discovered in another experiment, but fawn strains crossfed them exactly as did olive strains, that is to olive and black respectively. One can therefore conclude that the bands are not due to heterokaryosis. Their presence depends on the block(s) responsible for the mutant phenotype of the crossfeeding strain, but their colour depends on the genotype of the crossfed strain. So far it has neither been possible to dissociate the ability of being crossfed from the light spore colour with which it was isolated, nor has it been possible to link crossfeeding to any growth requirement.

When it was realized that crossfed colour mutants might have been missed or discarded as unstable in previous mutagenic treatments, mutagenesis was repeated and isolation of spore colour mutants was done before contact had occurred between colonies. Among 18 phenotypically different spore colour mutants isolated under these conditions 2 showed crossfeeding, 1 of them in a gene different from *w7*. Both mutants showed the same basic crossfeeding reactions as *w7*. Details will be reported elsewhere.

It may be of interest to note that all *A. niger* spore colour mutants obtained so far, whether showing crossfeeding or not, show *p*-diphenol oxidase activity, while in *A. nidulans*[4] this enzyme has been identified as the crossfeeding material of yellow-spored mutants by wild-type green-spored strains. Another difference in the crossfeeding of the 2 species is that in *A. nidulans* crossfeeding is limited to the spores at the junction of the colonies[4].

The mechanism of the reaction is being investigated and it is hoped that the availability of crossfed mutants will help the study of spore pigmentation, pigment metabolism and perhaps conidiation.

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Photostimulation of ATP production, in cell-free extracts of insect integument

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Summary. Cell free extracts of *Pieris brassicae* integument or its 220,000 × g sonicated supernatant exhibit a light-dependent ATP production system (LAPS). ADP and Pi seem to be the substrates of the reaction. LAPS cannot be located in intact mitochondria.

One of the standard white-light experimental rhythms is 8 L:16 D (8 h white light followed by 16 h of dark). Under these conditions *Pieris* larval development lasts 23 days and 100% of pupae diapause[1-8]. In earlier experiments under the same conditions of quantic energy and rhythm we replaced, the white light with red light at the wavelength of the absorption maximum of pterobilin, the integumentary pigment. Larval development lasts 16 days without any diapausing pupae. Animals and their progeny are normal[9-11]. Red light has a particulary positive effect on the development of *Pieris*, since it prevents diapause and accelerates larval development[11].

These two spectacular changes in metabolism led us to assume that light, especially red light, can transfer its energy into some molecules such as ATP, which the integument can use directly.

In a previous paper we confirmed 1) that in vivo, the ATP content of the integuments increases together with the illumination time and 2) that in vitro, the ATP concentration in acellular integument-extracts also rises as a function of the illumination time[12].

This light-dependent ATP production system (LAPS) which is present in cell-free extracts of *Pieris* integuments is still far from being understood at the molecular level. In this paper we show that the LAPS can be split into small subunits which maintain their capacity for light-dependent ATP production, and that the substrates for such ATP production are probably phosphate and ADP.

Materials and methods. Animals. The 5th instar larvae of *Pieris brassicae* were chosen under equivalent physiological conditions[13] and grown under red or white lights as previously described[11]. Temperature was 19 ± 1 °C. Photoperiod was 8 L:16 D.

Preparation of acellular integument extracts. Animals were selected 30 min before the onset of the light period. Acellular extracts were prepared in the darkness at 4 °C. Integuments were homogenized (0.25 M sucrose, 2.5 mM $CaCl_2$ and 50 mM tris pH 7.2). Homogenates were centrifuged for 30 min at 60,000 × g. Certain supernatants were sonicated at 40 V for 40 sec and again centrifuged either at 100,000 × g for 60 min or at 220,000 × g for 30 min. The resulting supernatants were used for the experiments.

Acellular extract illumination. Extracts obtained in the dark were incubated in a large dish, so that a maximum surface

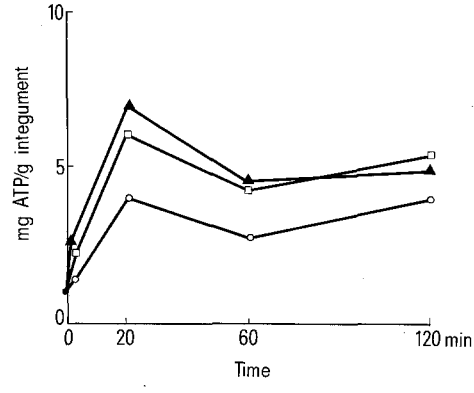

Fig. 1. LAPS activity in 60,000 × g supernatant after sonication (▲). For comparison, ATP concentrations were measured under the same conditions in non-sonicated 1000 × g supernatants (○) and in non-sonicated 60,000 × g supernatants (□).

was exposed to the light. The temperature of the controls or samples was 19 °C. At time t=0 extracts were illuminated by the red light[11]. Variation in ATP with time was determined by periodical sampling of 250 µl extracts which were added to 1 ml of 0.6 N cold perchloric acid to stop all enzymatic reactions.

ATP-assay. The perchlorate mixture was centrifuged at 10,000×g for 30 min, the supernatant was neutralized and the ATP concentration measured with a Boehringer-Mannheim-ATP-Kit[12-14].

Results. In our earlier work we showed that LAPS was present in 60,000×g supernatants devoid of intact mitochondria, but disappeared from supernatants after additional 100,000×g centrifugation. We concluded that LAPS was part of a particle system. This led us to investigate the effect of sonication on LAPS, particularly as regards the resistance of LAPS to this process.

LAPS activity is resistant to sonication. As shown in figure 1, LAPS activity can be detected in the 60,000×g supernatant even after sonication.

After sonication, LAPS activity is still present in 220,000×g supernatants. Figure 2 shows that when sonicated 60,000×g supernatants were centrifuged at 220,000×g, LAPS activity was not detectable. This result is consistent with the presence of LAPS included in particles. However,

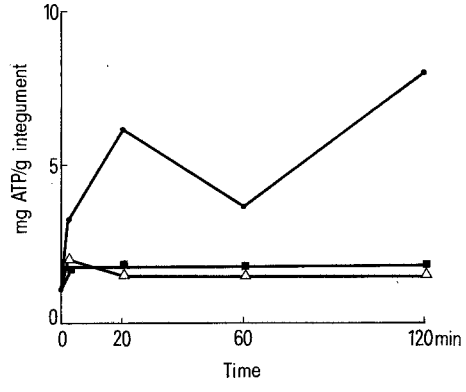

Fig. 2. Detection of LAPS activity in a 220,000×g supernatant. The 220,000×g supernatants were illuminated for increasing periods either after addition of 10 mM KPO_4 and 1 mM ADP (●) or without KPO_4 and with ADP (■). 1 sample of the 220,000×g supernatant containing phosphate and ADP was kept in the dark (△).

LAPS activity became detectable when phosphate and ADP were added to these 220,000×g supernatants. The persistence of this activity led us to conclude that LAPS can be split into smaller sub-units by sonication without losing its capacity to produce ATP when they are stimulated by light after prior addition of ADP and phosphate.

This last condition prompted us to investigate the possibility that ADP and phosphate might be substrates for the light-dependent ATP production in the LAPS sub-units.

The presence of ADP and phosphate is necessary for ATP production stimulated by light. Different concentrations of ADP were added to samples of a 220,000×g supernatant which all contained a 0.1 M final concentration of KPO_4 buffer (pH 7.2). The ATP concentrations were measured after 20 min of illumination.

As shown in figure 3 ATP light-dependent production increased as a function of the ADP concentration. Thus ATP production was zero when no ADP or 0.01 mM ADP was added. After addition of 0.1 mM ADP this production was measurable and reached a maximum after addition of 10 mM ADP. The half-maximum was obtained for an ADP concentration somewhere between 1 and 10 mM.

After defining the optimal ADP concentration (10 mM) we were able to compare the light-stimulated ATP yields in the 220,000×g supernatants in the absence or in the presence of 1, 10, 50, 100 mM KPO_4 for a concentration of 10 mM ADP. The KPO_4 enhanced light-dependent-ATP production from 2.5 for 10 mM to 12 for 100 mM (figure 4).

We may thus conclude that phosphate and ADP are the most probable substrates for light-dependent ATP production.

Discussion. This paper describes some of the characteristics of the light-dependent ATP production system (LAPS) present in cell-free extracts of insect integuments.

One of the surprising characteristics of LAPS is that it remains functional in sonicated 220,000×g supernatant. This observation and others[12] rule out the possibility that the LAPS system is located in the intact mitochondria.

The identity of the light-collecting element is still unknown. Pterobilin, an integumentary pigment biosynthesized in situ is a possible photoreceptor[11,15-17].

The precise mechanism producing the increase in ATP remains open to question. It might be that light has an inhibitory effect on the ATP degradation system, but this would require a continuous flow of ATP into the acellular extracts which in terms of energy is impossible. Thus, in our

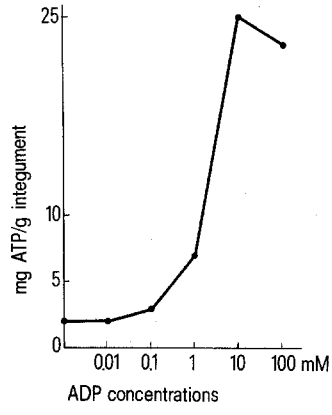

Fig. 3. Increase in the light-dependent ATP production as a function of ADP concentrations. 0–100 mM ADP were added to a 220,000×g supernatant containing 0.1 M KPO_4. Each sample was illuminated for 20 min and the ATP concentration measured.

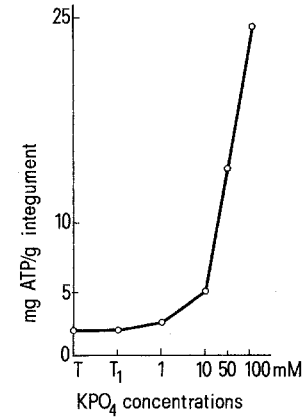

Fig. 4. Increase in the light-dependent-ATP production as a function of KPO_4 concentrations. 0–100 mM KPO_4 were added to a 220,000×g supernatant containing 0.01 M ADP. T represent the control in dark; T_1 the control after 20 min of incubation under red light. An other samples were illuminated for 20 min.

view, the simplest explanation for the increase in ATP seems to be the existence of an enzymatic activity which produces ATP from phosphate and ADP under light stimulation. This hypothesis is consistent with the results presented here. Additional arguments in favor of this enzymatic mechanism include the sensitivity of the cell free extracts to heat on detergents, and the fact that they are protected by low temperature and SH bonds.

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Plasma albumin patterns of the species *Rana ridibunda* in Greece

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Summary. Serum electrophoretic studies on the species *Rana ridibunda* displayed one new albumin band D and a total of 6 albumin patterns B, BC, C, BD, CD, BCD. The albumin patterns found are compared with those reported from Central and Northern Europe.

The species *Rana ridibunda* Pallas 1771, which occurs in great abundance in Greece[2], occurs in Central Europe in broad sympatry with the species *Rana lessonae* and *Rana esculenta*. During recent years breeding experiments among these 3 forms[3] have revealed that *R. ridibunda* and *R. lessonae* breed true and that *R. esculenta* is a hybrid between them. Moreover, the hybrid origin of *R. esculenta* was confirmed by electrophoretic comparisons of serum proteins[4]. From the above facts it is obvious that the species *R. ridibunda* belongs to a very interesting systematic group. Our study also indicates that in Greece this species shows a great external polymorphism.

The purpose of this paper is to study the serum albumins of 2 frog populations.

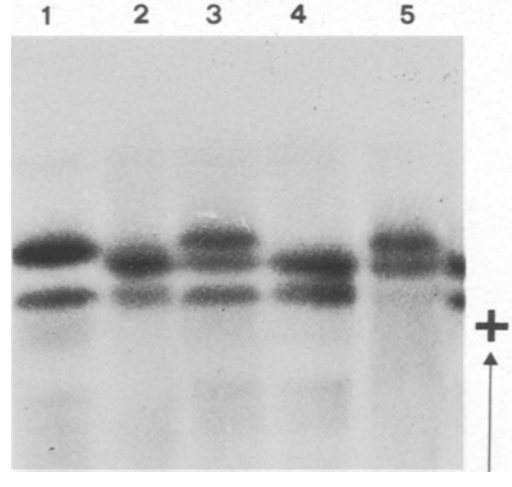
Materials and methods. Adult individuals of *R. ridibunda* (a total of 94 frogs) were collected from 2 localities (Chalastra and Gallikos river) at a distance of about 40 km around the city of Thessaloniki. The frogs were anaesthetised and blood was taken from the heart ventricle. Serum was separated from the clotted blood the next day and frozen immediately at −20 °C. Sera were mixed with 20% sucrose solution before use. Then horizontal starch gel elctrophoresis was carried out, according to the method of Weitkamp et al.[5]. After electrophoresis, gels were stained with 1% amido-black. As standards for the different albumin patterns we used sera which were kindly provided by Dr H. Wijnands.

Results and discussion. Serum electrophoresis showed 6 albumin patterns B, BC, C, BD, CD and BCD (figure). Apart from the bands B and C which are already known in the Netherlands[6] and in Central Europe[4] one new band was found, which we called D. The band D moves more slowly than the C band, and bands C and D have relative mobilities compared with B of 0.77 and 0.93 respectively (figure). The distribution of our 6 patterns is shown in the table. There is an obvious difference between the 2 populations examined in the distribution of the albumin patterns. Moreover, our frequency distribution of the known patterns B, BC and C is quite different from that of Wijnands[6]. He states that in France the frequency of the pattern B is greater than that of pattern BC and that in the Netherlands he found only 1 specimen with the albumin pattern C.

It is a problem to explain the pattern with the 3 albumin bands BCD. Perhaps we can adopt the same hypothesis as

Frequency distribution of the albumin patterns of frogs from two different localities

Collecting station	Albumin pattern B	BC	C	BD	CD	BCD	Total
Chalastra	17	30	22	1	1	–	71
Gallikos river	3	6	4	4	3	3	23



Electrophoretic albumin patterns of the species *Rana ridibunda* (1 = BD, 2,4 = CD, 3 = BCD, 5 = BC).