

Cytochemical comparison between fetal and maternal tissues

Fetus and mother constitute a temporary biological association, separated only by the placenta. They have different functions though in the fetal period the organs are largely differentiated so that there are no fundamental structural differences between the maternal and fetal organs. To find out whether and how far these functional differences within a bio-economic association can be identified cytochemically in the single cell or in the cellular association, was the purpose of this study. Two points are of importance:

1. The preparation of chemical substrates of various cell associations, in qualitative and semiquantitative respect, and the control of the activities in these associations by selective tests on certain substrates and enzymes.
2. On the basis of this cytochemical analysis, the question for differences in the biochemistry of fetal and maternal tissues is studied.

Methods

The starting materials were pregnant sheep, whose fetuses were won by Caesarean section in the twentieth week of gestation. Ten kinds of corresponding tissues of the mothers and fetuses were examined by means of microscopic smears. In question are tissues from the stomach (abomasum), pancreas, brain (cortex), liver, bone-marrow, spleen, muscle, kidney, omentum, lung. These preparations were stained with:

4 nuclear or nucleic-acid preparations

(haemalaun-eosin; ferric haematoxylin; Feulgen; methyl green pyronin); 3 substrates (fat: scarlet red; glycogen: Best's carmine; alpha-amino-acid groups: ninhydrin); 2 enzymes (dopa-oxydase, alkaline phosphatase).

To avoid random results, 4 series of every animal preparation were examined with these nine stainings so that 720 preparations were obtained, of which 800 microphotographic pictures were taken.

Differences between fetal and maternal tissues

Stomach

The conspicuous differences between fetal and adult stomachs is due to the dispersion of nuclear plasma. The cytoplasmatic areas prevail in the fetal preparation, and the nuclear structures are exactly outlined. In the adult preparation, however, the constituents of cytoplasm stand back behind the nuclear structures (fig. 60, 61). The RNA concentration in the adult preparation is correspondingly smaller, with about an equal concentration of DNA. Fat and glycogen are traceable only in minute quantities, partly extracellular, in all stomach preparations (fig. 62, 63). Striking is the high concentration of the alpha-amino-acids, especially in the fetal stomach. The enzymes are more active in the preparation of the adult animal.

Pancreas

All four nuclear stainings depict the nuclei of the fetal tissue stronger than those of the adult preparation (fig. 64, 65). The cytoplasm of the fetal tissue has more structures, which corresponds also to the ample concentration

and dispersion of RNA whereas in the pale colour of the cytoplasmatic space of the adult pancreas only a few particles can be traced. Fats can scarcely be identified in the adult preparation though their concentration is quantitatively higher. In the fetal tissues, however, the glycogen staining prevails. Only small quantities of alpha-amino-acids, and no alkaline phosphatase can be found. The rather strong activity of the dopa-oxydase, especially in the fetal preparation, is worth mentioning.

Brain

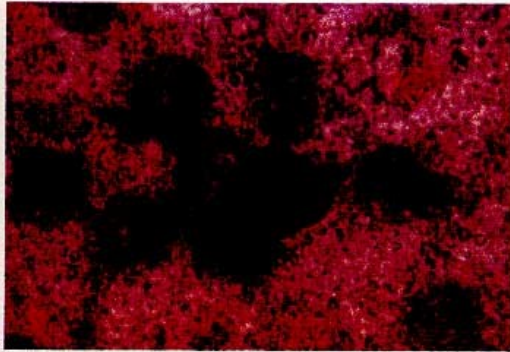
Characteristic of the cerebral tissues are the strong nuclear structures with their fibrous branches, which probably correspond to dendrite fragments and occur more in the fetal tissue (fig. 66, 67, 68, 69). The DNA is intensely stained, only traces of RNA are found.

Fatty substance and glycogen are contained in all preparations, in the adult tissue if the staining is quantitatively increased (fig. 70, 71). Alpha-amino-acids are amply traceable in both series; the activity of ninhydrin prevails in the fetal

Fig. 60:
Stomach-fetal, ferric hematoxylin-eosin 1:1250.
Intense colouration of the cytoplasm.

Fig. 62:
Stomach-fetal, scarlet (1:1250). Good colouration
of nucleus, no *fat* traced.

Fig. 64:
Pancreas-fetal, Feulgen (1:1250)
High concentration of *DNA*, structures hardly
differentiable.



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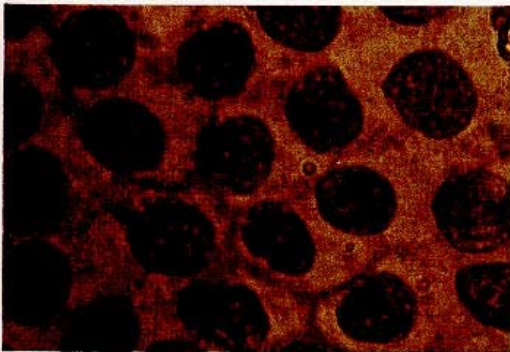
Fig. 61:
Stomach-adult, ferric haematoxylin (1:1250).
abvious appearance of the nuclear structures.

Fig. 63:
Stomach-adult, scarlet (1:1250). Good coloura-
tion of nucleus, little *fat* traced.

Fig. 65:
Pancreas-adult, Feulgen (1:1250). *DNA* nuclear
colouration less intense than in fig. 64.



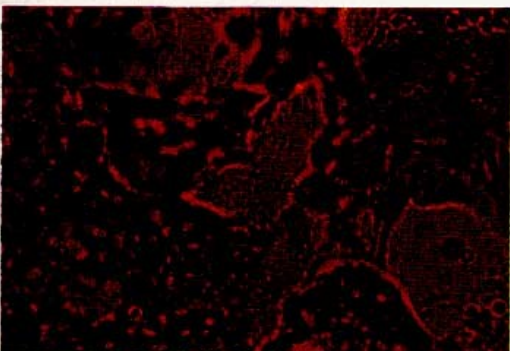
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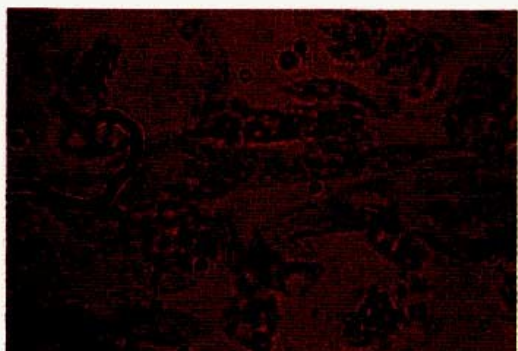
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64



65

preparation. The activity of dopa-oxy-
dase is specially spectacular in the adult
preparation where nuclei, membranes

and fibre structures are coloured. The al-
kaline phosphatase is little active in fetal
and adult tissues.

Fig. 66:
brain-fetal, hemalaun-eosin (1:1250). Little intense cytoplasm colouration, distinct colouration of nucleus.

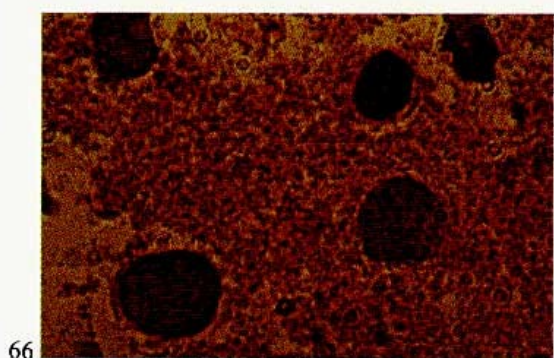
Fig. 68:
Brain fetal, ferric hematoxylin-eosin (1:30). Many fibriform structures.

Fig. 70:
Brain-fetal, Best-carmin colouration (1:1250). Slight *glycogen* colouration.

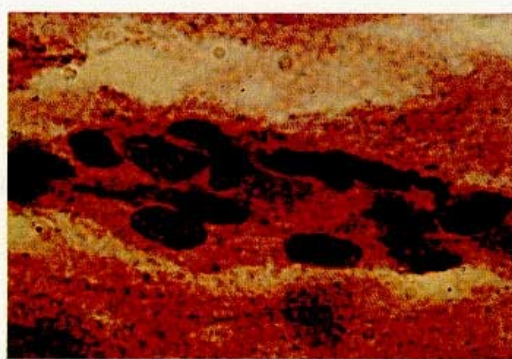
Fig. 67:
Brain-adult, hemalaun-eosin (1:1250) Intense cytoplasm colouration of longitudinal structures.

Fig. 69:
Brain-adult, ferric hematoxylin-eosin (1:1250). Intense cytoplasm colouration. Fragment of medullary sheath.

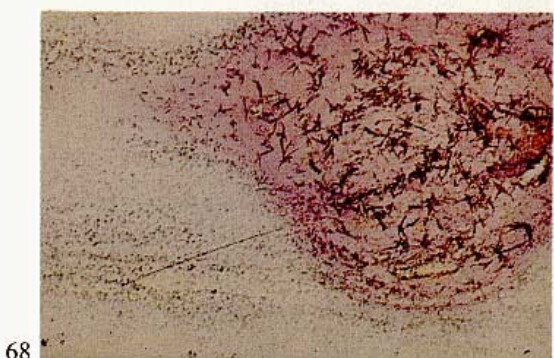
Fig. 71:
Brain-adult, Best-carmin colouration (1:1250). Abundant, island-like concentration of *glycogen*.



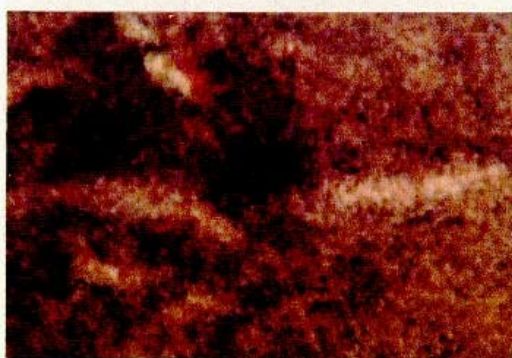
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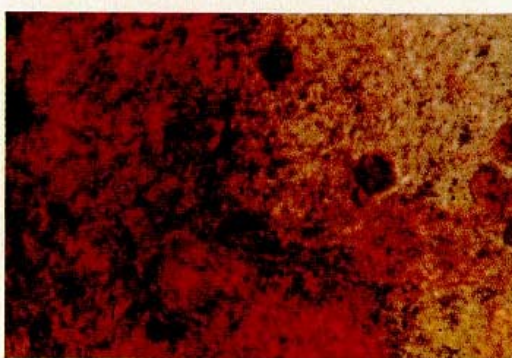
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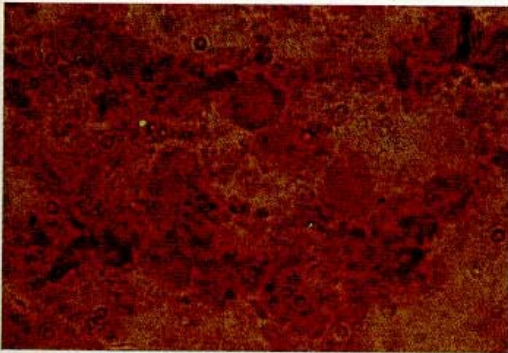


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Fig. 72:
Liver-fetal, Feulgen (1:1250).
Nuclei smaller than in the adult preparation.

Fig. 74:
Liver-fetal, ninhydrin (1:30).
Small amount of *alpha-amino-acids*.

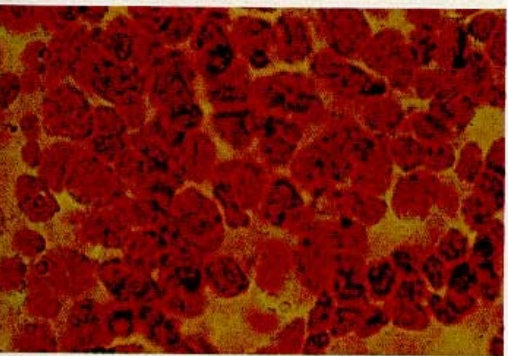
Fig. 76:
Bone-marrow-fetal, Feulgen (1:1250). Round
nuclei prevail.



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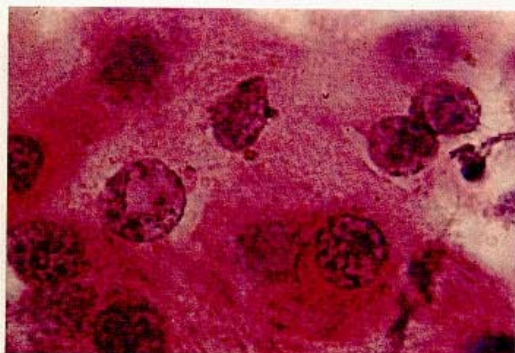


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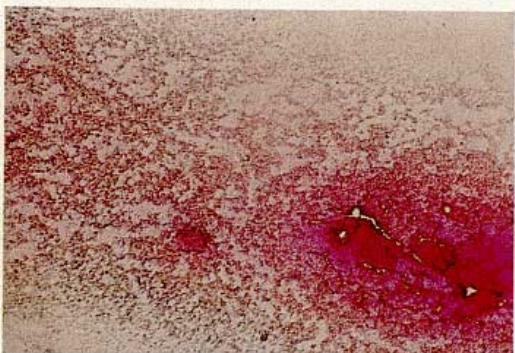
Fig. 73:
Liver-adult, Feulgen (1:1250)
Larger nuclei. Good cytoplasm colouration.

Fig. 75:
Liver-adult, ninhydrin (1:30). Areas abundant in
ninhydrin.

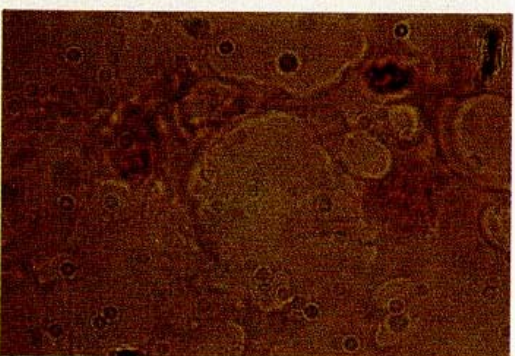
Fig. 77:
Bone-marrow-adult, Feulgen (1:1250).
Fat drops. Reticular structures. Moderate DNA-
colouration within the meshwork.



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Liver

The nuclear structures of the liver preparations come out well, the small nuclei of the fetal tissues appear more intensely

coloured than the very large nuclei of the adult preparations. The cytoplasm takes dye readily but, corresponding to the smaller nuclei, is comparatively better

Fig. 78:
Bone-marrow-fetal, ninhydrin (1:1250). Intense
colouration of the cytoplasm.

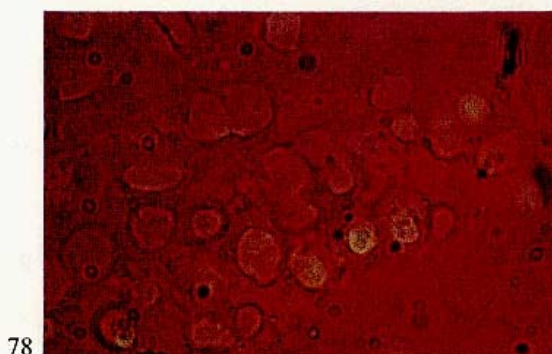
Fig. 80:
Spleen-fetal, methyl green-pyronin (1:1250). In-
tense DNA-colouration of the nuclei. Small
amount of RNA as reddish fundamental tone of
the ground-substance.

Fig. 82:
Spleen-fetal, scarlet (1:1250). Nuclei clearly
structured. Erythrocytes, yellowish. No *fat* traced.

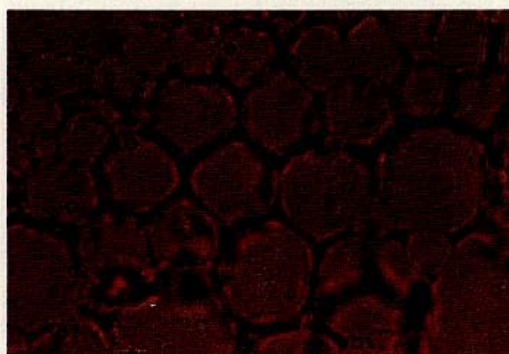
Fig. 79:
Bone-marrow-adult, ninhydrin (1:1250). Con-
centration of ninhydrin to trace the alpha-ami-
no-acids in the seams of the meshwork.

Fig. 81:
Spleen-adult, methyl green-pyronin (1:1250). Fi-
brous structure and nuclei colourable with met-
hyl green. RNA fundamental tone and partic-
les.

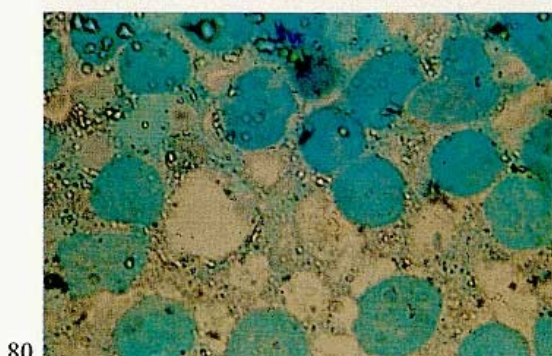
Fig. 83:
Spleen-adult, scarlet (1:1250). Clear nuclear and
fibrous structures. More intense colour of ery-
throcytes. Small amounts of *fat* particles.



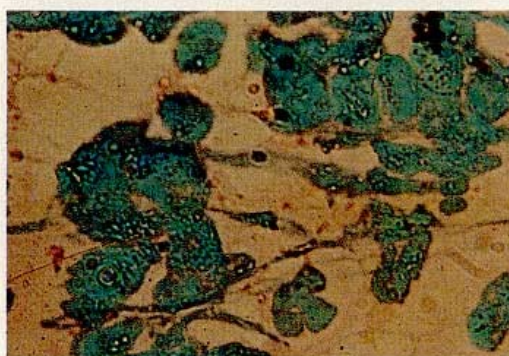
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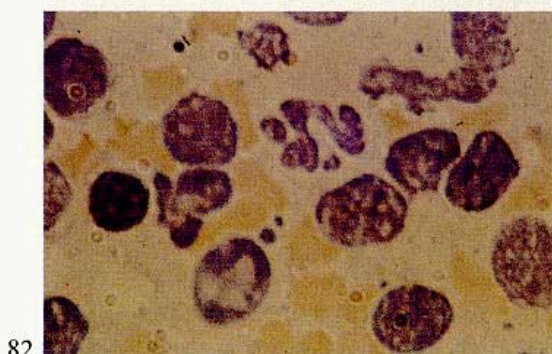
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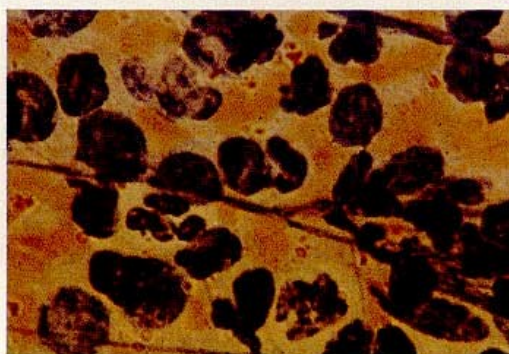
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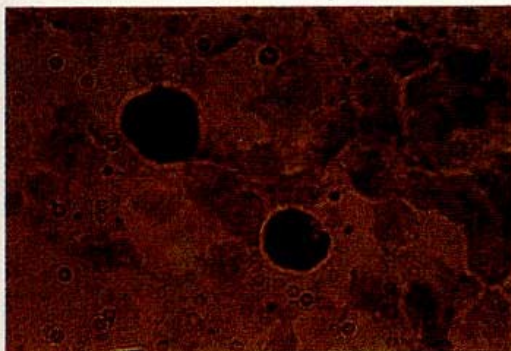


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Fig. 84:
Spleen-fetal, dopa-oxydase (1:1250).
Small amount of dopa-oxydase.

Fig. 86:
Muscle-fetal, ferric hematoxylin (1:1250).
Reticular structure. Roundish-oval nuclei.

Fig. 88:
Muscle-fetal, phosphatase (1:1250). Longitudi-
nal striation accentuated by phosphatase
colouration.

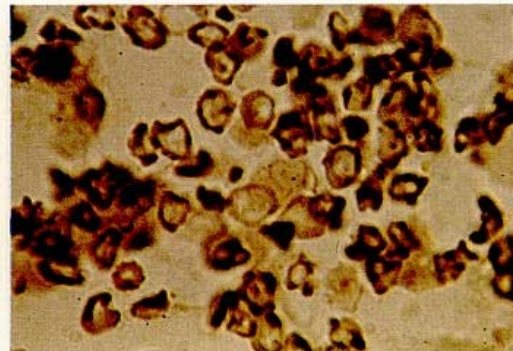


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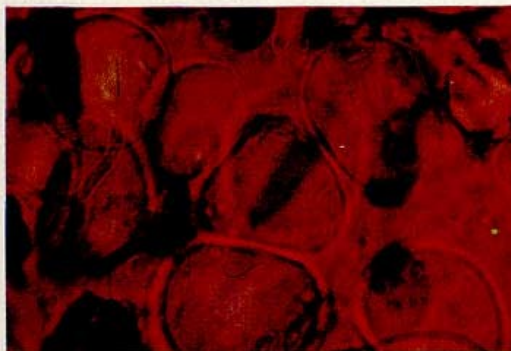
Fig. 85:
Spleen-adult, dopa-oxydase (1:1250). Erythrocy-
te membranes and cytoplasm spaces accentua-
ted.

Fig. 87:
Muscle-adult, ferric hematoxylin (1:1250).
Intense striation with oblong, fusiform nuclei.

Fig. 89:
Muscle-adult, phosphatase (1:1250). Transverse
striation indicating activity.



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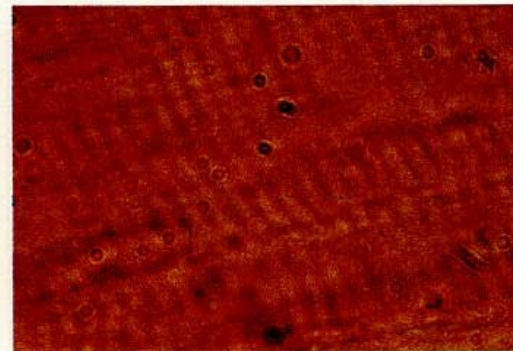
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accentuated in the fetal tissue (fig. 72, 73). These findings correspond to the identification of DNA with rela-

tively slight RNA activity. Fat can be demonstrated only by traces. The adult, unlike the fetal, animal shows a great con-

Fig. 90:
Kidney-fetal, hemalaun – eosin (1:1250). Round, intensely coloured nuclei. Tubular areas.

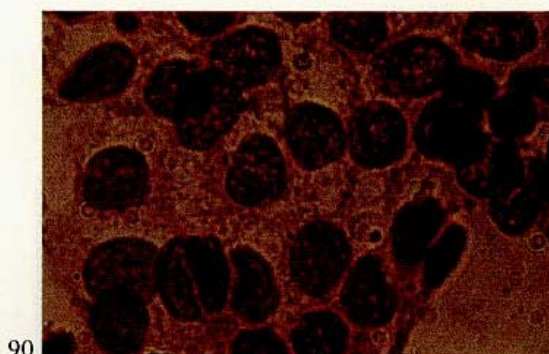
Fig. 92:
Kidney-fetal, ninhydrin (1:1250). Small amounts of ninhydrin.

Fig. 94:
Kidney-fetal, phosphatase (1:1250). Intense colouration. Particles surround the nucleus in the form of crayfish-claws.

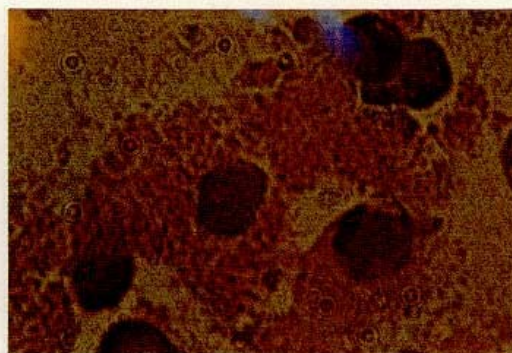
Fig. 91:
Kidney-adult, hemalaun – eosin (1:1250). Less intense colouration of nucleus. Only sporadic deeply coloured cytoplasm.

Fig. 93:
Kidney-adult, ninhydrin (1:1250). Clear colouration of cytoplasm and nuclear membranes.

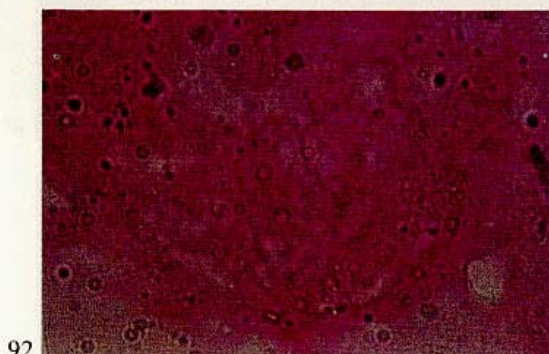
Fig. 95:
Kidney-adult, phosphatase (1:1250). Colouration of the ground substance.



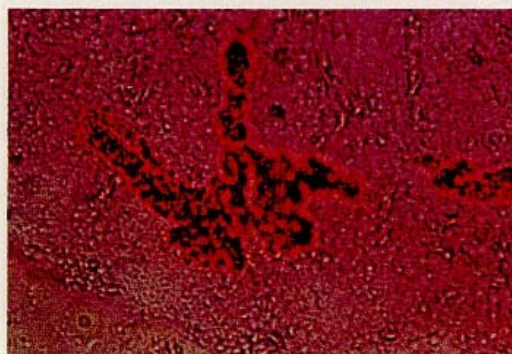
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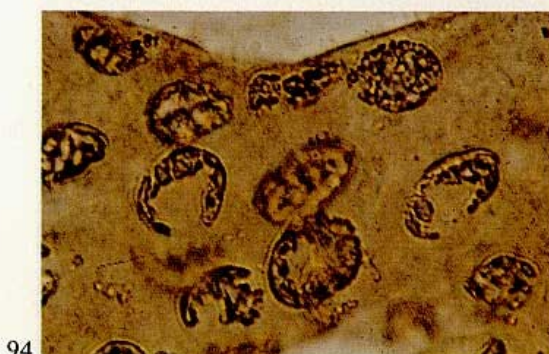
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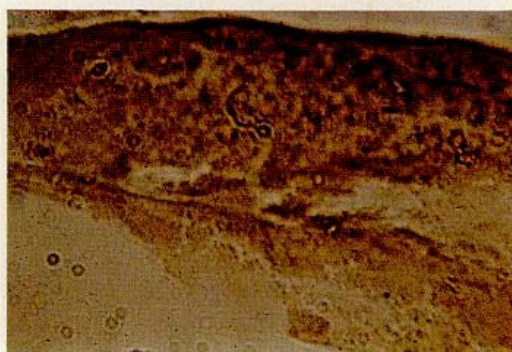
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centration of glycogen, which is dispersed into large cells with wide spaces of cytoplasm but attains the most intense

colours. Just so, the concentration of alpha-amino-acid is much higher in the adult tissue (fig. 74, 75). The colouring of

the adult tissues prevails for both enzymes, and enzymatic activities can clearly be shown in all preparations.

Bone-marrow

The structures of the bone-marrow preparations alone are very different. Whereas in the fetal preparation many round cells prevail and thus the cytoplasm and ground substance are of less importance, fibres and reticular structures with few incorporated cells and much fat predominate in the adult preparation (fig. 76, 77). Consequently, the concentration of DNA in the fetal tissues is high. The colouring of RNA in the adult tissue is very pale but somewhat more concentrated than in the fetal tissue. A higher fat-content can be found in the tissues of the adult animals, in contrast to the fetal tissue. The fetal preparation contains very large amounts of glycogen and alpha-amino-acids (fig. 78, 79). The activity of the alkaline phosphatase can be called strikingly low. The dopa-oxydase is very active and brings about a remarkably intense colouring of the fetal tissues.

Spleen

The fetal tissue is distinguished for its roundish nuclei whereas the adult preparation has much more ground substance, which is transversed by fibriform branches of the nuclei. As the fibres, same as the nuclei, contain much DNA, the preparation of the maternal animal appears richer in nuclei acids. Also the amount of RNA in the adult tissue exceeds that of the fetal preparation (fig. 80, 81). In contrast to the adult splenic tissue slightly interspersed in the nuclei and fibres, no fatty substances can be detected in the fetal tissue (fig. 82, 83). The concentration of alpha-amino-acid

of the spleen corresponds in the mother and fetus; this substance is bound mainly to the reticular ground substances. The rather strong activity of dopa-oxydase concentrates in the cytoplasm space in the adult splenic tissue, and it must be taken into consideration that the intensely coloured erythrocytes of the non-specific dopa-oxydase reaction may easily give a false impression. The fetal preparation contains very little dopa-oxydase (fig. 84, 85). A mean phosphatase activity is in the cells and ground substance of both tissues.

Muscle

Muscle preparations of the mother and fetus differ by the structure alone. Whereas in the fetus longitudinal structures with roundish-oval nuclei possess the picture, the preparations of the adult animal show an intense transverse striation with oblong, fusiform nuclei. The cytoplasm is less coloured in the fetal tissue than in the adult (fig. 86, 87). All nuclei contain much DNA; the concentration in the large nuclei of the adult tissue is lower. Altogether, the intensity of pyronin is high, which is reflected especially by the transverse striation of the adult muscles. Only small quantities of fat can be found in the spaces between the muscle-fibres of the adult preparation. Glycogen occurs in the cytoplasm and ground substance of the fetal and adult muscular tissue. The concentration of alpha-amino-acid is very high in either preparation and is bound to the above-mentioned longitudinal structures in the fetal tissues and to the transverse structures in the adult tissues. The dopa-oxydase stains very intensely in both, the fetal and the adult tissues. The mean activity of phosphatase receives expression very clearly in the longitudinal and transverse structures (fig. 88, 89).

Fig. 96:

Omentum – fetal, hemalaun – eosin (1:1250). Syncitial structure. Dense colouration of nucleus. Obvious eosin colouration of the cytoplasm.

Fig. 98:

Omentum – fetal, scarlet (1:30). Different amounts of fat in arteries and veins.

Fig. 100:

Omentum – fetal, methyl green – pyronin (1:1250). Distinct majority of the pyronin-colouration, which proves the presence of RNA. Island-like formation.

Fig. 97:

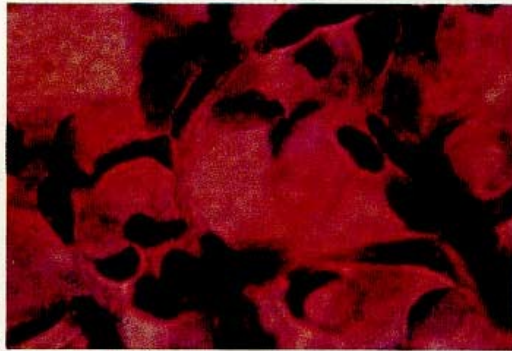
Omentum – adult, hemalaun – eosin (1:1250). Fusiform nuclei in longitudinal formations.

Fig. 99:

Omentum – adult, scarlet (1:30). Distinct proof of fat.

Fig. 101:

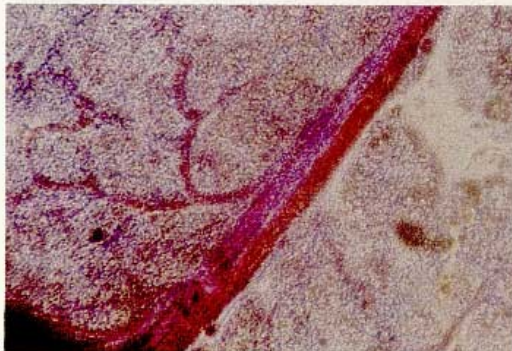
Omentum – adult, methyl green – pyronin (1:1250). DNA colouration of the nuclei at the connective points of the reticular structure.



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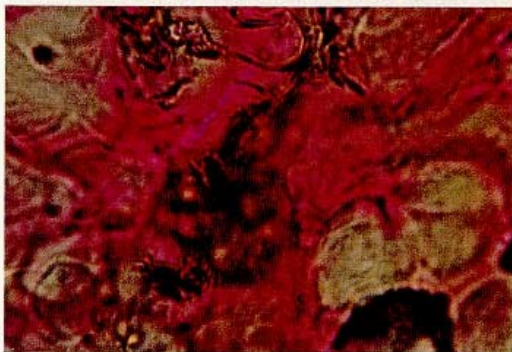
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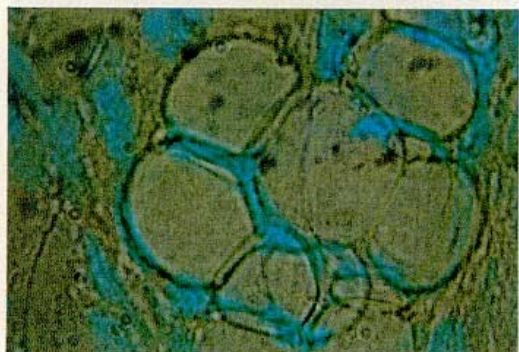
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Fig. 102:

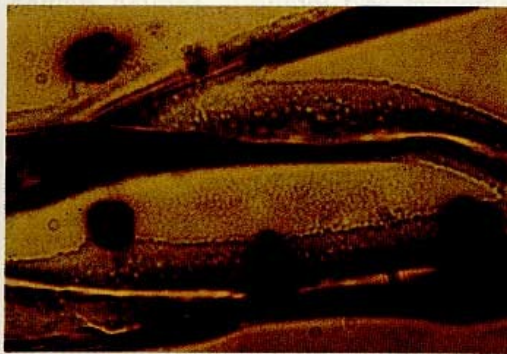
Omentum – fetal, dopa-oxydase (1:1250). Intense activity of dopa-oxydase.

Fig. 104:

Lung – fetal, methyl green – pyronin (1:1250). Strong RNA concentration in the cytoplasm.

Fig. 106:

Lung – fetal, Best-carmin colouration (1:1250). Small amount of glycogen.



102

Fig. 103:

Omentum – adult, dopa-oxydase (1:1250). Distinct fibrous structure with less intense colouration.

Fig. 105:

Lung – adult, methyl green – pyronin (1:1250). Good nuclear colouration. Relatively homogeneous RNA superposition (reddish tone).

Fig. 107:

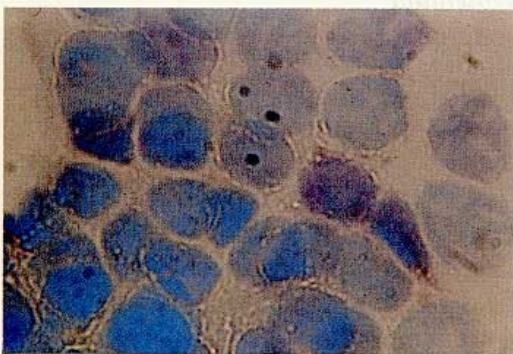
Lung – adult, Best-carmin staining (1:1250). Only traces of glycogen.



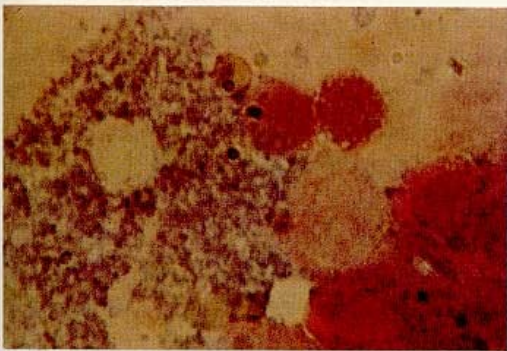
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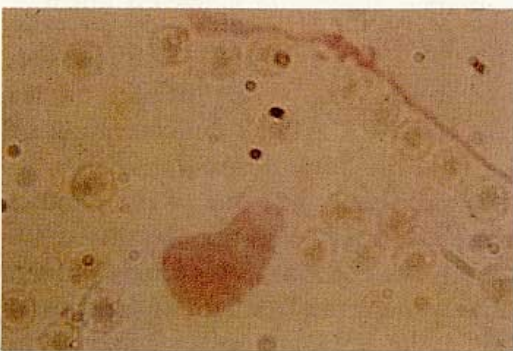
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Kidney

The roundish to roundish-oval nuclei of the renal tissue have a compact structure and can be stained deeply with DNA according to their consistency; depending on the quantity of nuclei, the fetal tissue shows a deeper colouring (fig. 90, 91). Traces of RNA can be identified in the adult preparation alone. Fat and glycogen have been found only by traces both in the fetal and in the adult tissues. Quite different is the concentration of the alpha-amino-acids, found highly intense in the adult tissue, chiefly in the cytoplasm, ground substance and nuclear membranes (fig. 92, 93). The dopa-oxydase stains deeply the fetal and adult tissues, without taking into account the effect of the non-specific dopa-oxydase staining of the erythrocytes. The activity of phosphatase is considerably high in both categories of the renal tissue, especially in the space of cytoplasm of the fetal kidney (fig. 94, 95).

Omentum

The fetal omentum preparation is distinguished for alveolar, syncitial, pentagonal to hexagonal structures. The roundish to oval nuclei are localized in the intersections of the syncitium. In contrast thereto, the adult tissue has a fibrous, net-like structure in which oblong, fusiform nuclei prevail (fig. 96, 97). Both tissues, especially the fetal, are interwoven with nets of vessels, veins and arteries mostly running parallel side by side (fig. 98). DNA and RNA, forming islet-like groups, can be seen in both tissues (fig. 100, 101). A high concentration of fat is in the adult tissue (fig. 98, 99). In the fetal tissue, just one of the parallel vessels attracts attention by more intensely stained fat. Glycogen is stained mainly in the fetal tissue. The concentration of the alpha-amino-acids prevails in

the fetal preparation and is massive in the area of the vascular network and islet-like regions, which are rich in ground substance whereas the adult preparation shows the deepest staining on the membranes of the fibre systems. The dopa-oxydase disperses uniformly on the islet-like complexes in the fetal and adult smears (fig. 102, 103). The activity of phosphatase prevails in the fetal tissue but is rather high in both.

Lung

The nuclei of the fetal lung are roundish to oval, poor in structures, very compact and intensely enough stained with DNA whereas the about three times larger, more loosely but more differently structured nuclei of the adult lung show correspondingly smaller concentrations of DNA. The cytoplasm is well stained. The fetal lung contains much RNA in branches of cytoplasm and reticular structures. In the mothers, however, only low concentrations of RNA are found outside the cell nuclei (fig. 104, 105). Fat did not occur in either tissue. Traces of glycogen were identified in a few storage cells, above all in fetal tissue (fig. 106, 107). Alpha-amino-acids are found in both tissues, but the adult tissue has more. High concentrations of dopa-oxydase are there in both cases. A low activity of phosphatase bound to the membrane systems can be marked for the fetal animal.

The descriptions of the organs have given just a concise survey of the points to be answered. Results can be seen from the summarizing synoptic fig. 108.

Morphologically and cytochemically, there are more differences between fetal and maternal tissues than could be anticipated in any bio-economic association. The morphological structures of the fetal and adult tissues are largely similar, with the following exceptions:

1. The fetal tissues have more and smaller nuclei, which appear round to roundish-oval and much more concentrated, more distinct and thus stronger than the voluminous, often oblong-fusiform, loosely structured nuclei of the adult tissues.

Fig. 108:

Synopsis of the cytochemical comparison between fetal (F) and maternal (E) tissues.

TABLE: QUANTITATIVE IDENTIFICATION OF THE SUBSTANCES IN TISSUES		HAEMAL. EOSIN	FERRIC HAEMATOX.	SCARLET	BEST-CARMINE	NINHYDRIN	FEULGEN	DOPA-OXYDASE	METHYL-PYRON.	A-PHOSPHATASE
STOMACH	• F	●	●	●	●	●	●	●	●	●
STOMACH	• E	●	●	●	●	●	●	●	●	●
PANCREAS	• F	●	●	●	●	●	●	●	●	●
PANCREAS	• E	●	●	●	●	●	●	●	●	●
BRAIN	• F	●	●	●	●	●	●	●	●	●
BRAIN	• E	●	●	●	●	●	●	●	●	●
LIVER	• F	●	●	●	●	●	●	●	●	●
LIVER	• E	●	●	●	●	●	●	●	●	●
BONE-MARROW	• F	●	●	●	●	●	●	●	●	●
BONE-MARROW	• E	●	●	●	●	●	●	●	●	●
SPLEEN	• F	●	●	○	●	●	●	●	●	●
SPLEEN	• E	●	●	●	●	●	●	●	●	●
MUSCLE	• F	●	●	○	●	●	●	●	●	●
MUSCLE	• E	●	●	●	●	●	●	●	●	●
KIDNEY	• F	●	●	●	●	●	●	●	●	●
KIDNEY	• E	●	●	●	●	●	●	●	●	●
OMENTUM	• F	●	●	●	●	●	⊗	●	●	●
OMENTUM	• E	●	●	●	●	●	⊗	●	●	●
LUNG	• F	●	●	○	●	●	●	●	●	●
LUNG	• E	●	●	○	●	●	●	●	●	●

- identified in large quantities
- identified in medium quantities
- identified in small quantities
- no identification possible
- ⊗ technical impossibility of preparing the product

2. The cytoplasm of the fetal tissues is more uniform and homogeneous. The extranuclear areas of the adult preparations are veined with a network of fibrous, reticular structures so that even in preparations, in which the cytoplasm prevails in proportion to the nucleus: plasma relation in the fetal tissue (e. g. spleen), the compactness of the ground substance appears lower than in the fetal tissues.

3. A syncitial-alveolate formation prevails in fetal omentum and bone-marrow, and a reticular-fibrous formation in the corresponding preparations of the adult animal.
4. The fetal muscle preparations show a longitudinal structure, the adult muscle preparations a distinct transverse striation.

As regards the cytochemical composition, the following is worth mentioning:

- a) Fat occurs always in higher concentration in the adult tissue.
- b) The proportion of the alpha-amino-acids: glycogen in the fetal and adult tissues depends on the tissue and cannot be generalized.
- c) The concentration of nucleic acid, especially the nuclear DNA, is higher in the fetal tissues (exception: spleen) than in the mothers.
- d) A uniform tendency of the cytochemically traceable enzymatic activities in the fetal and adult tissues has not been found as the enzymes apparently depend more on the organs than on the age.