Table 2. Reactivity of anti-human lung elastin peptide sera with enzymatic digests (20 mg/ml) of different elastins

Elastin source	Guinea-pig antiserum (1:8)	Rabbit antiserum (undiluted)
Human lung	4+	2+
Human aorta	4+	±
Rabbit lung	4+	–
Rabbit skin	+	–
Rat lung	4+	–
Dog lung	4+	–
Dog skin	+	–
Ox lung	4+	–
Sheep lung	4+	–

route of injection. These findings are consistent with the results of Plescia et al.[12] that rabbits produced antibodies to both DNA and methylated BSA, whereas mice did not produce antibodies against either. Similarly, different patterns of specificities in different animal species were described for collagen, another connective tissue protein. In this case rabbits, guinea-pigs, rats, mice and chickens elicited antibodies to different antigenic sites[11].

Anti-elastin peptide guinea-pig serum showed a faint precipitin reaction with 2.5 mg/ml immunogen, whereas the rabbit antiserum did not give a detectable reaction with 10 mg/ml dilution of immunogen. Substitution of the incomplete form of Freund's adjuvant did not increase the titer.

The guinea-pig antiserum did not react with any of the enzymes used in the preparation of the immunogen, i.e. trypsin, elastase or collagenase, at 10 mg/ml.

There are some apparent differences in specificity and crossreactivity between the guinea-pig and rabbit anti-elastin peptide serum, as shown in table 2. The increased cross-reactivity of the antibodies produced in guinea-pigs may be due to the higher antibody titer or to production by the rabbit of antibodies of lower titer but with higher specificity. Recently, the production of species-specific rabbit antibodies to canine lung elastin has been reported by Damiano et al.[13].

Although immuno-double diffusion against the human lung elastin peptides in the present study showed a reaction of identity between rabbit and guinea-pig antiserum, the possibility of a response against different antigenic sites cannot be completely ruled out. The guinea-pig antiserum-elastin peptide reaction produces a second precipitin line, which may arise from a different sequence. The association of antigenicity with more than one peptide sequence is also supported by the results obtained by the present authors in parallel experiments with elastin peptides from other species. Thus, peptides derived from adult sheep lung elastin elicited in rabbits antibodies which had a higher titer (1:8) than those obtained with the same amount of lung elastin peptides from humans (1:2). Unlike the anti-human rabbit sera but analogous to the anti-human guinea-pig sera, the anti-sheep rabbit sera cross-reacted with lung elastin peptides from other species, including man. Experiments designed to define the immunogenic peptide sequence(s) are in progress[14].

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Moult inhibition in the insect *Dysdercus cingulatus* (Insecta: Heteroptera) by the cerebral glands of the millipede *Jonespeltis splendidus* (Myriapoda, Diplopoda)

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Summary. Implantation of 2 pairs of cerebral glands of the diplopod *Jonespeltis* into the newly-moulted 4th or 5th instar nymphs of the insect *Dysdercus* delayed the moulting of these insects for a fairly long time. Implantation of cerebral glands into 1-day old 5th instars postponed the subsequent moult for a shorter period, whereas implantation of cerebral glands into 2-day old 5th instars had no effect. These observations suggested that a neurosecretory factor from the cerebral glands inhibited moulting in this insect, and in the case of 5th instar nymphs there was a critical period before which implanted glands were effective in moult inhibition.

Among myriapods, especially in chilopods, there is some experimental evidence that cerebral glands are the source of a moult-inhibiting principle[2,3]. Cerebral glands of the millipede *Jonespeltis splendidus* consist of swollen axonal terminations of neurosecretory cells of brain and visceral ganglia[4]. Earlier observations by the present author involving bilateral ablation of cerebral glands from well-tanned (intermoult) animals of *Jonespeltis* resulted in an acceleration of moulting which suggested that the cerebral glands are the source of a moult-inhibiting principle[5]. In the

Effects of cerebral glands of *Jonespeltis splendidus* in the moulting of 4th and 5th instar nymphs of *Dysdercus cingulatus**

Group	Number of insects	Day of moulting and the number of insects moulted														
		4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th
1. a) 2 pairs of CG's implanted into newly moulted 4th instar	60	(15)	2	2	5 (2)	4 (4)	2 (1)	6	7	4 (1)	3	2				
b) Controls	52	38 (14)														
2. a) 2 pairs of CG's implanted into the newly moulted 5th instar		73			(32)	4 (1)		5 (2)	1 (1)	6 (1)	2 (4)	3 (2)	3 (3)	2		(1)
b) Controls	60				14 (38)	8										
3. a) 2 pairs of CG's implanted into a 1-day old 5th instar	20				(2)	6	1	11								
b) Controls	12				8	4										
4. a) 2 pairs of CG's implanted into 2-day old 5th instar	20				10 (4)	3 (3)										
b) Controls	11				5 (4)	1 (1)										

Figures given in brackets are the number of insects that died. CG's - Cerebral glands.

present study, cerebral glands of *Jonespeltis* were implanted into the nymphs of *Dysdercus cingulatus* to find out whether these glands affect the moulting of these insects.

Materials and methods. The insect *Dysdercus cingulatus* (Insecta, Heteroptera) and the millipede *Jonespeltis splendidus* (Myriapoda, Diplopoda) were taken from stock raised and standardized in the laboratory. *Dysdercus* has 5 immature instars in its life history, the 5th instar moulting into the adult. The newly-moulted 4th and 5th instar nymphs were easily recognizable in the colony because of their size, nature of pigmentation, etc. The 4th instar nymphs took 4 days to moult into the 5th instar and the 5th instar 6-7 days to moult into the adult. Well-tanned millipedes were decapitated and the whole nerve ring, consisting of the brain, the cerebral glands and the suboesophageal ganglion, was dissected out and kept in a cavity block with ice-cold Ringer solution[7]. The cerebral glands were then separated from the nerve ring and kept in cavity-blocks containing ice-cold Ringer solution. The 5th instar nymphs used as hosts for implantation belonged to 3 age groups: those immediately after emergence, and 1 day and 2 days after emergence. Tissues were introduced into the body cavity of mildly anaesthetised insects by means of tweezers (No.5) via a small window made on the abdominal tergite. Corresponding control insects received muscle tissue implants of approximately the same size. Experimental and control insects were fed ad libitum on soaked cotton seeds.

Results and discussion. The present study clearly showed that implantation of cerebral glands inhibited moulting of these insects to a large extent (Table). Of the 60 newly-moulted 4th instar insects, in which 2 pairs of cerebral glands were implanted, 45 animals survived for 5 days or more (group 1). 37 animals moulted as normal 5th instars on different days ranging from the 5th-14th. In group 2, in which 73 newly-moulted 5th instar nymphs were implanted with 2 pairs of cerebral glands, 41 survived for 7 days or more. 26 animals moulted normally as adults, whereas the remaining 15 animals died as 5th instars. One survived up to the 18th day, and died as a 5th instar. Thus there was considerable inhibition of moulting in experimental animals when compared with controls. These experiments suggest that cerebral glands are the source of a moult-inhibiting principle which inhibits moulting in the insect *Dysdercus cingulatus*. When 2 pairs of cerebral glands were implanted into 1-day-old 5th instar nymphs, moulting was postponed for 3 days, whereas these glands were ineffective when implanted into 2-day old nymphs (group 3 and 4). It was interesting to find that inhibition was more pronounced when cerebral glands were implanted into newly-moulted 5th instar nymphs (group 3 and 4). It was thus apparent that there was a critical period before which cerebral glands were effective in the inhibition of moulting in 5th instar nymphs. The preceding experimental observations suggest that cerebral glands possess some kind of moult-inhibitory principle which is active not only in *Jonespeltis* but also in the insect *Dysdercus*. The present evidence of a neurosecretory factor from the cerebral glands of a diplopod inhibiting moulting in insects is the 1st study of its kind in arthropods. The above finding also raises some important evolutionary implications, in view of the fact that diplopods are supposed to be closely related to insects.

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