birth and are depleted gradually to adult levels over the next 1–2 weeks. The disappearance of lipid material occurs first from cells in the central regions of the lobules, as noted by Deane (1944) and by Leeson & Cutts (1972). It is possible that deposition of lipid material in hepatic cells during the postnatal period may also occur in other species. White (1939) noted that liver cells in

the newborn pig possess a very vesicular cytoplasm, and that shortly thereafter the cells diminish in size and the cytoplasm becomes more granular, and Sarrut & Nezeloff (1959) described a prominent vacuolation of liver cells in a stillborn human fetus. The latter authors ascribed the vacuolation to pathological changes, but they did not specifically investigate liver lipids.

Du Bois (1963) has reviewed earlier investigations concerning the presence of lipids within hepatic cells, and has pointed out that the fats demonstrated by means of routine histological sections form only a portion of the total lipid reserves contained within the liver. Although Du Bois did not comment upon the possible significance of lipid deposition within the liver during postnatal development, he did suggest that hormonal control of lipid metabolism may involve the thyroid. Thyroidectomy in the 23 day old rabbit fetus does not prevent growth but it does increase the body's total lipid content, including some increase in hepatic lipids.

Haematopoiesis in the liver has been the subject of study by several other investigators (Gilmour, 1941; Emery, 1956; Sorenson, 1960; Thomas *et al.* 1960). The results of such studies generally have indicated a predominance of erythrocyte formation as a feature of this process: Thomas *et al.* (1960) could find no evidence of granulocytopoiesis in the human liver. On the other hand, granulocytes do develop in the fetal rat liver (Sorenson, 1960) and in the liver of the neonatal rabbit (Leeson & Cutts, 1972), but their formation is subordinate to the production of erythrocytes. Both granulocytes and erythrocytes are formed postnatally in the liver of the opossum, and at birth these elements are present in almost equal numbers. However, a greater production of erythrocytes, relative to granulocytes, results in their subsequent dominance as the haematopoietic cell type. This is particularly evident by the 6th day after birth, but is then maintained throughout the following stages of development.

Loss of haematopoietic cells from the liver occurs through the same orderly sequence of maturational changes that are seen in the bone marrow. Loss of granulocytes from the liver occurs earlier than loss of the erythrocytes, but the process of production and decline is essentially the same for both types of cells. Following a period of intense proliferative activity (mitotic index 40/1000 haematopoietic cells at birth), the total number of haematopoietic cells is increased. There then follows a lengthy period of maturation with little or no replacement of the primitive or 'blast' cells. This is similar to the sequence in the rabbit, where Leeson & Cutts (1972) reported an increased number of haematopoietic units through the first 5 days postnatal, followed by a rapid decline of blood-forming cells thereafter.

The granulocyte and erythrocyte elements show a somewhat different distribution throughout the liver. The erythrocyte elements tend to occur as large groups of the same or closely related stages, whereas the granulocyte precursors are more scattered, or at most form small islets in which the cells may be of only distantly related stages. The more mature forms of granulocytes often are scattered among the developing erythrocytes. The differences in their distribution probably reflect the more motile nature of the granular leukocytes.

In their study of the mouse liver, Nichols & Simmons (1970) have shown that the decrease in haematopoietic cells is concomitant with an increase in hepatic cells from birth to the age of 30 days. A similar event occurs also in the liver in the post-

natal opossum. From birth to the postnatal age of 60 days (10 cm), there is a progressive increase in the number of hepatic cells in this species: during this same period, the number of haematopoietic cells declines. Greengard *et al.* (1972) have shown that haematopoietic elements occupy one-third of the liver volume, and comprise more than 50 % of the total cells, in the liver of the rat during gestation (15–18 days). They continue to form a significant part of the cell population of the liver up to 12 days after birth. A similar finding is evident in the opossum up to 9 days after birth where the haematopoietic cells form 70–80 % of the cells of the opossum liver: 15 days after birth they comprise nearly 50 % of the cells and are still present in appreciable numbers 20 days after birth.

Megakaryocytes are prominent in the liver up to the 20th day of postnatal development and, although their subsequent decline is rapid, they can very occasionally be found even in the adult. Maturation of these cells occurs during the first twenty days, as indicated by the progressive increase in the number of nuclei they possess. Unlike those of many other mammals, the opossum megakaryocyte only rarely develops the large convoluted form of nucleus, and the more usual occurrence is the development of up to four discrete nuclei, which may at times overlap. Cells with more than four nuclei were extremely rare.

The presence of stem cells in developing liver appears to be in some doubt. Thomas *et al.* (1960), although unable to recognize any granulocytopoiesis in the human, did identify cells 'with the features of myeloblasts'. Sorenson (1960), on the other hand, was able to identify the development of heterophil granulocytes in fetal rabbit liver, but could not identify stem cells. He commented that even the most immature haematopoietic elements exhibited cytoplasmic granulation. Cells that resemble primitive blood cell precursors (haemocytoblasts) are present in the liver of the postnatal opossum, but occur in small numbers, and only in the earliest stages. These cells soon disappear and none is found after the 6th day of postnatal development. Coincident with their decline is an increase in the number of progranulocytes and erythroblasts, indicating that the primitive cells are the precursors of the more definitive cells of the haematopoietic lines. However, as has been noted previously, the time at which the numbers of primitive cells are diminished (6th day after birth) also corresponds to that stage of liver development in which the sinus-lining cells of the hepatic sinusoids are first clearly defined. Possibly some of the primitive cells contribute to the formation of the sinus-lining cells. The haemocytoblast is a cell that is capable of self-perpetuation, and its differentiation along a second line to form sinus-lining cells would constitute one means by which the liver is depleted of this element: with continued differentiation of the haematopoietic cells previously derived from the haemocytoblast, decline and extinction of haematopoiesis in the liver would result.

SUMMARY

The postnatal development of the liver has been examined in ten groups of young opossums. At birth, the liver is very immature in appearance: hepatic cells show little organization into plates and large islands of haematopoietic cells occur between the hepatic cells. The hepatic cells are small, and contain numerous lipid droplets. During the first 20 days after birth, hepatic cells are organized into plates

and the lobular arrangement becomes quite definite. Liver growth results principally from hyperplasia: peaks of mitotic activity precede subsequent increases in the total number of hepatic cells. There is a gradual loss of lipid material from hepatic cells up to the 20·0 cm (juvenile) stage.

Haematopoiesis is a prominent feature of the liver up to 15 days after birth. Loss of haematopoietic cells from the liver follows an orderly sequence of maturational changes similar to those seen in the bone marrow. After a period of intense proliferative activity, the total number of haematopoietic cells is increased. This is followed by an extended period of maturation with little or no replacement of the primitive 'blast' cells. The time at which primitive cells disappear (6 days after birth) corresponds to that stage of liver development in which lining cells of the hepatic sinusoids first become clearly defined. The decrease in haematopoietic cells occurs concomitantly with the increase in hepatic cells.

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