Fourteen adult New Zealand White does (3–5 kg body weight) were randomly allocated to either control or experimental groups. One doe was mated per day; she was taken to the cage of the buck and removed immediately after copulation. The time of mating was considered as 0 h of pregnancy. The experimental group was treated daily with thalidomide (α-phthalimido glutarimide) in a dose of either 150, 200 or 250 mg/kg from the 8th to 12th day inclusive. Immediately before administration, the drug was suspended in 10 ml 0.5% carobxymethyl cellulose and 5% glucose by vigorous agitation for 1–3 min at room temperature and given by gavage. Control animals were given 10 ml of the suspending medium on the corresponding days of gestation.

Animals were killed on day 13, 15, 17 and 21. Each doe was anaesthetized with i.v. sodium pentobarbital, the abdomen opened and the foetuses and membranes exposed in utero. The numbers of viable, dead and resorbed foetuses were recorded. From each foetus in turn, the membranes were removed and Karnovsky's combined aldehyde fixative injected into the subdural space at the base of the skull. The cervical vertebral column including the intact spinal cord and ganglia, were excized without delay and agitated in Karnovsky's solution overnight at 4 °C. The dorsal root ganglia at the level of C6 and C7 were then dissected away from the vertebral column and spinal cord and transferred to 0.1 *M* cacodylate buffer pH 7.4. The ganglia were post-fixed in 2% osmium tetroxide for 1 h and then embedded in Spurr's epoxy resin. Sections were cut with a LKB ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Six control does produced a total of 49 viable foetuses of which none were deformed but there were 5 resorptions. 3 does treated with 150 mg/kg thalidomide produced 9 resorptions and 15 foetuses, of which 6 were malformed. 3 does treated with 200 mg/kg produced 6 resorptions and 17 foetuses, of which 9 were deformed. 2 does treated with 250 mg/kg produced 5 resorptions and 12 foetuses, one of which was dead and 6 were malformed.

Ultrastructural changes were found in axons, Schwann and satellite cells as well as ganglion cells of cervical posterior root ganglia in 15-, 17- and 21-day-old foetuses exposed to thalidomide which exhibited marked reduction deformities of the relevant dermatomes, as well as in 13-day-old foetuses from treated does. Abnormalities of axons were the commonest lesions which included loss of microfilaments and degeneration of microtubules, together with vacuolation and distension of mitochondria (Figure 1). More severely damaged axons were represented by irregular whorls of laminated, electron dense configurations or 'myelin figures', many of which were related to either the cytoplasms of Schwann cells or to immediately adjacent axons (Figure 2). Ganglion cells exhibited only minor abnormalities, comprizing an overall increase in number of mitochondria and free ribosomes, but without concomitant increase in endoplasmic reticulum. The nuclear envelopes of ganglion cells at 13 days and to a significantly and progressively lesser extent in 15-, 17- and 21-day-old foetuses were irregularly indented.

Ultrastructural changes are therefore present in relevant cervical posterior root ganglia at 13 days, i.e., preceding the appearance of thalidomide-induced fore limb malformations and subsequently with increasing frequency and concomitant with progressive maldevelopment. It is therefore likely that these changes represent the primary lesion evoked by thalidomide and are not secondary atrophic neural changes resultant from deformity of the peripheral segments.

Pseudomonas aeruginosa Causes Epidemic Disease in the Milkweed Bug, *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera)

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Summary. *Pseudomonas aeruginosa* was recognized as the causative organism of an epidemic disease occurring in a laboratory breed of *Oncopeltus fasciatus*. The infection probably occurs peroral and is favoured by high temperature and humidity. *Pseudomonas aeruginosa* destroys the fat body of the bug.

Oncopeltus fasciatus has become a widely used laboratory animal[2]. Therefore the occurence of a severe epidemic disease, as observed in this and other laboratories, seems of interest. The illness breaks out rather suddenly and unpredictably affecting almost exclusively animals of the 5th (last) larval instar, never imagines. If the sickness appears in a culture jar, within 1 to 3 days almost all bugs of the 5th and – to a lesser degree – 4th larval stage are killed. Dead bugs have dark blue to black swollen abdomens and spread a characteristic unpleasant odor, which reminds one of trimethylamine. First signs of the illness are hard to diagnose, since dark gut content shining through the cuticle can be mistaken with the onset of the pathological abdomen darkening. If the symptoms, i.e. discoloration of extended body areas and paralization, are clearly recognizable, the animal perishes normally within 24 h; recovery was never observed.

Light and electron microscopic studies on specimens from independently diseased populations revealed that bacteria were present in the hemocoel, together with tissue debris, and extra- and intracellularly in the fat body, obviously dissolving it (Figure 1). Other tissues seemed intact and never invaded by bacteria. In several experiments, at least 5 different strains of bacteria (Pl 3, Pl 4, Pl 5, Pl 11, Pl 1/12) were isolated. It is highly probable that the majority of the strains is derived from gut content[3], for despite careful preparation of the fat body a damage of the gut could not always be avoided. To establish the disease causing bacterium, young 5th larval instars were punctured with a needle contaminated

[1] For her interest and discussion I thank Dr. G. Hausner. Miss I. von Graevenitz and Miss H. Schilling gave techincal support.

[2] D. Feir, A. Rev. Entom. *19*, 81 (1974).

[3] D. Feir, Ann. entom. Soc. Am. *56*, 829 (1963).

with the testing culture (Pl 3, Pl 4 etc.). The mortality in experiments with Pl 3, Pl 4, Pl 5 and Pl 11 was not significantly higher than in controls (totally untreated or punctured with a sterile needle). Pl 1/12, in contrast, caused death of all treated specimen, not later than 4 days after puncturing, but mostly within 24 h. The larvae vaccinated with Pl 1/12 – and only these – offer the same symptoms as spontaneously diseased animals. Electron microscopic preparations reveal the same pathological picture as shown in Figure 1. Also the fine structure of agar cultures of Pl 1/12 appears to be identical with that of bacteria found in spontaneously sick bugs (Figure 2).

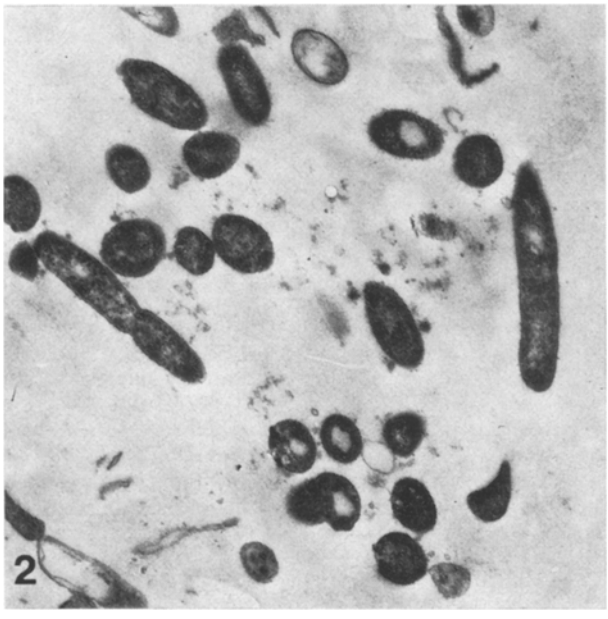

Fig. 1. Part of fat body from a spontaneously diseased bug. Remnants of destroyed tissue between the bacteria. × 4,000.

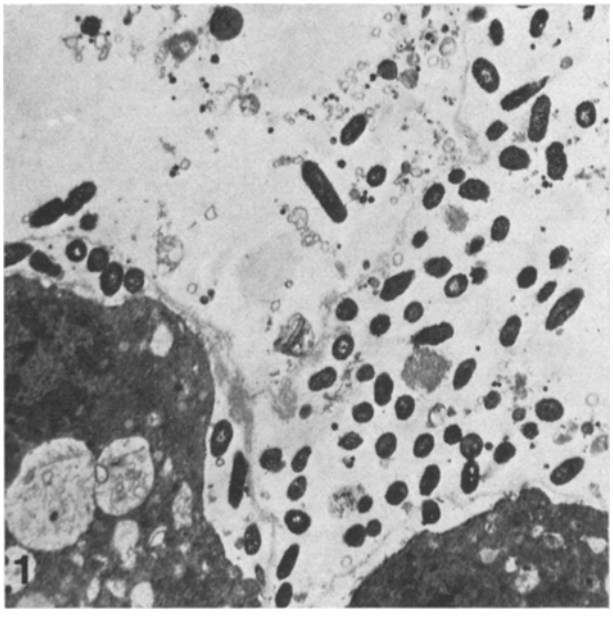

Fig. 2. *Pseudomonas aeruginosa* isolated from sick bugs and cultivated on agar culture medium. × 6,000.

Based on these results, and the facts that Pl 1/12 was not isolated and microorganisms were never found in tissue from healthy bugs, Pl 1/12 is considered to be the causative organism of the epidemic disease in milkweed bugs.

Pl 1/12 grew well in all used media (Standard I-, and Standard II-Nähragar, and Sabouraud-Glucose-Agar from Merck, Darmstadt) but was cultivated mainly on Standard I. Newly inoculated cultures maintained at room temperature released after 2 to 4 days a pale blue, bluegreen dye into the substrate, which soon turned brown. A smell was noticed very similar to that of ill bugs. In the Institut für Medizinische Mikrobiologie[4], Pl 1/12 was identified as *Pseudomonas aeruginosa*.

The pathogenity of Pl 1/12 was compared with that of a laboratory strain (A 24) of *P. aeruginosa*[5] in respect to different developmental stages of *Oncopeltus* and to the guinea-pig. Larvae of the 3rd, 4th, (early and late) 5th instar and young (less than 4 days) and older (more than 30 days) adults were punctured with Pl 1/12. All test animals died within 4 days after treatment, except old imagines, of which 10% survived day 4 and 2% day 10. Thus it seems that immunity can be gained after adult ecdysis, which would explain that the disease does not normally affect bugs of this stage. Immunity against *P. aeruginosa* was reported in other insects[6]. A 24 tested on 5th larval instars showed a decreased pathogenity in comparison to Pl 1/12: 10% survived and moulted to adults. Whereas a s.c. injection of A 24 in Ringer's solution in guinea-pigs was followed by a noticable reaction (locomotor activity was lowered and eating ceased for 2 days), injections of Pl 1/12, and extracts of diseased and healthy bugs had no visible impact. In conclusion, *P. aeruginosa* isolated from sick milkweed bugs shows increased pathogenity in the insect *Oncopeltus* and a decreased pathogenity in the guinea-pig in comparison to the laboratory strain A 24.

In a number of insects *P. aeruginosa* was detected in gut content or feces, and had caused no defects[3,6,7]. But when the bacterium was injected into the hemocoel, the test animals died within 48 h, even if very low doses were given. Whereas some species showed no reaction after peroral infection, others died within 7 to 21 days after feeding[6]. The occurrence of a spontaneous laboratory epidemic caused by *P. aeruginosa* is only reported from *Schistocerca gregaria*, where the bacterium was isolated from the body fluid[8]. But later it was found that *P. aeruginosa* was a secondary invader to the infestation of the fungus, *Aspergillus flavus*[9]. In order to discover the factors leading to the manifestation of the disease, and the mode of its transmission in a population of *Oncopeltus*, a series of experiments was carried out.

Food, strongly and repeatedly contaminated with Pl 1/12 and kept moist, was supplied to imagines and larvae of all stages. The test animals were kept till all individuals had reached adult stage, or were dead. Whereas exposed adults and larvae of late last instar showed no significantly increased death rate in comparison to controls, in all other tests mortality was about

[4] I wish to express my appreciation to Prof. Dr. M. Loos, Institut für Medizinische Mikrobiologie, Universität Mainz, for his help.

[5] Friendly supplied by Prof. Dr. B. HACCIUS, Institut für Spezielle Botanik, Universität Mainz.

[6] A. KRIEG, *Grundlagen der Insektenpathologie* (Dr. Dietrich Steinkopff Verlag, Darmstadt 1961).

[7] E. A. STEINHAUS, *Insect Microbiology: An Account of the Microbes Associated with Insects and Ticks* (Hafner Publishing Company, New York and London 1967).

[8] P. LEPESME, C. r. Soc. Biol. Paris *125*, 492 (1937).

[9] P. LEPESME, Bull. Soc. Hist. Nat. Agric. *29*, 372 (1938).

25% above normal (10 to 20%) within 10 days. Nevertheless, the induced sickness had not the characteristics of an epidemic disease, for the death rate in the experiments was rather constant till imaginal moult. A sudden increase of mortality (as was expected after reaching last larval stage) was not observed. Thus one or several additional factors must be of influence.

It is known that mucin increases the pathogenity of *P. aeruginosa*[6]. The rôle of *Aspergillus flavus* in the epidemic of *Schistocerca* was already mentioned. For the disease in *Oncopeltus fasciatus* previously described by BEARD[10], a mycotoxin from *Aspergillus flavus var. columnaris* was made responsible[11,12]. Fungi frequently grow on the normal food (sunflower seeds), but normally the bugs do not seem to mind it. Nevertheless the influence of *Aspergillus flavus* (D2)[5] was tested. Sunflower seeds on an agar culture of D2, and seeds on which D2 was actually cultivated, were given as nutrition to 3rd, 4th and 5th larval instars. The results showed that in no case had D2 a significant impact on larvae. Also when the food was additionally contaminated with Pl 1/12, no synergistic effect of D2 was observed. This, of course, does not exclude that a different strain or another fungus may facilitate the manifestation of the epidemic.

The illness occurred most frequently when humidity and breeding temperature were high (30 °C). Larvae of 4th and 5th stage and imagines reared under these conditions received with Pl 1/12 contaminated food. In all cases 90% of the test animals died within 2 weeks, the majority between day 5 and 10. The sick and dead bugs showed the symptoms of spontaneously diseased populations. The incubation period seems to be about 1 week. This is also supported by the observation that culture jars with healthy larvae which are brought into an incubator with experimentally diseased populations, are invaded by the bacterium after about 10 days. Imagines are obviously susceptible to the bacterium but have probably built up some immunity (see above), which may protect them under normal conditions, where they would hardly come in contact with as much bacteria as in the experiment.

Thus at least three factors are responsible for the manifestation of the epidemic disease: The presence of *P. aeruginosa*, high temperature (optimal temperature for development of *P. aeruginosa* is 37 °C[6]), and high humidity (probably necessary for the bacterium to survive[6]). It is assumed that the disease spreads by peroral infection, while feeding on contaminated seeds. Since cannibalism is not seldom, especially when starving, this could also be of importance for transmission. The bacterium apparently does not damage the gut epithel while passing through the hemocoel. Death is obviously caused by destruction of the fat body.

[10] R. L. BEARD, J. econ. Entom. *52*, 177 (1959).
[11] R. L. BEARD, J. Invert. Path. *10*, 438 (1968).
[12] R. L. BEARD and G. S. WALTON, J. Invert. Path. *14*, 53 (1969).

Influence of Cyclic Nucleotides on Protein Synthesis in Vascular Smooth Muscle

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Summary. The incorporation of leucine-^{14}C into protein in bovine mesenteric arteries was augmented by cyclic GMP (10^{-3} *M*) and decreased by cyclic AMP (10^{-3} *M*). There was no effect of 5'AMP (10^{-3} *M*). The phosphodiesterase inhibiting drugs theophylline (10^{-3} *M*) and papaverine (5×10^{-5} g/ml) both decreased the leucine-^{14}C incorporation.

In atherosclerotic aortas of pigs, the synthesis of proteins has been reported to be increased[2,3]. There was a reduction of the cyclic AMP and an elevation of the cyclic GMP level in atherosclerotic pieces of the muscle in comparison with normal parts of the vessel[3]. There are reports that cyclic AMP inhibited the proliferation of myogenic cells in tissue culture[4], whereas cyclic GMP was found to stimulate the in vitro synthesis of thyroidal proteins[5]. Considering these observations, we thought it of interest

[1] We are indebted to Mrs. LENA BURLIN for her assistance. Financial support has been provided by the Swedish State Medical Research Council (No. 04X-101; 04X-4498).
[2] D. N. KIM, K. T. LEE and W. A. THOMAS, Expl molec. Path. *8*, 263 (1968).
[3] L. LUNDHOLM, L. JACOBSSON, H. ARNQVIST and R. ANDERSSON, in preparation.
[4] J. P. WAHRMAN, R. WINAND and B. LUZZATI, Nature New Biol. *245*, 112 (1973).
[5] PH. NAYER, Biochimie *55*, 1507 (1973).

Table I. Influence of some drugs on ^{14}C-leucine incorporation in bovine mesenteric arteries

	Control	Drug	Difference
Cyclic AMP (1×10^{-3} *M*) ($n = 6$)	0.257 ± 0.015	0.123 ± 0.015	− 0.135 ± 0.019[c]
Cyclic AMP (5×10^{-4} *M*) ($n = 6$)	0.174 ± 0.020	0.131 ± 0.010	− 0.042 ± 0.010[b]
Cyclic AMP (1×10^{-4} *M*) ($n = 4$)	0.202 ± 0.029	0.227 ± 0.048	0.025 ± 0.019
5'AMP (1×10^{-3} *M*) ($n = 6$)	0.239 ± 0.035	0.235 ± 0.019	0.004 ± 0.015
Theophylline (1×10^{-3} *M*) ($n = 10$)	0.212 ± 0.014	0.168 ± 0.014	− 0.044 ± 0.012[b]
Cyclic AMP (5×10^{-4} *M*) ($n = 6$) + theophylline (1×10^{-3} *M*)	0.218 ± 0.015	0.189 ± 0.018	− 0.029 ± 0.017
Papaverine (5×10^{-5} g/ml) ($n = 7$)	0.174 ± 0.020	0.074 ± 0.012	− 0.100 ± 0.016[c]
Cyclic AMP (5×10^{-4} *M*) + papaverine (5×10^{-5} g/ml) ($n = 7$)	0.174 ± 0.020	0.053 ± 0.012	− 0.121 ± 0.012[c]
Cyclic GMP (1×10^{-3} *M*) ($n = 6$)	0.204 ± 0.027	0.244 ± 0.028	0.040 ± 0.010[b]
Cyclic GMP (1×10^{-5} *M*) ($n = 6$)	0.197 ± 0.022	0.210 ± 0.033	0.013 ± 0.023

Incorporation in μmole/g protein/180 min. Significance of the effects is denoted [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.