creased about 2-fold in the submandibular and sublingual glands and increased slightly in the serum compared with the activity in unoperated rats. The activity did not, however, increase in the submandibular or sublingual glands or serum of the stimulated rats with pilocarpine. Amylase activity in the pancreas of the parotidectomized rats was almost the same as that of unoperated rats and decreased to 57% after the injection of pilocarpine. Stimulation by pilocarpine led to 51% and 55% depletions of amylase from the parotid gland and pancreas and a 7-fold increase in the activity in the serum of normal rats, respectively (table 1), but the depletion from the pancreas did not lead to an increase in serum amylase activity of parotidectomized rats (table 2). These results indicate that depletion of amylase from the parotid gland caused an increase in amylase activity in the serum and that the increase of serum amylase concomitantly produced considerable rises in amylase activity in the submandibular and sublingual glands. In the parotidectomized rats, sodium pentobarbital was used for anesthesia of the rats. However, sodium pentobarbital alone had no influence on the amylase activity in the salivary glands.

Shear et al.[6] reported that the rise in amylase activity in the rat submandibular gland after stimulation by pilocarpine was due to an increased activity of the enzyme in the blood vessels distributed around the glands. But they did not identify the origin of the increase of the enzyme activity in the blood. It is unlikely that the contamination of the blood causes the increase in amylase activity in the submandibular and sublingual glands because the activity was still high in these glands after perfusion with saline.

A primary step in stimulation of amylase release from the parotid gland by cholinergic agents is an increased influx of Ca^{2+} into the gland cells[2]. Driesbach reported that pilocarpine increased Ca^{2+} transport through membranes in vitro[7]. The administration of pilocarpine increased amylase activity in saliva (data not shown). These observations indicate that the stimulation by pilocarpine leads to a marked increase in amylase activity in saliva secreted through secretory ducts from the parotid gland. Our results suggest that pilocarpine may increase not only the secretion of amylase into the secretory ducts but also permeability of the enzyme into the blood stream. We are unable to demonstrate how amylase in the blood is taken up into the submandibular and sublingual glands. The mechanism of uptake of amylase from the blood into the salivary glands remains to be further elucidated.

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Vitellogenin and lipovitellins in *Orchestia gammarellus* (Pallas) (Crustacea, Amphipoda); labelling of subunits after in vivo administration of ^{3}H-leucine

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Summary. The distribution of ^{3}H-leucine between the various polypeptide components of *Orchestia gammarellus* vitellogenin and lipovitellins, separated by SDS-PAGE, has been studied after in vivo injection of this amino-acid. The results corroborate the view that the heaviest components, or native polypeptide subunits of vitellogenin, are progressively transformed into lighter ones.

In the amphipod *Orchestia gammarellus* (Crustacea, Amphipoda) vitellogenin (VTG), the precursor of vitellus synthesized by the sub-epidermal adipose tissue[1,2], lipovitellin I (LPV I), the main component of that vitellus, and lipovitellin II (LPV II) were impossible to differentiate on the basis of polyacrylamide gel electrophoresis (PAGE) and immunological studies[3].

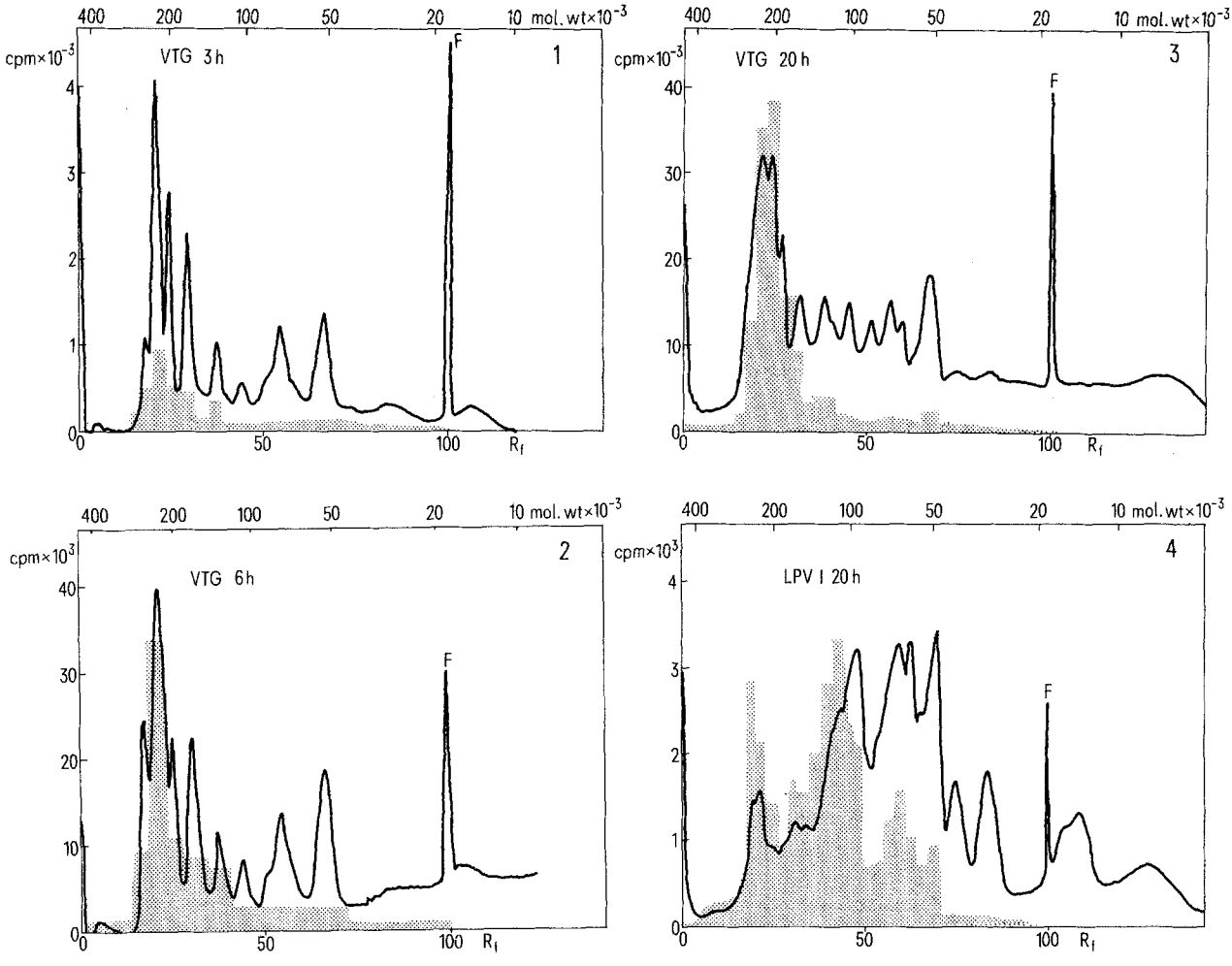
The question arises whether the VTG undergoes any chemical modification during vitellogenesis. Electrophoresis performed in a denaturing medium has revealed differences in the composition of VTG and both LPVs[4]. VTG is composed of a large number of components (8–11), the molecular weights of which range from 31,000 to 235,000. In the course of vitellogenesis, during which VTG is transformed into LPV I and LPV II, there is a gradual disappearance of the largest subunits (mol.wt > 127,000). When egg-laying occurs, those with a mol.wt above 200,000 are totally absent from the lipovitellins[5].

We have postulated that VTG is synthesized in a form which only includes 2 components with high molecular weights, i.e. 217,000 ± 17,000 and 235,000 ± 10,000. These *sensu stricto* subunits would then undergo a process of proteolytic cleavage starting even before the entrance of VTG into the oocytes. Analogous phenomena seem to occur in certain insects[6,7].

In the present paper, the process was studied by following the distribution of label among the various components of VTG and the LPVs 3, 6 and 20 h after injection of tritiated leucine.

3 groups of animals were formed, each including 30–65 females in vitellogenesis. Each female was first injected with 2.5 µCi tritiated leucine, and samples of a few µl of hemolymph were taken after 3 h (1st group), 6 h (2nd group) and 20 h (3rd group). The specific activity of the tritiated leucine injected was 48–50 Ci/mM for the 1st 2 groups, and 146 Ci/mM for the 3rd (L-(4–5 ^{3}H) leucine, Radio-Chemical Centre, Amersham, England).

The VTG and LPVs were isolated by preliminary PAGE followed by electrodialysis, and were then submitted to SDS-PAGE according to previously described techniques[4].



Distribution of labelling between the various components, as separated by SDS-PAGE of vitellogenin (VTG) and lipovitellin I (LPV I) of the Amphipod Crustacean *Orchestia gammarellus* (Pallas). The histograms indicate radioactivity, expressed in cpm; they have been superimposed on densitometric recordings of the electrophoreses.

Figure 1. Vitellogenin extracted from 196 µl hemolymph taken from females which had received 2.5 µCi leucine ^3H (sp.act.: 48 Ci/mM) 3 h earlier.

Figure 2. Vitellogenin extracted from 108 µl of hemolymph taken from females which had received 2.5 µCi leucine ^3H (sp.act.: 50 Ci/mM) 6 h earlier.

Figure 3. Vitellogenin extracted from 280 µl of hemolymph taken from females which had an injection of 2.5 µCi leucine ^3H (sp.act. 146 Ci/mM) 20 h before.

Figure 4. Lipovitellin I extracted from 8 ovaries undergoing vitellogenesis, taken from females in stage $D_{1'a}$ that had been injected with 2.5 µCi leucine ^3H 20 h earlier.

It should be noted that the VTG and LPVs did not at any time undergo freezing nor lyophilisation.

Subsequent to densitometric recording in order to calculate the molecular weight from the R_f-values, the gels were cut into 2 mm thick discs which were then dissolved individually in 1 ml H_2O_2 (30%) at 60 °C for about 16 h. After the addition of 10 ml of 'Instagel' (Packard), radioactivity was measured using a 'Packard Tri-Carb' liquid scintillation spectrophotometer. All samples were treated in an identical manner; the results are given in cpm.

Examination of figures 1, 2 and 3 shows that after 3, 6 and 20 h of incubation, most labelling was concentrated in the heavy components of the VTG, particularly in the subunits with mol.wts of 217,000 ± 17,000 and 235,000 ± 10,000. This distribution did not vary significantly with the different incubation periods used in our experiments.

Results concerning LPV I after 20 h of incubation differ greatly. Indeed, there was strong radioactivity in all 3 constituent groups. The 1st was made up of elements whose mol.wt was above 200,000, corresponding to those found in VTG; the 2nd group comprised components with mol.wts between 175,000 and 85,000, and the 3rd group contained elements with a mol.wt between 85,000 and 45,000.

As compared to VTG, therefore, there was a shift in labelling to the light LPV I elements. This shift was correlated with a change already seen in the proteinogram[4]: the light components became much more abundant than the heavy ones. Data on LPV II, not presented here, were analogous to those on LPV I.

The strong labelling of the heaviest VTG components seems to support our hypothesis that these elements represent the native polypeptide subunits of VTG. The fact that the radioactivity does not spread to the lighter components when the incubation period is increased from 3 to 20 h. This might indicate that after 3 h of incubation, an equilibrium is already established between: 1) the synthesis of radioactive VTG in the form of heavy subunits; 2) the slow transformation of these heavy subunits into lighter elements within the subepidermal adipose tissue and/or in the hemolymph; 3) the uptake of VTG from the hemolymphatic compartment by oocytes undergoing vitellogenesis.

The distribution of labelling among the components of

LPV I, on the other hand, indicates transformation into smaller components within the oocytes during vitellogenesis. It should be recalled that by the time of egg-laying the heaviest elements had completely disappeared. It is unlikely that the ovary participates in the synthesis of all or part of the LPVs, as it has been proposed in some decapods[8]. In fact, LPV labelling happens very late, only starting to appear in vivo 2–2.5 h after injection of ^{3}H-leucine, that is, much later than VTG labelling (about 45 min)[5]. It is highly probable that the labelling of LPVs is the consequence of the entrance of labelled VTG into oocytes.

Finally, it is notable that these rearrangements are intramolecular: they do not affect the mol.wt of LPV I, which remains stable throughout vitellogenesis (350,000) and hardly differs from that of VTG (400,000)[3]. The physiological significance of this process remains unknown.

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Cytoprotection – organoprotection by somatostatin: gastric and hepatic lesions*

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Summary. In rats, the hemorrhagic gastric erosions produced by ethanol, and the fatal hemorrhagic hepatic necrosis induced by phalloidin, were significantly reduced by regular somatostatin, but not by derivatives devoid of -SH containing cysteines. These effects of the hormone were abolished in animals which received, in addition, the sulfhydryl blocker n-ethylmaleimide before the toxic chemicals. Thus, somatostatin exhibits organoprotection dependent on endogenous sulfhydryls.

Prostaglandins (PG) were recently shown to exhibit gastric cytoprotection, that is to prevent various gastric lesions such as those induced by ethanol, aspirin, acids or alkali[1,2]. The cytoprotective effect (i.e., inhibition of superficial, hemorrhagic mucosal erosions) of PG differs from the well known anti-ulcerogenic action (i.e., directed against well-formed gastric and duodenal ulcers) of these compounds in that only the latter effect is associated with inhibition of gastric acid secretion[1,2]. The mechanisms of the cytoprotective effect of PG are unknown[3–5]. Local cytoprotection is also offered by other drugs, e.g., cimetidine, probanthine[6] and sulfhydryl-containing chemicals[7].

Somatostatin, besides exhibiting an anti-ulcerogenic effect[8–11], also reduces intoxications involving other organs, e.g., pancreas[12–14], liver[15,16], adrenals and lungs[17,18]. We reported that endogenous sulfhydryls (e.g., glutathione) might mediate the cytoprotective effect of PG[7], and have now recognized that -SH groups are also present in somatostatin, which exerts potent local gastric cytoprotection. We hereby present data and a hypothesis that somatostatin, due to its inherent structure, may provide a systemic or generalized cytoprotection (i.e., histoprotection, organoprotection).

Materials and methods. In the present studies Sprague-Dawley rats with an initial b.wt of 200 g had free access to Purina lab chow and tap water. Each control and experimental group consisted of 3–5 animals; every experiment was performed at least twice and the results were pooled. Somatostatin (Serono) was either dissolved in the original, commercially provided (Serono) protamine sulfate and $ZnCl_2$ for s.c. injection to delay absorption and to prolong the biological half-life of the hormone, or solubilized in distilled water for i.p. administration and for rapid, short-time elevation of blood somatostatin levels. The general design of experiments is presented in table 1.

In the 1st study following an overnight fast, rats recieved s.c. somatostatin, 10 µg/100 g as a 0.2 ml of protamine sulfate and $ZnCl_2$ suspension, 30 min before 1 ml of 100% ethanol p.o. The sulfhydryl blocker N-ethylmaleimide (Sigma) was injected s.c. at the dose of 5 mg/100 g, 10 min after somatostatin (or 20 min before ethanol). A derivative of somatostatin (bis-S-Acm Somatostatin, L361, 728, Merck or di-S-tBu-Somatostatin, Serono) in which the -SH groups (cystein) were replaced, was also injected at 10 µg/100 g s.c., 30 min before ethanol. The animals were killed 1 h after ethanol administration and the lesions in the stomach

Table 1. General design of experiments