In order to compare the electrophoretic mobilities of the protein fractions from different species on the same gel, the split-gel technique was used[17]. A small piece of cellulose acetate foil was inserted into the upper surface of the sample gel to prevent the mixing of protein samples applied to the same gel. The electrophoretic conditions were otherwise the same as stated above.

Results and discussion. As illustrated by the electropherograms in Figure 1, the protein patterns of paragonial proteins in the different species differ greatly in both the numbers and concentrations of individual bands. Striking differences in the anodal mobilities of the major bands are particularly evident. The age of the flies employed for analysis varied from 10 to 23 days post emergence. There is a progressive accumulation of secretion in the paragonial glands when the males are prevented from mating. However, our experience showed that this accumulation has no effect on the protein pattern. We estimated that in the paragonial glands of flies aged 10–15 days the secretory proteins account for approximately $^3/_4$ of the total soluble protein[18]. This means that the electrophoretic patterns shown in Figure 1 represent mostly the secretory proteins, including the needle-like 'crystals'[19] and the 'filamentous bodies'[20,21] secreted by the glandular cells. By using 10% SDS-gels VON WYL[12] estimated that the paragonial proteins in *D. melanogaster* have molecular weights from 12,000 to 120,000. Although corresponding data for the other species are not available, they are probably within the same range.

In order to determine the electrophoretic mobilities under more comparable conditions we employed the split-gel method by using the protein pattern of *D. melanogaster* as a standard. In each electrophoretic run, the paragonial extract from one species and that from *D. melanogaster* were absorbed on to the same gel without, however, the two samples being mixed. Since in this procedure only half as much protein could be applied to the gel, the comparison had to be limited to the major bands. The results are summarized in the diagram in Figure 2. As can be seen, the elctrophoretic pattern of *D. simulans* shows the greatest similarity to that of *D. melanogaster*. From a total of 7 bands of *D. simulans* detected on the split-gel 4 have identical mobilities as bands 2, 5, 6 and 8 in *D. melanogaster*. On the other hand, not a single band in *D. ananassae* shows such an identity, though this species, as *D. simulans* and *D. melanogaster*, also belongs to the *melanogaster* group of the subgenus *Sophophora*. Among the remaining species only *D. subobscura, virilis, americana* and *nigromelanica* each exhibit one band with the same anodal migration as band 6 or 7 in *D. melanogaster*. From these results it may be concluded that there is no correlation between the similarity of the paragonial protein patterns and the taxonomic relationships of the *Drosophila* species examined in this study. Admittedly, electrophoretic mobility itself is no proof for the identity of the proteins. For a definite conclusion more crucial evidence from two-dimensional gel electrophoresis or immunoelectrophoresis is needed.

With regard to the functions of the paragonial proteins no definite information is available. Many suggestions have been made, without, however, convincing experimental support. The secretions of the paragonial glands are considered to play a role in the transfer, storage and utilization of the sperm (references in FOWLER[3]). They may also influence the behavior and reproductive physiology of the female following copulation. Transplantation of the paragonial gland or injection of its secretion into virgin females resulted in a distinct increase in egg deposition[22]. A similar response of the host females following transplantation of these glands from different species has also been reported[23]. The stimulation of such heterologous transplants is, however, difficult to be understood in view of the high species-specificity of the protein patterns, assuming, of course, that some protein component(s) in the paragonial secretion serves indeed as a trigger to stimulate fecundity. A meaningful discussion about the functions of the paragonial proteins must await future experiments with purified preparations of individual proteins.

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Effect of Cycloheximide on Germination-Induced Isocitritase Development and Decline in Intact and Excised Flax Cotyledons

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Summary. Removal of embryo axis before germination markedly prevents the development as well as subsequent decline in Flax cotyledon isocitritase. Cycloheximide inhibited the development and decline of the enzyme in intact cotyledons but prevented only the former in excised cotyledons.

It is well known that many events that occur in the cotyledons of germinating seeds are triggered and controlled by embryo axis. Numerous cotyledonary enzyme activities either fail to develop or develop only partially if the embryo axis is excized early during germination, and one or more auxins or cytokinins usually substitute for the axis[2,3]. Maximal development of isocitritase in squash cotyledons also requires the presence of embryo axis and benzyladenine replaces in part this requirement[4]. However, despite the strong evidence indicating the importance of both synthesis and degradation in maintenance of cellular enzyme levels in animal[5] and plant systems[6], no attempts appear to have been made to investigate the role of embryo axis, or substances

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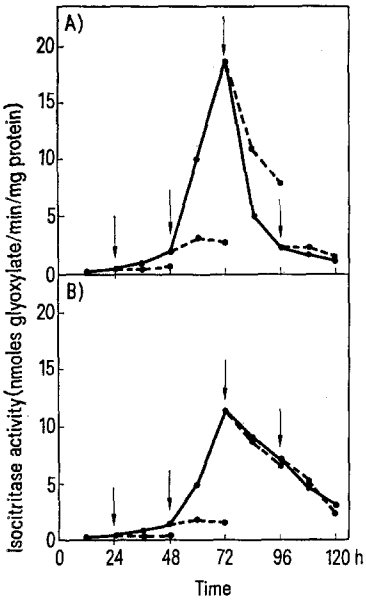
synthesized therein, on disappearence of enzyme activities from cotyledons. The aim of this investigation was to study the influence of embryo axis on isocitritase formation and subsequent disappearance from these cotyledons.

Flax seeds were surface sterilized before germination. The intact or embryo axis excised seeds were germinated at 30 °C in sterile petri dishes lined with filter paper and containing suitable quantities of water. At the time indicated, they were immersed in 10^{-3} *M* cycloheximide (Calbiochem; USA) for 8 h. Isocitritase activity in the cotyledons was determined by the combined procedures of CARPENTER and BEEVERS[7] and ROCHE et al.[8]. Protein concentration was determined as described by LOWRY et al.[9].

Flax cotyledons, either of the intact seedlings or excised from the embryo axis, develop isocitritase with activity peak on 3rd day of germination (Figure A and B). However, as compared with the intact cotyledons, the excised cotyledons develop only 50% activity, and loss of enzyme activity from these cotyledons is markedly slow. PENNER and ASHTON[3] have likewise demonstrated an inhibition in the formation and subsequent decline of protease activity in excised squash cotyledons and have attributed this to a lack of supply of a factor required for the synthesis of the protease inhibitor. As evident from the Figure, while cycloheximide prevented the development of isocitritase both in intact and excised cotyledons, suggesting de novo synthesis, decline of only the former was inhibited. Similar data has been interpreted to favour the existence of specific degrading enzyme[10] or inactivating protein[11]. Thus, the cycloheximide sensitive, rapidly turning over, specific inactivating system responsible for the accelerated decline of isocitritase, whether a degrading enzyme or inactivating protein, appears to be absent from excised cotyledons. Presumably, the slow decline of isocitritase in excised cotyledons is due to the lack of such inactivation mechanism.



Effect of cycloheximide on germination induced changes of isocitritase in intact (A) and excised flax cotyledons (B). The arrows indicate the time of cycloheximide treatment. Each value represents the mean of at least 3 separate determinations.

During germination of several oil-rich seeds, isocitritase is detectable only during conversion of fats to carbohydrates; and it is interesting to note that a specific inactivation mechanism exists for this enzyme in flax cotyledons, which seems to be regulated by embryo axis. Obviously in cells with long cell cycles, like those of the cotyledons, it is advantageous to have the ability specifically to destroy unwanted proteins in response to the environment. Hormonal regulation of protein levels via altered synthesis and inactivation has been demonstrated in animal systems[12,13], but no such data appears to be available in plants. The present study indicates that embryo axis, presumably by substances synthesized by it, influences not only the development but accelerates decline of isocitritase in cotyledons. We failed, however, to observe any effect of indoleacetic acid, gibberellic acid, benzyladenine and kinetin on decline of isocitritase, although the latter 2 compounds stimulate isocitritase development (data not given). Further studies to identify the factor(s) responsible for accelerated decline of isocitritase are in progress.

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Octopamine, Dopamine and Noradrenaline Content of the Brain of the Locust, *Schistocerca gregaria*

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Summary. The octopamine, dopamine and noradrenaline content of the brain of the locust, *Schistocerca gregaria* has been determined using sensitive radiochemical-enzymatic assays. Octopamine and dopamine are present in high concentration but the noradrenaline content is only 1/25 that of octopamine. Both reserpine and fusaric acid (a dopamine-β-hydroxylase inhibitor) produce a significant depletion of the octopamine stores.

Dopamine has long been recognized as the major catecholamine in insects; noradrenaline is present only in small amounts[2-5]. Histochemical studies using the technique of FALCK and HILLARP have demonstrated that catecholamines and 5-hydroxytryptamine (5-HT) occur intraneuronally in the insect central nervous system[5,6]. The ultrastructural localization of catecholamines in small granular vesicles in insects has also been described[7-9]. Dopamine has been applied iontophoretically to single neurons in the insect brain where it inhibits spontaneously active neurons[10].

Octopamine, the phenolic analogue of noradrenaline, is also a major amine in the insect central nervous system[11]. It is also found in the nervous systems of various