Die stabilisierende Wirkung von 5 mg Rinderalbumin/Ansatz kann durch 2 mg Rinder-γ-Globulin ebenfalls erzielt werden. Bei Zusatz grösserer Mengen γ-Globulin (bis 5 mg) wird bei gleichbleibender O_2-Aufnahme die Phosphataufnahme wieder geringer und demgemäss sinken die P/O-Quotienten (Abb.). Humanalbumin hat einen wesentlich geringeren Effekt als Rinderalbumin: 1–2 mg haben keine Wirkung, 3–5 mg steigern zwar die Phosphataufnahme, aber nicht in dem Masse wie Rinderalbumin. Die Untersuchungsergebnisse beweisen damit eine überlegene Schutzwirkung des arteigenen Albumins. Unter Berücksichtigung der pro Warburg-Ansatz eingesetzten Mitochondrienmenge (als Mitochondrien-N) ist im Querschnitt aller Versuche ein optimaler Effekt bei Zugabe von Rinderalbumin im Verhältnis 3 mg/mg Mitochondrien-N zu beobachten.

Nach heutigen Erkenntnissen beruht die Albuminwirkung auf der Bindung des Chromoproteids «Mitochrom», das von älterierten Mitochondrien freigesetzt wird und die oxydative Phosphorylierung hemmt[8]. Die molekulare Konfiguration des Albumins soll bei der Bindung des Mitochrom eine geringere Rolle spielen als spezifische Endgruppen in den Peptidketten, da partiell denaturiertes Albumin seine positive Wirkung auf die Phosphorylierung behält. Da die Artspezifität ebenfalls in den Hauptvalenzen der Peptidketten verankert ist[9], kommt dadurch möglicherweise auch die bessere Wirkung arteigenen Albumins zustande.

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Summary

Addition of 3 mg bovine serum albumin/mg mitochondrial-N enhances and stabilizes the phosphorylation capacity of isolated beef liver mitochondria. Albumin reduces the fluctation of the resulting P/O-rates and brings about a better reproducibility of the entire method. The same effect is produced by addition of bovine γ-globulin in half the concentration of albumin, while the effect of heterologous proteins (e. g. human albumin) is inferior.

Secretory Cycle and Disappearance of the Prothoracic Glands in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

Cyclic changes have been observed in the prothoracic glands of several larval instars of *Tenebrio molitor*. In each instar, the gland is much attenuated shortly after moulting, the web of cells stretching between the tracheae is inconspicuous and the layer of cells normally spread over the dorsal tracheal trunk is observed with difficulty[1]. The cells have hyaline cytoplasm with very few and small vacuoles and a few minute granules at the periphery, but without secretion droplets. The nuclei are compact and filled with numerous small chromatin granules.

In the two-week old instar, the gland is considerably developed. The layer of gland cells over the dorsal tracheal trunk is prominent and uniform. The cytoplasm is uniformly granular and minute secretion droplets can be observed in fresh preparations. The nuclei become larger and block-like, elongated, and sometimes lobulated, and chromidial substance is concentrated beneath the nuclear membrane.

In the four-week old instar, 3 or 4 days before the next moult, all the parts of the gland show maximum development and it extends somewhat onto the ventral tracheal trunk also. The cytoplasm has numerous fine granules and sometimes fibrils arranged along the axis of the gland, as reported by ARVY and GABE[2] in the Ephemeroptera. Secretion droplets are now observed in fresh preparations.

Two days or less before the next moult, the gland is again much reduced, and the sheath over the dorsal tracheal trunk is difficult to discern. The cytoplasm is almost free from granules and secretion droplets, and empty vacuoles are seen. The nucleus is also smaller, thin and elongated and darkly staining.

In the last instar also, similar changes occur. The gland shows maximum development about the time when the larva stops feeding, and about 24 h later, it shows great reduction. The band-like gland assumes the shape of a strand and the elongated nuclei are arranged length-wise in it, but the cells over the dorsal tracheal trunk can still be seen. In the 16 h old pupa, the gland has already lost its discrete nature and the web between the tracheal trunks is disintegrating. Soon after, the gland is represented only by a few cells attached to the dorsal tracheal trunk; and by the time the first traces of eye pigmentation appear, the gland has lost its identity completely.

Thus in each instar of *Tenebrio*, the prothoracic glands undergo maximal development about two days before moulting, when it discharges its secretion to become reduced again. Hence, the critical period occurs about two days before the next moult. In *Anisotarsus*, NUNEZ[3] described the critical period to occur about 20 h before moulting. In *Dysdercus*, WELLS[4] found that the volume of nuclear increase at the peak period of the development of the gland is about 5 times its original volume. Such marked increase in nuclear volume does not occur in beetles. In *Rhodnius*[5] and *Dysdercus*, degeneration of the gland starts about two days after the completion of metamorphosis, but in *Platysamia* WILLIAMS[6] found that the degeneration commences soon after eye pigmentation has started. Both in *Tenebrio* and *Anisotarsus*, the gland disappears long before metamorphosis is completed, and in *Tenebrio*, it is already disintegrating when eye pigmentation appears, showing that the completion of metamorphosis does not need secretory activity of the prothoracic gland throughout the process. WIGGLESWORTH[7] suggested that the disappearance of the gland is due to some change (metamorphosis) of the gland itself during the preceding moult, and to some humoral stimulus acting during the last moult. In *Tenebrio*, occurrence of initial changes in the last larval instar, which are similar to those occurring in the earlier ones, indicates that the first factor is not a very important one, while the influences working during the last moult are strong and decisive.

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Zusammenfassung

Bei Tenebrio-Larven werden cyclische Veränderungen der Prothoraxdrüsen beschrieben. Sie bestehen in Vergrösserung der Drüse, verbunden mit Bildung von Körnchen, Fibrillen und Sekrettröpfchen im Cytoplasma. Etwa zwei Tage vor der Häutung wird das Sekret ausgestossen und die Drüse wieder verkleinert. Im letzten Larvenstadium liegt diese kritische Phase 24 h nach Aufhören des Fressens. Die Drüse degeneriert in der Puppe bei Beginn der Augenpigmentierung.

Activation of Pancreatic Trypsinogen by Liver and Kidney Mitochondria

Following upon observations made in our Institute (in publications for the 2nd Italian Congress of Histochemistry, at Florence, 1959) on the inhibition of succinoxidasic activity of liver mitochondria by pancreatic granules and mitochondria, I decided to investigate whether such inhibition might be related to a tryptic action of the isolated pancreatic structures on liver mitochondria. For the first time, I was able to demonstrate that, like pancreatic granules, so also pancreatic mitochondria can develope tryptic activity when activated by enterokinase[1].

Afterwards SCHEPOWALNIKOW[2] demonstrated the existence of enterokinase, several discussions occurred between the various authors about its identity and action. PAVLOV, BAYLISS[3], ZUNZ, VERNON, HAMBURGER, STASSANO, and others concluded that there is more than one proteolytic enzyme of pancreatic production. VERNON stated that, to activate trypsinogen into trypsin, enterokinase was not indispensable, as the simple addition of trypsin to trypsinogen was enough to catalyse the reaction. Finally KUNITZ[4–6] observed that it was possible to obtain activation of trypsinogen by a kinase of Penicillium. NORTHROP and KUNITZ[7] concluded that reaction trypsinogen-trypsin can be catalysed by the same trypsin, by kinase of Penicillium, and by enterokinase. Considering that such a reaction could perhaps be catalysed by isolated structures of liver, and or of other organs, I considered the action of liver and other organs mitochondria on pancreatic trypsinogen.

Material and Method. For my research I used eight guinea pigs having an average weight of about 300 g each. Immediately after having killed each animal by beheading, I collected its pancreas and the other organs to be examinated; after having homogenized each organ in sucrose solution 0.25 *M*, I made fractionated centrifugation in cold room for 10 min at 2000 g on a first time, and then for 15 min at 10000 g; by this method I obtained isolated mitochondria of the various organs (liver, kidney, muscle, heart, lung, spleen, suprarenals) by 1 g of fresh organ.

In the meantime I homogenized pancreas, and by the method described by NOVELLI[8] I isolated in sucrose solution 0.88 *M* granules and mitochondria of pancreas that were suspended for a second time together in distilled water. While mitochondria isolated by 1 g of fresh organ were put into 2 ml of distilled water, a suspension of pancreas was prepared separately and total N_2 was titred by micro-Kjeldahl method. 1 ml of pancreas titred suspension was put with 1 ml of isolated mitochondria in test-tubes containing denaturated hemoglobin, and were incubated for 10 min at 25°C according to the method of ANSON[9]; in the meantime a control was prepared for each test. Complete description of this method has been already reported by myself in another work[1].

	Milliequivalents of tyrosin $\times 10^{-4}$ per 1 mg of pancreatic N_2	Tryptic Action
Liver mitochondria + pancreas	550.38 ± 103	Present
Kidney mitochondria + pancreas	284.80 ± 44	Present
Muscle mitochondria + pancreas	—	Absent
Heart mitochondria + pancreas	—	Absent
Suprarenals mitochondria + pancreas	—	Absent
Spleen mitochondria + pancreas	—	Absent
Lung mitochondria + pancreas	—	Absent

Tryptic activity was titrated by Beckman spectrophotometer on the basis of the tyrosin developed, and milliequivalents of tyrosin $\times 10^{-4}$ were calculated on the basis of 1 mg of pancreatic N_2.

Conclusions. The results of this research permit the conclusion that liver mitochondria contain an activator of pancreatic trypsinogen that acts like the activators previously described, that is by producing the reaction trypsinogen-trypsin. Kidney mitochondria were demonstrated to have the same capacity to activate trypsinogen, although at a lower rate.

As to what concerns the nature of the activator contained by liver and kidney mitochondria, the hypothesis that it could be the same enterokinase appears the most probable, although it still needs specific research for confirmation.

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Riassunto

Si dimostra la possibilità di catalizzare la trasformazione del tripsinogeno in tripsina mediante aggiunta di mitocondri isolati dal fegato e dal rene; i mitocondri del muscolo, polmone, cuore, surrene, milza non catalizzano tale reazione. La significatività dei risultati viene accertata calcolando il «*t*» di Fisher.

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