concentrations higher than 1.8 M sucrose the yield will decrease progressively; a compromise must be reached according to specific purification needs. If the nuclei content of the homogenate is known, the thickness of the nuclear pellet at the rotor wall can be calculated considering that at 1 mm from the rotor wall a 1 mm band will hold about 41 ml of nuclear pellet. At the conditions described above, processing 10 l of homogenate would result in the formation of a band only about 2 mm thick. Alternatively, a cushion of a denser sucrose solution could be introduced between the 1.8 M sucrose layer and the rotor wall; in this case no pelletting would occur and nuclei, concentrated in a narrow band at the interface with the 1.8 M sucrose layer, could be collected by emptying the rotor with the same procedure employed when a gradient experiment is performed. However, in order to avoid the time-consuming gradient elution step and a second passage of nuclei in concentrated suspension through the rotating seal of the rotor which might lead to damage, pelletting to the rotor wall was preferred.

Amino acid composition and sequence of kassinin, a tachykinin dodecapeptide from the skin of the African frog Kassina senegalensis[1]

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Summary. Methanol extracts of the skin of the African amphibian Kassina senegalensis contain a dodecapeptide, kassinin, belonging to the family of tachykinins or physalaemin-like peptides. Kassinin, like all other natural tachykinins, possesses the characteristic C-terminal tripeptide Gly-Leu-Met-NH_2 and a phenylalanine residue in position 5 from the C-terminus. However, the amino acid sequence in the N-moiety of the molecule differs sharply from that of the other tachykinins.

A new peptide which can be included in the tachykinin family has been traced in the skin of the African frog Kassina senegalensis. The peptide, called kassinin, has been isolated in a pure form and its structure identified as follows: + Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2.

It may be seen that kassinin possesses the C-terminal tripeptide -Gly-Leu-Met-NH_2 and the Phe residue at position 5 from the C-terminus which are characteristic for all known natural peptides of the tachykinin group (eledoisin, physalaemin, phyllomedusin, uperolein, substance P), and are essential for the physalaemin-like activity.

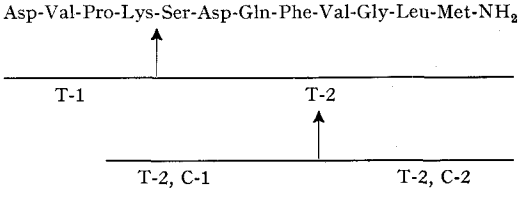
However, kassinin differs from all the other tachykinins in that it possesses 12 amino acid residues, and strikingly differs from the amphibian tachykinins in the amino acid composition of the N-moiety of the molecule. Moreover, like substance P, kassinin shows a free N-terminus, instead of the usual pyroglutamyl residue.

Materials. The fresh skins of 1200 specimens of Kassina senegalensis, collected in Kenia and South Africa during the period 1971–1974 were used in this study. The material weighed 659 g (average 0.55 g per fresh skin). The skins were removed from the frogs immediately after killing and extracted twice with a volume of methanol 5 times the weight of the tissue. The methanol extracts were combined and filtered and then stored in the refrigerator.

Isolation procedure. Samples of pure peptide were obtained by submitting the extracts to the following purification steps: a) washing of the evaporation residue with petroleum ether in order to eliminate fat contaminants; b) 120-tubes countercurrent distribution with the system n-butanol:acetic acid:water (80:12:108); c) gel filtration through Sephadex G 10 columns eluted with 0.01 M acetic acid; d) gel filtration through Sephadex G 25 columns eluted with 0.01 M acetic acid. Further purification could be obtained (when needed) by chromatography on alumina columns and/or preparative electrophoresis on paper.

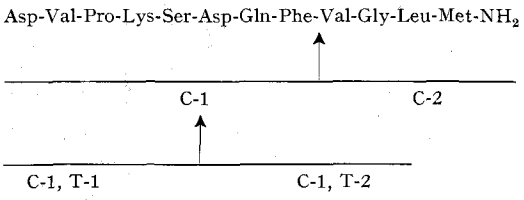
Purification of kassinin could be followed, step by step, by bioassay (guinea-pig ileum) and by paper electrophoresis. At pH 1.2 the peptide showed an electrical mobility of 0.55 Glu, but no mobility was observed at pH 5.8. Rf values on paper chromatograms were 0.54 in the system n-butanol:ethanol:acetic acid:water (80:16:16:128) and 0.21 in the system n-butanol:ethanol:water (96:16:128). The spot of kassinin could be revealed by ninhydrin and chlorine.

Structure. The occurrence in the kassinin molecule of a lysyl and a phenylalanyl residue (demonstrated by total acid hydrolysis) was the premise for the treatment of the molecule with trypsin and chymotrypsin. By digestion of kassinin with trypsin, 2 fragments were obtained, containing 4 and 8 amino acid residues, respectively. Submitting the octapeptide to chymotrypsin digestion, it split into 2 tetrapeptide fragments, as shown below.



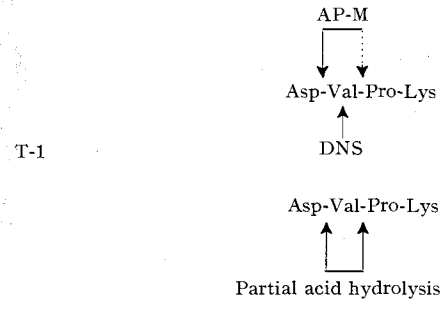
The same 3 tetrapeptide fragments were obtained by reversing the succession of enzymes.

1 Supported in part by grants from the Consiglio Nazionale delle Ricerche, Roma.

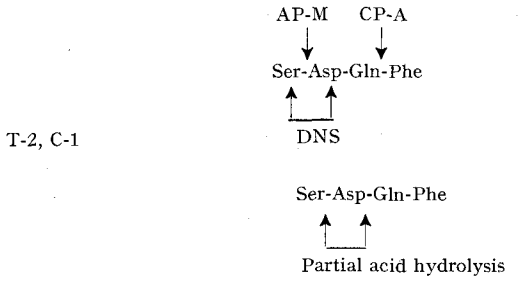


The sequences of the 3 tetrapeptides were elucidated by sequential experiments.

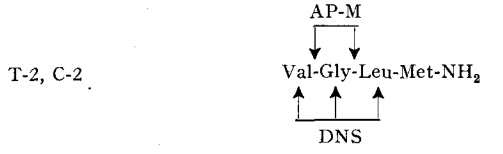
N-terminal tetrapeptide. Digestion with aminopeptidase M(AP-M) liberated aspartic acid and traces of valine, the position of which was confirmed by dansylation (DNS) on the remaining tripeptide. Partial acid hydrolysis liberated aspartic acid and the tripeptide Val-Pro-Lys. Splitting of small amounts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