The earthworms used were *Eisenia foetida*, isolated from known locations and maintained in the laboratory for at least 2 years, and *Dendrobaena veneta* collected from the wild. The worms were cultured in a peat-chalk mixture (100:1), in petri dishes and maintained at 15 °C. Grafts were made between clitellate worms of similar age (120 days) and size (60 mm). The worms were anaesthetized for 5 min in 7% ethanol in earthworm saline and grafts cut from the dorsal body wall (3 × 2 mm) with fine scissors. The graft bed of the host was not swabbed out to remove excess coelomic fluid, the graft was placed in this maintaining its original posterior anterior orientation after some trimming if necessary to allow for a good fit. No sutures were used to hold the graft in place. Each operation was carried out whilst the animal was laid on a cold plate maintained at 2 °C. The worms were then transferred to individual petri dishes containing damp filter paper. The experimental material was observed at 2 hourly intervals for the first 6 h, after which time they were moistened again, they were then observed at 24 hourly intervals. Grafts were made between the donor-recipient combinations shown in the Table which also shows the percentage of hyperacute rejections for each combination. Autografts showed a hyperacute rejection rate of about 20% and as there should be full compatibility, this rate probably represents a true base level for technical failure. The hyperacute rejection rate for primary allograft and xenograft combinations is in all cases considerably greater and χ^2 analysis shows the differences to be significant at 0.05% level. With second set grafts there was marked hyperacute rejection when the time interval between first and second graft was 5 and 8 days. Published work suggests this time interval between first and second grafts produces the greatest accelerated rejection of second grafts[4].

Summary of graft combination and statistical analysis

Donor		Host	Hyperacute rejection (%)	χ^2
ET	→	EU	53	4.3796[a]
EU	→	EU	39	
EU	→	ET	55	7.7815[b]
ET	→	ET	35	
ET	→	ET	35	0.2556
EU	→	EU	20	
ET	→	EU	53	0.3070
EU	→	ET	55	
EU	Autograft		20	3.8788[a]
EU	→	EU	39	
ET	Autograft		24	1.5568
ET	→	ET	35	
EU	Autograft		20	10.2546[b]
ET	→	EU	53	
ET	Autograft		24	9.5085[b]
EU	→	EU	55	
EU	Autograft		20	7.2180[b]
DV	→	EU	52	
ET	Autograft		24	4.0614[b]
DV	→	ET	50	
EU	→	EU		
Same population				
5 day interval between grafts				
1st set graft			29	21.5666[b]
2nd set graft			50	
8 day interval between grafts				
1st set graft			28	4.4979[a]
2nd set graft			75	

EU, *Eisenia foetida unicolor*; ET, *Eisenia foetida typica*; DV, *Dendrobaena veneta*. [a] $0.05 > p > 0.01$. [b] $0.01 > p > 0.001$.

It seems likely then that this excess of hyperacute rejections seen in allografts and xenografts does reflect the incompatibility of donor and recipient. Graft rejection within 24 h in such combinations can no longer be ascribed solely to technical failure, and the underlying mechanisms, whether cellular or humoral, deserve further study.

Zusammenfassung. Das Phänomen der hyperakuten Transplantat-Abstossung (innerhalb von 24 h) wurde bei Invertebraten untersucht. Diese rasche Abstossungsreaktion wird signifikant häufiger bei Allo- oder Xeno- als bei Autotransplantationen beobachtet. Dies spricht für das Vorliegen einer Inkompatibilitätsreaktion.

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The Influence of Cyclic Nucleotides (c-AMP and c-GMP) on the Nitrous Blue Tetrazolium Reduction by Human Polymorphonuclear Leucocytes

The participation of Adenosine 3′:5′-cyclic monophosphate (cyclic-AMP) in various metabolic processes accompaning phagocytosis of particles by polymorphonuclear (PMN) leucocytes is the subject of controversy in recent literature[1–3]. SCHELL-FREDERICK[4], recently reported that addition of cyclic-AMP or related substances does not influence the glucose oxidation and CO_2 production, the two basic processes stimulated by particle ingestion. QUALITIONE et al.[5] demonstrated an inhibition of hexose-monophosphate shunt activity following administration of cyclic-AMP, dibutyryl cyclic-AMP and caffeine.

These contradictory reports prompted us to investigate the influence of cyclic-AMP and substances known to

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The influence of cyclic nucleotides and its relatives on the quantitative nitrous-blue-tetrazolium reduction test

Substance and amount	No. of tests	NBT reduction (Δ OD)	
		Mean	Range
Dibutyryl cyclic-AMP (5×10^{6} *M*)	17	0.01	0.00–0.07
Cyclic-GMP (3×10^{-3} *M*)	14	0.15	0.09–0.30
Theophyllin (10^{-5} *M*)	25	−0.02	0.00–(−)0.06
Carbachol (10^{-5} *M*)	8	0.09	0.05–0.12
Endotoxin (20 γ)	30	0.18	0.13–0.24
Ascorbic acid (5×10^{-4} *M*)	11	0.26	0.22–0.31
Methylene blue 0.22×10^{-3}*M*	16	0.32	0.26–0.37
Dibutyryl c-AMP + endotoxin[a]	17	0.01	0.00–0.01
Dibutyryl c-AMP + ascorbic acid[a]	11	0.01	0.00–0.02
Dibutyryl c-AMP + methylene blue[a]	16	0.30	0.25–(−)0.33
Theophyllin + endotoxin[a]	18	−0.02	0.00–(−)0.06

[a] The amount of each substance as when separately added.

increase its intracellular concentration (such as theophyllin) on the nitrous blue tetrazolium (NBT) reduction test.

According to the Yin Yang hypothesis, cyclic-AMP and guanosine 3′:5′-cyclic monophosphate (cyclic-GMP), have antagonistic effects in the bidirectionally controlled systems[6], with accumulating evidence that this is true also for phagocytizing cells. We decided to investigate the influence of Cyclic-GMP on the NBT reduction in PMN leucocytes.

Material and methods. Samples of 20 ml of venous blood of healthy young medical students were collected in 30 ml disposable plastic tubes containing 50 units of heparin per ml (Organon). 5 ml of 6% Dextran solution (Fluka A.G.) were added to each tube and the mixture was allowed to settle for 1 h at room temperature.

The next steps were as described by BAEHNER and NATHAN[7], with the following modifications. To the test tubes with 0.1 ml of leucocytes suspension (approximately 2.5×10^{6} leucocytes), we added instead of latex the following reagents: 1. 20 γ of Endotoxin (B_4 lipopolysaccharide W, Difco Detroit). 2. Dibutyryl adenosine 3′:5′-cyclic monophosphoric acid (Sigma). 3. Guanosine 3′:5′-cyclic monophosphoric acid (Sigma). 4. Theophillin (Sigma). 5. Ascorbic acid. 6. Methylene blue (MB). The amounts of these substances were similar to those used by other investigators studying other leucocyte functions.

In addition to the preincubation with the substances mentioned, another addition was made as in the combinations listed in the results (Table). After addition of 0.2 ml of 0.1% NBT (Sigma), the mixtures were incubated for 30 min at 37 °C. The reaction was stopped by addition of 10 ml 0.5 *N* HCl and centrifuged. The reduced NBT (Formazan) was extracted once in 3 ml pyridine (BDH) in a boiling water bath. The colour intensity of the pyridine was determined by a spectrophotometer (Unicam 800). The results were expressed as Δ OD of reduced NBT per 2.5×10^{6} cells per 30 min calculated as the difference in OD between stimulated tube and a blank). If the effect of a tested material was diminution of Δ OD, then the difference was expressed as a negative value.

Results. The mean values of Δ OD of all experiments are summarized in the Table. It is clear that addition of cyclic-AMP to the leucocyte suspension has no stimulatory effect on the NBT reduction and the 'stimulating' values are near the 'resting' (very low Δ OD). The inhibitory effect of cyclic-AMP is accentuated when cells are preincubated with cyclic-AMP and then endotoxin or ascorbic acid are added. The Δ OD for endotoxin falls from 0.18 to 0.01, and for ascorbic acid from 0.26 to 0.01 ($p < 0.001$). Theophyllin added to leucocyte suspension lowers even the resting values and the Δ OD becomes negative. The same effect is seen when the cells are preincubated with theophyllin and then endotoxin added (negative Δ OD). Cyclic-GMP when added to a cell suspension acts as a stimulator and a Δ OD of 0.15 is calculated without statistical significant difference from the Δ OD obtained when endotoxin is used as a stimulator (0.18). When leucocytes are pre-incubated with cyclic-AMP and then MB is added, there is no significant reduction in the Δ OD.

Discussion. Our results show clearly the inhibitory effect of dibutyryl cyclic-AMP on the NBT reduction by human polymorphonuclear leucocytes. The same effect characterizes theophyllin which is known to increase the concentration of cyclic-AMP by inhibition of its diesterase.

On the other hand, cyclic-GMP increases the reducing ability of NBT by human leucocytes as does carbamylcholine (Carbachol). The inhibitory effect of cyclic-AMP on hexose-monophosphate shunt was demonstrated by QUALITIONE et al.[5]. They found that dibutyryl c-AMP depressed the hexose-monophosphate shunt activity of resting phagocytes by 20%. 1 m*M* theophyllin inhibits the hexose-monophosphate shunt by over 50%. FLYER and FINCH[8] found that PGE, which increases the intracellular content of cyclic-AMP, impairs glucose oxidation and NBT reduction in human granulocytes during phagocytosis. WEISSMANN et al.[9] demonstrated inhibition of lysosomal enzyme release from polymorphonuclear leucocytes during phagocytosis in the presence of 3′:5′-cyclic-AMP. SCHELL-FREDERICK and VAN SANDE[4] recently reported that the intracellular concentrations of cyclic-AMP change little during phagocytosis of latex particles, and that there is no effect of cyclic-AMP, dibutyryl c-AMP and related substances on glucose oxidation, oxygen consumption and iodide organification during phagocytosis.

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In contrast with rapidly accumulating contradictory reports concerning the action of cyclic-AMP on phagocytic events, there are practically no reports about the influence of its counterpartner cyclic-GMP.

Other parameters of phagocytic activity are known to be influenced by cyclic-GMP. ESTENSEN et al.[10] reported recently that cyclic-GMP and cholinergic agents enhance degranulation of PMN and increase its leucotactic activity. Cyclic-AMP inhibits both leucotaxis[11] and degranulation[12]. These antagonistic activities of both nucleotides were also clearly demonstrated in our system. The effect of MB (a known stimulator of hexose monophosphate shunt) in increasing the NBT reduction was previously demonstrated[13]. It was further shown that ascorbic acid, another stimulator of hexose monophosphate shunt[14], also increases the NBT reduction ability of PMN[15].

In this investigation it is shown that when leucocytes are pre- incubated with cyclic-AMP and then endotoxin, ascorbic acid or MB are added, the stimulating effect of endotoxin and ascorbic acid is markedly reduced, while that of MB is unchanged. This might suggest that we are dealing with different mechanisms of stimulation of NBT reduction. Although our conclusions were made indirectly, they support the previous suggestions[6] about the influence of cyclic nucleotides on the intracellular mechanisms during phagocytosis and the antagonistic activities of cyclic-AMP and cyclic-GMP.

Résumé. On a étudié l'influence des nucléotides cycliques (AMP-cyclique et GMP-cyclique) sur la réduction du NBT. L'AMP-cyclique ainsi que la théophylline inhibent cette réduction, tandis que la GMP-cyclique l'active. La préincubation de leucocytes en présence de l'AMP-cyclique annule l'activation de la réduction par l'acide ascorbique mais ne l'influence pas par le bleu de méthylène. On peut en conclure que dans ce système, comme dans les autres, ces deux nucléotides jouent un rôle d'antagonistes.

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Relative Oxygenase Activities in Juvenile Hormone Biosynthesis of Corpora Allata of an African Locust (*Schistocerca gregaria*) and American Cockroach (*Periplaneta americana*)

All known insect juvenile hormones contain a 10,11-oxirane ring in the sesquiterpenoid moiety of the molecule[1], and it is believed that this ring is introduced at the last enzymatic step in biosynthesis by mono-oxygenation of the corresponding sesquiterpenoid olefinic ester[2]. In this report we concern ourselves with the activity of this terminal oxygenase in corpora allata of 2 insect species, as revealed by the application of short-term in vitro radiolabelled assay procedures. Work in this laboratory[2] has shown that when corpora allata are taken from adult female *Schistocerca gregaria*, addition of sesquiterpenoid acid to the medium results in a large increase in the rate of biosynthesis of juvenile hormone, and that the corresponding olefinic ester is detectable within the glands. The kinetics of incorporation of (methyl-^{14}C) methionine and (C-2 ^{3}H) *trans,trans*-farnesenic acid into both the olefinic ester (methyl farnesoate) and the 10,11 epoxy ester (C_{16}JH) confirm that methyl farnesoate is the immediate precursor of this juvenile hormone in *S. gregaria*[3]. Here we have compared the rate of epoxidation with the intracellular amount of methyl farnesoate over a wide range of epoxidation rates by utilizing both the natural variations in the biosynthetic capacity of the glands in reproductively active female locusts during the course of ovarian maturation (TOBE and PRATT, in preparation) and the effects of graded additions of farnesenic acid to the incubation medium[3]. For comparative purposes, we have also examined the relationship which obtains in corpora allata of reproductively active *Periplaneta americana*, whose principal juvenile hormone has also been identified as C_{16}JH (PRATT, unpublished data)[4]. We shall show that there is a large difference in the observed oxygenase activity of the glands from the 2 species and that in the case of the locust, there is no evidence that this is normally a rate-limiting step in juvenile hormone biosynthesis.

Methods. Animals were reared as previously described[2,5]; the female locusts employed were of known age between 5 and 20 days old, female cockroaches were of unknown age taken at different intervals throughout the oviposition cycle. The procedures for preparation of radio-labelled incubation media, incubation of the glands, extraction, separation and quantitation of the products by radio-TLC and liquid scintillation spectrometry were identical to, or minor modifications of, those described elsewhere[2,3]. In many of the experiments, tissue culture medium 199 (without glutamine, bicarbonate; with HEPES buffer 20 m*M*, pH 7.2) (Flow Control Laboratories Ltd.) served as the basis of the radio-labelled incubation medium. L-methionine was always present at a final concentration of 0.29 m*M* and (methyl-^{14}C) methionine (Amersham-Searle) was present at final specific radioactivities of 10.5 to 36.6 mCi/mmol in different experiments. When present, (C-2 ^{3}H) *trans,trans* farne-

[1] C_{16}JH: methyl, 10,11-epoxy-3,7,11-trimethyl-*trans,trans*, 10R-2, 6-dodecadienoate; C_{17}JH: methyl 10, 11-epoxy-3, 7, 11-trimethyl-*trans, trans, cis* 10R, 11S-2,6-tridecadienoate; C_{18}JH: methyl 10, 11-epoxy-3,11-dimethyl-7-ethyl-*trans, trans, cis* 10R, 11S-2,6-tridecadienoate.
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