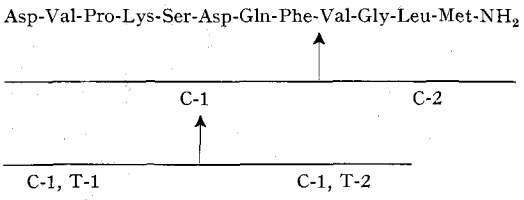
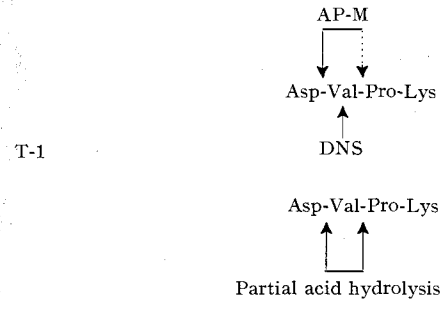
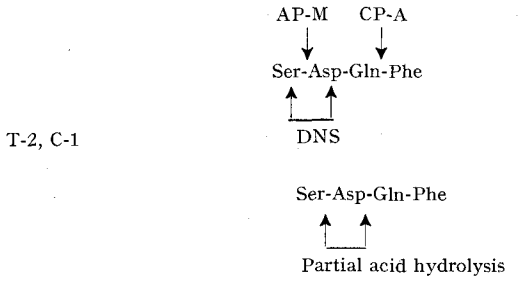


The sequences of the 3 tetrapeptides were elucidated by sequential experiments.

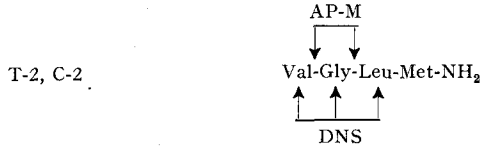
N-terminal tetrapeptide. Digestion with aminopeptidase M(AP-M) liberated aspartic acid and traces of valine, the position of which was confirmed by dansylation (DNS) on the remaining tripeptide. Partial acid hydrolysis liberated aspartic acid and the tripeptide Val-Pro-Lys. Splitting of small amounts of lysine and of the dipeptide Val-Pro was also observed.



Central tetrapeptide. The position of serine was assessed with aminopeptidase M(AP-M) and by dansylation; carboxypeptidase A (CP-A) in its turn liberated phenylalanine from the C-terminus. The position of the aspartyl residue was determined by dansylation on the tripeptide liberated with aminopeptidase. Partial acid hydrolysis confirmed the above results.



C-terminal tetrapeptide. Sequence of amino acid residues in this peptide was elucidated by aminopeptidase M digestion and by dansylation.



It is probable that kassinin or kassinin-like peptides occur in the skin of other African amphibians such as Hylambates maculatus and Phlyctimantis verrucosus.

Influence of pregnancy and fibrosarcoma on hepatic mitochondrial proteins of mice

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Summary. Non-pregnant, pregnant and fibrosarcoma-bearing non-pregnant mice differ in their total hepatic mitochondrial protein content, as well as the electrophoretic pattern following separation on SDS acrylamide gels.

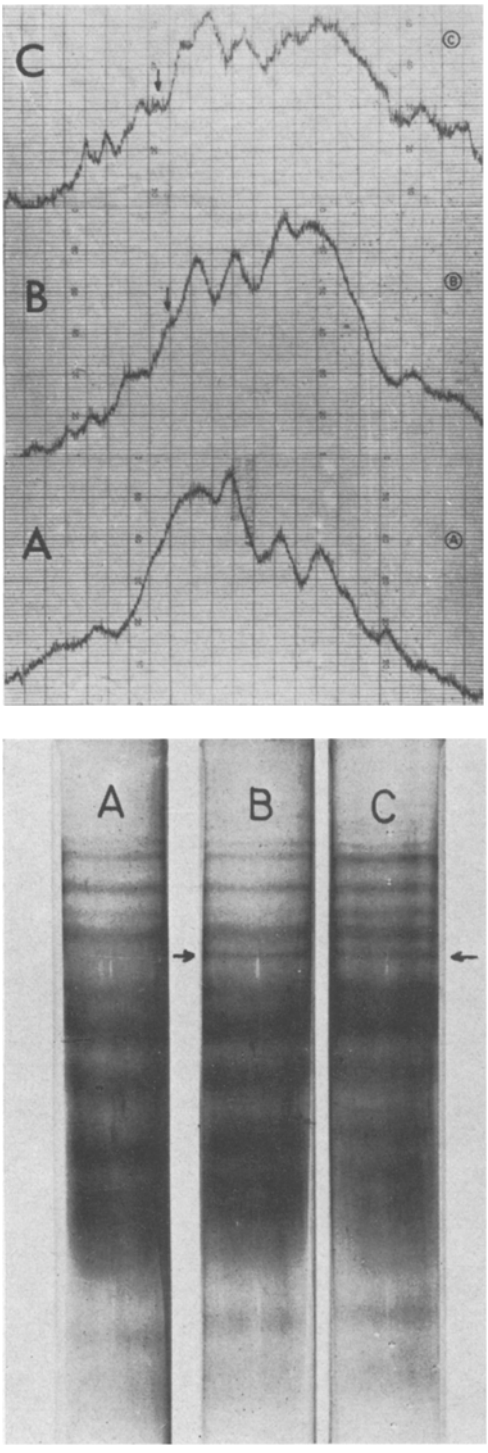
Mitochondria undergo physiological changes during cellular development and differentiation[1]. An earlier investigation showed certain differences in the incorporation of an amino acid in vivo into hepatic mitochondrial protein of non-gravid and gravid mice[2]. A recent electron microscopic study demonstrated the effect of pregnancy and fibrosarcoma on mitochondrial morphology[3]. The present report is on qualitative and quantitative studies of hepatic mitochondrial proteins in non-gravid, gravid and fibrosarcoma-bearing non-gravid mice.

Materials and methods. Adult (75 ± 5 days) Swiss mice, weighing 22–25 g, were used in all experiments. Pregnant mice were obtained as reported earlier[2]. A chemically-induced (dimethyl-benzdithionaphthene) fibrosarcoma was transplanted into normal female mice of comparable age 15 days prior to sacrifice. All animals were fed ad libitum on a balanced laboratory diet. Gravid mice were sacrificed by cervical dislocation on the 15th day of gestation along with non-gravid ones and those bearing fibrosarcoma. Mitochondria were isolated at 0–4 °C in the following manner. Liver homogenates (10% w/v) were prepared in ice-cold 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA and spun in the cold at 600 × g for 10 min to sediment nuclei and cell debris. The supernatant was spun at 10,000 × g for 10 min to obtain the mitochondrial pellet. The mitochondria were washed thrice with the homogenizing medium before the biochemical assays and electrophoresis. Hepatic mitochondrial protein content in the homogenate and pellet was calculated from succinic dehydrogenase activity as described by Gross[4]. Protein was estimated according to Lowry et al.[5].

Total hepatic mitochondrial proteins of non-pregnant and fibrosarcoma-bearing non-pregnant mice

Status	fresh tissue (mg/g)
Non-pregnant mice	68.96 ± 3.04 (8)
Pregnant mice	110.62 ± 4.02 (8)
Fibrosarcoma-bearing non-pregnant mice	92.90 ± 3.08 (8)

Values are mean ± SEM. Number of animals in each group is given in parenthesis.



Densitometric tracings and photograph of gels showing electrophoretic separation of hepatic mitochondrial proteins in non-pregnant *A*, pregnant *B* and fibrosarcoma-bearing non-pregnant mice *C*. The arrows indicate a new band.

SDS polyacrylamide gel electrophoresis of mitochondrial proteins was carried out according to Weber and Osborn[6]. Proteins were solubilized at a concentration of 2 mg/ml in a solvent containing 10% glycerol, 1% SDS and 1% mercaptoethanol in 0.01 M sodium phosphate buffer (pH 7.0). Monomerization of the proteins was achieved by keeping the mixture in a boiling water-bath for 2 min[7]. 50 µg of mitochondrial proteins were applied to 7.5% acrylamide gels containing SDS and electrophoresed with a current of 8 mA per tube. Standard marker proteins of known mol.wt were electrophoresed along with the samples to calibrate the approximate mol.wt of proteins at each band.

Results and discussion. A quantitative difference is discernible in the total hepatic mitochondrial proteins of the 3 groups of animals studied (table). The highest amount is present in the mitochondria of pregnant mice followed by in that of fibrosarcoma-bearing non-pregnant and normal non-pregnant mice. The advantage in employing the succinic dehydrogenase method to measure mitochondrial proteins quantitatively has been elaborated by Gross[4]. Our earlier electron microscopic observations[3] lend support to the view that the differences noticed in total mitochondrial proteins are related to the size of the mitochondria. This in turn would reflect enhanced synthesis, and it is reasonable to attribute this altered turnover of mitochondrial proteins to the metabolic status of the animal.

Acrylamide gel electrophoresis of hepatic mitochondrial proteins from the 3 groups of animals results in a number of bands (figure). Qualitative differences are apparent between the mitochondrial proteins of the non-pregnant control mice on the one hand, and those of the pregnant and fibrosarcoma-bearing mice on the other. For example, a band of protein with a computed approximate mol.wt of 60,000 is discernible in all except the non-pregnant controls. Membrane bound as well as soluble cellular proteins take part in the continual replacement processes and mitochondrial proteins are no exception since they constantly turnover at different rates[8–10]. Although most of the mitochondrial proteins are synthetized on cytoplasmic ribosomes and transferred to the mitochondria[11], polysomes isolated from yeast mitochondria have been shown to be capable of active protein synthesis[12]. Although the exact nature of the changes remains to be elucidated, it is apparent that alterations in hepatic mitochondrial proteins occur during pregnancy and the tumour growth.

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