rocks. When it passes close to such effectively nondeformable and nondisplacable objects, the opposite wall of the tunnel will be compressed unevenly so that the final tunnel achieves its full if meandering diameter by extra asymmetric compression of the softer zones.

Whereas the posterior body musculature is capable of throwing the trunk into undulations, analysis of films shows clearly that (i) relatively little continuous force is exerted by this zone and (ii) this posterior half of the body is relatively inactive in propulsion. Furthermore, the shield-tailed snakes do not use their caudal tip to stem (that is, to absorb) the reaction forces obtained as their head starts to enter and extend crevices; thus they differ from worm snakes, families Typhlopidae and Leptotyphlopidae. The posterior vertebrae are relatively shallower than the anterior, which indicates that this portion of the trunk is not equipped to absorb major or continuous forces.

Consequently, the uropeltid body may be conceived of as analogous to a freight train. All its propulsive machinery is concentrated in the anterior portion, while the posterior trunk serves mainly for the indirectly powered transport of the viscera. In the same way that the caboose provides terminal protection for a string of freight cars, the uropeltid's modified caudal shield protects the snake's distal end (*8*). Movement along the tunnel is unidirectional; thus uropeltids cannot burrow backward and have trouble backing up unless the tunnel is smooth and well formed.

The adaptive pattern here shown is not only interesting as an ecological response to a particular set of environmental conditions but also documents the fact that the adaptive response of muscle is not restricted to mere hypertrophy or reduction. In this case, it involves a general modification of the enzyme system and fibrillar arrangement within regional groupings of muscles, though it is probable that the evolutionary change actually involved a simultaneous loss of oxidative capacity in the posterior portion of the trunk and its enhancement in the anterior.

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2. The energy that a compression burrower has to expend in generating a unit length of tunnel will increase at least with the first power of the tunnel's diameter. At the same time the burrower's mass will determine its metabolic rate and with this its absolute energy requirement. Other things (prey capture and locomotor techniques) remaining equal, a reduction of the relative diameter of the head forces a decrease in the size of prey objects that may be killed and ingested, and with this increases the number of prey-capture operations required per unit time.
3. The muscular and functional differentiation here described has been analyzed in detail in the Sri Lankan uropeltid species *Rhinophis blythi*, *R. drummondhayi*, *R. dorsimaculata*, *R.* sp., *R. philippinus*, *R. trevelyanus*, *Uropeltis melanogaster*, and *U. phillipsi*, and the Indian species *U. liura*. The differentiation is probably absent in the Indian genera *Brachiophidium*, *Melanophidium*, *Plecturus*, and *Platyplecturus*; it is definitely absent in the Indian *Teretrurus rhodogaster* and in the Sri Lankan genus *Pseudotyphlops*, the largest of the uropeltids. Although *Pseudotyphlops* represents a highly advanced set of character states, in such aspects as the nature of its caudal shield, it is apparently unable to tunnel in the low-country soils when these are dry and hard. It then remains in its deeper tunnels and only leaves or extends them when rain softens them, dropping penetrometer readings from 4.5 to 0.5 k/cm^2.
4. Muscles of mammals adapted to vigorous, prolonged exercise (such as long-distance running) have increased work capacity and ability to oxidize pyruvate and fatty acids. These capabilities are reflected in increased mitochondria and activities of a number of citric cycle enzymes, transaminases, and mitochondrial proteins [B. Parnow and B. Saltin, Eds., *Adv. Exp. Med. Biol.* **11** (entire issue) (1971); P. A. Mole, K. M. Baldwin, R. L. Terjung, J. O. Holloszy, *Am. J. Physiol.* **224**, 50 (1973)].
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6. This phenomenon accounts for several sets of misapprehensions in the taxonomic literature. One of these is the argument that uropeltids are generally characterized by carrying the head bent to one side ["Very frequently the longitudinal axis of the head is not the same as that of the neck, the head being impressed on one side, as if it had been dislocated during some effort of the snake to penetrate the soil"—A. C. L. G. Günther, *The Reptiles of British India* (Ray Society, London, 1864), p. 182]; this is the condition of a snake preserved with the axial musculature hypercontracted, thus forcing the head to one side or the other (unless restrained in a straight position by the tunnel wall). Another such artifact produces the descriptions "forebody swolled and knuckled" [F. Wall, *Ophidia Taprobanica or the Snakes of Ceylon* (H. R. Cottle, Colombo, Ceylon, 1921), p. 30] and "pronounced thickening of the nuchal area" [P. E. P. Deraniyagala, *A Colored Atlas of Some Vertebrates from Ceylon*, vol. 3, *Serpentoid Reptilia* (Government Press, Colombo, Ceylon, 1955), p. 8]; such descriptions are applicable to specimens that have the axial musculature of the neck slightly contracted so that the neck is curved and the integument appears widened.
7. This function probably provides one explanation for why the neck curvature method of tunnel widening is found in uropeltids and most caecilians but not in most members of the order Amphisbaenia except for *Agamodon compressum*. Most amphisbaenians bite pieces out of their prey rather than swallowing it entire as do uropeltids. The exceptional amphisbaenian *A. compressum* has a trunk of highly ribbon-shaped cross section and might presumably encounter some lateral limitation when harvesting seasonally abundant foods and when it is pregnant. The importance of pressure during pregnancy may be documented by the observation that in May 1976, at two localities, in a series of the uropeltid, *R. philippinus*, more than 20 percent of the individuals collected aborted fully formed but still immobile young that were estimated to be within 1 month of term. It is uncertain whether the abortion reflected only the trauma of being compressed while being dug up and shaken about, or also the general excitement.
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9. We thank the many friends and colleagues who facilitated the fieldwork, assisted in part by the Smithsonian Institution's Entomological project in Sri Lanka, the National Museum, Colombo, and aid from Professor R. J. Rajendran and Mr. P. Fernando. Drs. B. Carlson, J. A. Faulkner, G. Gorniak, and L. Maxwell commented on the manuscript. Preparative work involved the aid of Dr. D. F. Gartside and NSF grants BMS 71 01 380 (C.G.) and BMS 73 01 252 (H.C.D.). The drawings were prepared by R. Heberer.

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Intercellular Communication in Insect Development Is Hormonally Controlled

Abstract. *Cellular coupling in the insect epidermis changes in a characteristic way during metamorphosis. In vitro, β-ecdysone mimics the initial phase of these changes by increasing electrical coupling. Both adenosine 3',5'-monophosphate (cyclic AMP) and Ca^{2+} reverse natural and β-ecdysone–stimulated changes, which suggests that ecdysone could work on communication through changes in cyclic AMP and Ca^{2+} levels. The transient changes in intercellular communication before metamorphosis may reflect the timing of the signals that trigger proliferation and the generation of new spatial patterns in the epidermis.*

Growth regulation in a developing tissue requires that the component cells communicate with each other. Over short ranges, the transmission of growth-regulating and morphogenetically important molecules through specialized membrane junctions has been proposed as a likely means of intercellular communication (*1*). The cytosol of normal nonexcitable cells is connected by permeable pathways that traverse the plasma membrane at gap junctions (*2*) that remain open throughout the cell cycle (*3*) and, in postembryonic tissues, allow the intercellular transfer of molecules with molecular weights less than 1000 (*4*). The pathway is an obvious candidate for the transmission of cell division initiators and the feedback regulation of growth in multicellular tissues (*1*).

In the insect, cell growth is periodic and under hormonal control (*5*). The in-

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sect epidermis is a paradigm for the study of the role of intercellular communication in growth control. The epidermal cells are ionically coupled (*6*) and, in the larva of the beetle *Tenebrio*, gap junctions make up 20 to 30 percent of the junctional membrane (*7*).

I report here that a phase of increased epidermal communication in the prepupa may be stimulated by the molting hormone β-ecdysone in vitro. This effect may be neutralized by subsequent treatments designed to elevate intracellular Ca^{2+} or adenosine 3′,5′-monophosphate (cyclic AMP). The counteractive action of Ca^{2+} and cyclic AMP may serve to coordinate epidermal responses to ecdysone when signals influencing cellular proliferation and remodeling of tissues are believed to occur (*8*).

The ventral epidermis of abdominal segments II to VII of the mealworm were isolated in organ culture medium (*9*) at various times in the last-larval stage (*10*). The tissue was equilibrated in medium at 27°C for a period dependent on the specific experiment, as described below. Electrotonic coupling in the cell sheet was determined with intracellular electrodes that both pulsed current (6×10^{-8} amp) and recorded the resultant electrotonic potentials in selected cells in the sheet. The beetle epidermis is a monolayer of cells with uniform geometry (*7*). The resistivity (units, ohm centimeters) of the intercellular pathway (the resistance of the junctional membranes of the cells in the sheet in series combined with the epidermal cytoplasmic resistance) may therefore be calculated by means of a Bessel function describing electrotonic spread in a simple model in which a thin sheet of cytoplasm of infinite extent is bounded on both sides by plasma membrane (*11*). Intercellular resistivity is the product of epidermal sheet thickness (units, centimeters) and an intercellular resistance value (units, ohms) derived from fitting a theoretical curve to the experimentally recorded decay in electrotonic potential with increasing distance from the polarizing electrode. After each set of electrophysiological recordings was complete, the epidermis was fixed, embedded in plastic, and sectioned, and the thickness of the cell sheet in the recording area was determined. If the resistive properties of the cytoplasmic component of the intercellular pathway remain constant, changes in resistivity may be attributed to junctional effects.

Figure 1 illustrates the normal changes in intercellular resistivity in the epidermis during the preliminary phases of metamorphic activity in the larva. A phase of reduced resistance between 150 and 140 hours before pupation (P − 150 to P − 140) is terminated by a rapid and transient elevation in resistance that peaks at P − 130. This peak is followed by a return to intermediate levels of resistivity from P − 120 onward. These changes are particularly interesting in that they immediately precede cell division and pattern regulation (*12*).

Since ecdysone has been implicated in stimulating metamorphosis, I have investigated the effects of ecdysone on cell communication in vitro. Epidermis was dissected from larvae at about P − 170 (before the changes shown in Fig. 1 began), equilibrated in medium for 30 hours, and then exposed to medium containing $2 \times 10^{-6}M$ β-ecdysone (Rohto, Japan) for 12 hours. As a control, preparations from the same animals were incubated in normal medium for the same period. The intercellular resistivity of the hormone-treated preparations, 290 ± 17 ohm cm [ten experiments, mean ± standard deviation (S.D.)] was reduced by 32 percent when compared with that of control epidermis from the same larvae, 429 ± 33 ohm cm. This effect appears to be β-ecdysone–specific and is not mimicked by exposure to $2 \times 10^{-6}M$ α-ecdysone (Simes, Italy) which reduced resistivity by only 5 percent (control resistivity, 426 ± 42 ohm cm; resistivity after 12 hours of incubation with α-ecdysone, 406 ± 33 ohm cm; five experiments).

Table 1. The effect of $10^{-3}M$ cyclic AMP on intercellular resistivity in epidermis initially primed with $2 \times 10^{-6}M$ β-ecdysone for 12 hours in vitro. The results represent averages and standard deviations of six experiments.

Cells	Resistivity (ohm cm)	Change in resistivity* (%)
Nonprimed, control	434 ± 28	0
Primed with β-ecdysone	285 ± 17	−34
Primed; treated with cyclic AMP for 4 hours†	442 ± 66	+2
Primed; treated with $10^{-3}M$ 5′AMP for 4 hours†	339 ± 53	−22

*Relative to the control. †The nucleotide media were hormone-free

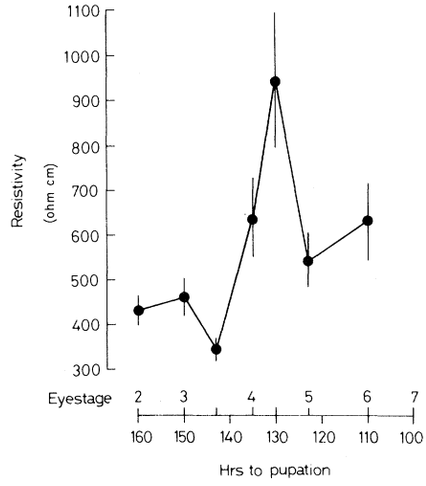

Fig. 1. Cell coupling fluctuates in a predictable manner during preparation for metamorphosis. After the prepupal animals had been ranked by the eyestage technique (*10*), the ventral epidermis of abdominal segment IV was isolated in medium, and electrical coupling was measured within 15 minutes after dissection. The corresponding time to pupation at 26°C for the eyestages is given. Eyestage 2 represents the larva as preparative events for metamorphosis begin. Each point is the mean and S.D. of at least three preparations. Two major features are the reduced intercellular resistivity between P − 150 and P − 135 and the resistivity peak at P − 130.

The effect of β-ecdysone in vitro is not due to changes in cell density. Density changes would alter the number of junctional interfaces per unit length of the epidermal sheet and influence the resistivity of the intercellular pathway without junctional conductance changes. To eliminate cell density effects, pairs of control and hormone-treated epidermis were fixed and Feulgen-stained, and the nuclei were counted from whole mount preparations. No cell division or cell death was observed. A direct comparison of density in each pair (four experiments) revealed a mean difference of less than 2 percent.

Thus, β-ecdysone lowers intercellular resistivity in vitro by elevating junctional membrane conductivity, either by stimulating the formation of new gap junctional channels (*13*), or by increasing the bore size of existing channels (*4*). In either event, the ecdysone-stimulated increase in intercellular communication could facilitate the transmission of morphogens within the segment (*14*). The initial decline in intercellular resistivity in vivo (Fig. 1) is presumed to be a response to blood ecdysone prior to pupation (*15*).

These results suggest that the high intercellular resistivity characteristic of the nonproliferative intermolt (> P − 150) and at P − 130 is modulated through the influence of ecdysone. Since a high intercellular level of cyclic AMP is detectable during the nonproliferative and differentiated state in many cell types (*16*), it was of interest to determine the effect of cyclic AMP upon cell com-

munication. To test this, epidermis was removed from larvae at P − 170 (at least four preparations from each larva) and subjected to β-ecdysone, as described earlier. After this in vitro priming period in hormone, some preparations were transferred to medium containing $10^{-3}M$ cyclic AMP for 4 hours. As a nucleotide control, a preparation was exposed to $10^{-3}M$ 5′-AMP. At each stage in this procedure, a preparation was removed, and electrotonic coupling was measured and then processed for thin-section analysis. Table 1 shows that the exposure to cyclic AMP negates the earlier effect of ecdysone on intercellular resistivity. The effects of ecdysone and cyclic AMP are antagonistic in the beetle epidermis, in contrast to the findings in *Drosophila*, where cell coupling in the salivary gland is raised by both cyclic AMP or β-ecdysone in the external medium (*17*). This discrepancy, which remains to be resolved, is further complicated by the fact that electrical coupling in the salivary gland of *Chironomus* drops dramatically in the prepupa in vivo (*18*) (presumably in response to blood ecdysone), whereas the opposite is seen in *Drosophila* cells exposed to β-ecdysone in vitro (*17*). In vertebrate cells in tissue culture, however, exposure to agents known to raise intracellular cyclic AMP levels or to cyclic AMP derivatives increases electrical coupling and stimulates the formation of gap junctions (*19*).

The elevated intercellular resistivity in response to cyclic AMP in the beetle epidermis is most likely a consequence of increased cytoplasmic Ca^{2+} activity. Insect cells are rapidly uncoupled by intracellular injection of Ca^{2+} (*20*) or by exposure to the Ca ionophore A23187 (Lilly) in the external medium (*21*). Both procedures raise the intracellular level of ionized Ca. If the transient peak in intercellular resistivity at P − 130 reflects a short-lived period of high intracellular Ca^{2+} activity, then exposure to a Ca ionophore should mimic this event in vitro. Epidermis from larvae at P − 150 to P − 140, after 2 hours of equilibration in control medium, was exposed to medium containing $2 \times 10^{-6}M$ A23187 for up to 4 hours. The intercellular resistance rose rapidly in all six experiments conducted, peaked between 30 and 75 minutes after exposure to A23187 started, but then recoupled to near-control levels within 3 hours in all cases, in the continued presence of the ionophore. In the example shown in Fig. 2, the cell sheet was completely uncoupled after 75 minutes of exposure, but the cells were recoupled by 120 minutes. The design of the experiment shown in Fig. 2 precluded processing the epidermal preparations for thickness determinations during the first 2 hours of exposure to A23187, since the preparations were used again later. However, the resistance changes shown in Fig. 2 directly reflect changes in intercellular resistivity. In an experiment where the epidermal thickness was measured after 60 minutes of exposure to A23187, a short-lived elevation in resistivity was seen (control resistivity at 60 minutes, 358 ohm cm; resistivity after 60 minutes of exposure to A23187 was 1563 ohm cm; resistivity after 3 hours of exposure to A23187 was 430 ohm cm).

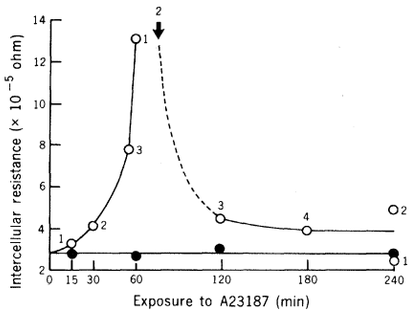

Fig. 2. Epidermal uncoupling caused by elevation of cytoplasmic Ca^{2+} activity is reversible in the continued presence of the Ca ionophore. Four epidermal preparations from a larva at about P − 150 were exposed to $2 \times 10^{-6}M$ A23187 at time zero, and intercellular resistance was recorded at various times later (open circles). Preparations 1 and 2 were read three times, preparation 3 was read twice, and preparation 4 was read only once, as indicated by the numbers next to the points. A steady loss of coupling over the first 60 minutes was recorded; at 75 minutes, coupling was undetectable, and the membrane potential was reduced transiently to about 30 percent that of the control preparation (−32 mv). Coupling was rapidly restored in all four preparations, however, and stabilized at an intercellular resistance generally slightly higher than that of control epidermis (closed circles) incubated in medium containing an equivalent amount of the solvent for the ionophore alone (0.25 percent dimethyl sulfoxide).

Hence the transient peak in intercellular resistivity before pupation at P − 130 may reflect a more central developmental event in the epidermis, characterized by a brief elevated cytosolic Ca^{2+} activity. What might this event be? It has been proposed that the primary mitogenic signal may be a net influx of Ca^{2+} into the cell (*22*). This concept, supported by evidence from such cells as lymphocytes (*23*) and fibroblasts (*24*), attempts to explain the much-debated role of cyclic nucleotides in growth control as secondary elements in a complex chain of feedback interactions that lead to cell division. In *Tenebrio*, extensive mitotic activity in the epidermis, leading to almost a doubling in cell density (*12*) starts some 30 hours after the peak in resistance, at about P − 100 (Fig. 1). Whether this temporary loss in cell coupling mimicked in vitro by A23187 and partially by cyclic AMP reflects the primary stimulus for cell division in the beetle epidermis has not yet been determined.

Finally it has been reported that, although cyclic AMP passes readily through junctional channels (*25*), Ca^{2+} does not (*21*). This suggests that cyclic nucleotides may coordinate metabolic and developmental activities stimulated by β-ecdysone and Ca^{2+} by diffusional transfer between the cells of the responding tissue.

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Hemipenile Preference: Stimulus Control of Male Mounting Behavior in the Lizard *Anolis carolinensis*

Abstract. *The urogenital system of squamate reptiles is represented by separate, bilaterally symmetrical tracts. Males alternate in their use of the right and left hemipenes. Sensory feedback from the hemipenis and, to a lesser extent, from the ipsilateral testis is important in determining which hemipenis the male will use for mating.*

The squamate reptiles (lizards and snakes) are unique among amniote vertebrates in having bilaterally symmetrical intromittent organs. These structures, known as the hemipenes, can be highly ornamented, possessing numerous papillae, calyces, and spines. Indeed, hemipenile structure has long been used by herpetological systematists as an aid in classifying snakes (*1*). The functional significance of the paired hemipenes is less well known, however. I now report several experiments which indicate that, in the lizard *Anolis carolinensis*, sensory feedback from the hemipenis is important both in the male's initial orientation during mounting and in the termination of copulation. Further, propioceptive feedback from the testes has a similar but less pronounced influence on male copulatory posture. These findings suggest that the hemipenile alternation observed in successive copulations in *A. carolinensis* is due to feedback from both the testes and hemipenis. A possible function of this process might be to monitor the ability of each testis to deliver mature sperm.

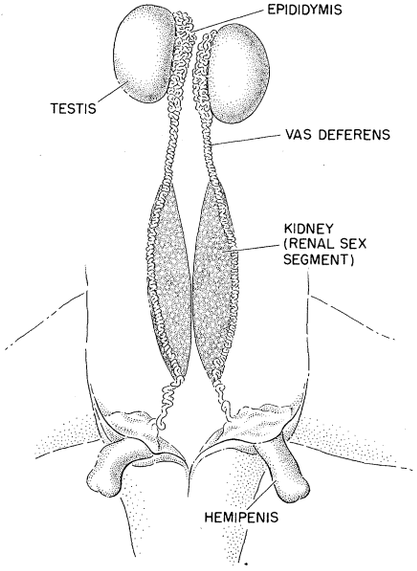

Fig. 1. Urogenital system of the male lizard *A. carolinensis*.

Courtship and copulation in the lizard *A. carolinensis* has been described in detail elsewhere (*2*). Briefly, the male advances toward the female, pausing to perform the courtship display. This display consists of a species-typical up-and-down bobbing movement of the body coordinated with extension of a brightly colored red throat fan or dewlap. If sexually receptive, the female will stand for the male, arching her neck as the male takes a neck grip (*3*). The male then straddles the female, swinging his tail beneath the female's to appose the cloacae. The male then inserts a single hemipenis into the female's cloaca; copulation lasts approximately 10 minutes, although individual males have characteristic mating times (*4*). If the male mounts on the female's right side, he will evert the left hemipenis and vice versa. Since the male curves his tail beneath the cloacal openings, it is possible to determine quickly which hemipenis he has inserted by the angle of deflection of his tail; for example, the male's tail will be curved to his right if he has intromitted with the right hemipenis.

In male lizards and snakes, the retracted hemipenes are paired membranous pouches in the base of the tail attached to the posterior wall of the cloaca (*1*). During mating, intromission is achieved with the eversion of one hemipenis through the male's cloacal opening. Dissections of the urogenital system of squamate reptiles reveal that the vas deferens from the paired abdominal testes do not enter into a common urogenital sinus as in other vertebrates. Instead, sperm are transported by each vas deferens to a seminal groove on the surface of the ipsilateral hemipenis (Fig. 1); thus, male lizards and snakes have two separate and functional reproductive tracts.

In my examination of the stimulus control of male copulatory behavior in this lizard, I have monitored the sexual behavior of individual males to determine whether they exhibit a preference for either the left or right hemipenis (*5*). In general, males alternate in their use of the right and left hemipenis (Table 1). Although some males exhibited a preference if given an extended series of tests (for example, males B and C), most males rarely mated with one hemipenis three or more times in succession [intercopulation interval (ICI): $\bar{X}$ = 1.28 days, standard error (S.E.) = 0.10]. Differences in copulation times for the right and left hemipenis were not significantly different within individuals, although males did show individually specific copulation times [see also (*4*)].

Removal of one hemipenis dramatically altered male mating patterns (Table 1) (*6*); unilaterally hemipenectomized males assumed only that posture which enabled them to use the remaining hemipenis. The transition from preoperative alternation behavior to a postoperative preference was immediate, without any obvious "trial and error" on the part of

Table 1. Pattern of alternation in use of right (R) and left (L) hemipenis exhibited by intact and unilaterally hemipenectomized *A. carolinensis* during successive matings.

Lizard	Hemipenis used	Total	
		Right	Left
	Intact normal		
2	LLRLRLRLRLR	5	6
3	LLRLRL	2	4
5	LRLLRLL	2	5
6	LLRLRRLR	4	4
A	RLRLRR	4	2
B	RRRLRR	5	1
C	RRRLRR	5	1
F	LRRRLR	4	2
G	RLRRLL	3	3
H	RLLRRL	3	3
J	RLRRLR	4	2
	Unilateral hemipenectomy		
3	RRRRRRRR	8	0
5	RRRRRRRR	8	0
6	RRRLRRRR	7	1
B	LLLLLL	0	6
C	LLLLLLLL	0	8
J	LLLLLLLL	0	8

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