MOTIVE FORCE OF THE MIGRATING PSEUDOPLASMODIUM OF THE CELLULAR SLIME MOULD DICTYOSTELIUM DISCOIDEUM

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SUMMARY

Motive forces of migrating pseudoplasmodia (slugs) of Dictyostelium discoideum were determined by application of a double-chamber method. The motive force of a whole slug was proportional to its volume, the value per unit volume being 5.85 × 10^8 dyne/cm³ (58.5 N cm⁻³). The motive force was independent of temperature (13.5 – 26 °C) and decreased during prolonged migration. Motive force per unit volume of an anterior isolate of a slug was much larger than that of a posterior isolate, their weighted mean being approximately equal to that of a whole slug. These results agree well with the predictions previously made using a model based on analyses of migrating velocities of slugs. The motive force per unit volume of either isolate was soon regulated to reach the normal value of an intact slug after several hours of isolation, concurrently with conversion of cell types between prestalk and prespore cells. The possibility that motive force of each cell is determined by its cell type is discussed in relation to cell sorting.

INTRODUCTION

Development of the cellular slime mould Dictyostelium discoideum is accompanied by a series of highly organized movements of a mass of cells from the time of aggregation up to formation of a fruiting body. Studies on the mechanisms of these movements are important in understanding their roles in morphogenesis and pattern formation. Migration of a pseudoplasmodium (slug) is relatively simple in that it is accompanied by no major changes in the shape of the cell mass. Several studies, both experimental and theoretical, have been made of this movement and some hypotheses as to its mechanism have been proposed (Bonner, Koontz & Paton, 1953; Shaffer, 1962, 1964; Francis: see Shaffer; Bonner, 1967; Garrod, 1969; Loomis, 1975; Inouye & Takeuchi, 1979).

In a preceding paper, we proposed a model for slug movement, on the basis of a dynamic equilibrium between the motive force generated by slug cells against their intrinsic resistance and the resistance of the slime sheath at the tip. It follows from this model that a linear relationship holds between reciprocals of the length and the migrating velocity of a slug, which was verified with slugs migrating on agar under different conditions. Analyses of experimental results by the use of the model led to the predictions that the motive force should be independent of temperature changes but should decrease during prolonged migration, and that the anterior cells of a slug have greater motive forces than the posterior cells. Although the size of the motive force is one of the most important factors to know in order to understand the mechanism of movement, little attempt has so far been made to ascertain it.
It is the purpose of this study to develop a method of measuring motive forces of slugs and their isolates migrating under various conditions. The method employed was in principle based on the double-chamber method of Kamiya, which was originally used for measuring motive forces of protoplasmic streaming in myxomycete plasmodium (Kamiya, 1940) and amoeboid movement of *Chaos chaos* (Kamiya, 1964). The results obtained in the present study agreed well with the predictions previously made on the basis of the model and measurements of migrating velocities (Inouye & Takeuchi, 1979).

**MATERIALS AND METHODS**

**Culture**

*Dictyostelium discoideum* NC 4 was used throughout this study. Amoebae were grown at 21 °C on *Escherichia coli* on a nutrient agar medium (Bonner, 1947). After about 42 h of incubation, amoebae were collected and washed free of bacteria by repeated centrifugation in chilled standard salt solution (Bonner, 1947). To obtain migrating slugs, washed amoebae were streaked on non-nutrient agar (2 %) and incubated at 21 °C in the dark. Usually, slugs which had migrated for several hours away from the original streak were used for the measurements. In some experiments, slugs were transferred to another plate and allowed to migrate for 2–4 days before use.

**Measurement of the motive force**

The measurements were conducted using a device schematically shown in Fig. 1. It consisted of an agar capillary, a rubber bulb, a manometer, a microscope and a time-lapse video tape recorder. An agar capillary was made by the method of Kamiya (1964); hot agar sol (4 % Difco purified agar) was sucked into a small plastic chamber containing a glass needle of appropriate diameter, which was pulled out after gelation of the agar. To introduce a slug into an
Motive force of Dictyostelium slug

agar capillary, a small piece of agar on which a slug was migrating was cut out of an agar plate and juxtaposed with the orifice of the capillary. When the slug entered it, as shown in Fig. 1, the chamber became airtight, and a desired pressure could be applied on the slug by controlling a rubber bulb. The pressure applied was read by a mercury manometer. Migration of the slug in the capillary was recorded by a time-lapse video tape recorder (National, NV-8030), with a time compression of 36 or 72. The migrating velocity, the length and the diameter of the slug were measured on a reproducing screen. All the measurements were conducted at 21 °C, unless otherwise stated. Temperature was kept constant during the measurements by the aid of a Nikon constant-temperature device for the microscope stage.

Preparation of anterior and posterior isolates

An anterior isolate of a slug was obtained by cutting a slug transversely at a point corresponding to one third of its length, measured from the anterior end, using a small piece of aluminium foil. To obtain a posterior isolate, the following method of isolation was used, unless otherwise stated. An agar capillary was cut into 3 blocks and then reconstructed, after which a slug was introduced into it. When the slug advanced so that its anterior one-third became located in the middle block, that block was removed and the 2 other blocks were immediately connected to re-form a capillary. The posterior isolate thus formed continued forward movement without interruption in the re-formed capillary.

RESULTS

Movement of a slug in an agar capillary

A slug introduced into an agar capillary of an appropriate bore continued to migrate with its entire lateral side in contact with the inner face of the capillary (Fig. 2). Unlike a slug migrating on agar, the rear end of the slug assumed a concave shape.

As the slug advanced, slime sheath was left behind around the whole inner face of the capillary. Accordingly, the concave rear end is most likely not covered by slime sheath. A slug in an agar capillary migrated continuously at almost a constant speed. No interruption of the movement, such as is observed with slugs migrating on an agar surface (Inouye & Takeuchi, 1979), was discernible.

We have previously shown that the migrating velocity of a slug depends on its
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length. Velocity \( (v) \) of a slug migrating in an agar capillary was determined and compared with the velocity \( (v^*) \) estimated for a slug of the same length migrating on agar, by use of the data shown in fig. 2 in the previous paper (Inouye & Takeuchi, 1979). It was found that the former was smaller than the latter in most cases and that the ratio \( (v/v^*) \) of the former to the latter varied according to the length of a slug, as shown in Fig. 3.

![Fig. 3. Ratios of migrating velocities of slugs migrating in agar capillaries to the estimated velocities on agar surface plotted as a function of slug lengths.](image)

The difference in migrating velocity between slugs on agar and in agar capillary does not seem attributable to a difference in resistance to the movement. Firstly, slime sheath on the lateral side is equally well fixed in both the cases (either to the agar surface or to the inner face of the capillary) and cells inside move over it as the substratum. Hence, it offers no resistance to forward movement of the cells in either case. The presence or the absence of slime sheath at the rear end likewise makes no difference in resistance, as evidenced by the previous study. On the other hand, resistance to the movement exerted by slime sheath at the tip must be the same with slugs both migrating on agar and in a capillary.

Assuming that there is no difference in resistance, the migrating velocity is then proportional to the motive force. It thus follows that the reduction in velocity in an agar capillary is due to a decrease in the motive force. The exact reason for such a decrease is unknown, but it is probably caused by certain changes in physiological conditions of a slug, for example a reduced oxygen tension, due to confinement in agar except for the ends. This interpretation agrees well with the fact that the rate of the decrease in migrating velocity depended on the slug length (Fig. 3). In any
event, when the motive force of a slug migrating on agar is to be estimated from the value measured with a slug in an agar capillary, the latter must be corrected by dividing by the above-described ratio \((v/v^*)\). The ratios for slugs of various lengths were obtained from a regression line drawn in Fig. 3. Motive forces of migrating slugs were henceforth calculated in this way.

*Motive force of a slug*

To determine the motive force, a slug was introduced into an agar capillary and its migrating velocities were measured under varied pressure differences between the ends of the slug, as described in the preceding section. Fig. 4 illustrates 2 such experiments. When the pressure applied to the front of the slug was lower than the atmospheric pressure, it migrated faster than it did without the pressure difference. As the applied pressure was increased, the slug migrated at progressively slower rates. In the case of the slug II, a pressure difference of 60 mm Hg (~ 8 kN m\(^{-2}\)) completely prevented forward movement. The pressure, which is referred to as the ‘balance pressure’ according to Kamiya (1940), should be equal to the motive force per unit cross-section of the slug. However, the balance pressure could not be directly determined in the case of the slug I, since application of a pressure difference large enough to bring about complete halt of the slug resulted in exudation of water from agar into

![Graph illustrating changes in migrating velocity of 2 slugs in response to pressures applied. Positive pressures represent the pressures applied against the direction of slug movement. Slug I: 432 μm in length, 69 μm in diameter, after several hours of migration. Slug II: 380 μm in length, 122 μm in diameter, after 4 days of migration. (Note: 1 mm Hg pressure = 133.3 N m\(^{-2}\).)
the capillary. Therefore, the balance pressure was determined by extrapolating the pressure-dependent changes of velocity. The balance pressures were corrected as described in the preceding section and then multiplied by cross-sections of slugs to obtain motive forces of whole slugs.

![Graph showing relationship between motive forces of whole slugs and their volumes.](image)

**Fig. 5.** Relationship between motive forces of whole slugs and their volumes.

When motive forces of a number of slugs thus determined were plotted against their volumes, a linear interrelationship held, as shown in Fig. 5. This indicates that the motive force of a slug is generated by all of its constituent cells. The mean motive force per unit volume of slugs was then calculated from this figure to be $5.85 \times 10^6$ dyne/cm$^3$ ($58.5$ N cm$^{-3}$). Since this value is independent of the size and shape of a slug, motive force of a slug was hereafter expressed on the basis of this unit.

**Effects of temperature**

The dependency of the motive force on temperature was examined by measuring migrating velocities of an identical slug under various pressure differences, both at 13.5 and 23.5 °C. As shown in Fig. 6, although the slug migrated faster at 23.5 °C than at 13.5 °C under any pressure difference applied, the balance pressure, and hence
Fig. 6. Effects of temperature on migrating velocity of a slug under various applied pressures. The measurements were first conducted at 13.5 °C (Δ) and then at 23.5 °C (〇) with one and the same slug (397 μm in length, 70 μm in diameter, after several hours of migration). (Note: 1 mm Hg pressure = 133.3 N m⁻².)

Fig. 7. Effects of temperature on motive force per unit volume of slugs. Slugs which had been migrating for several hours at 21 °C were introduced into an agar capillary and the motive forces determined at indicated temperatures. Vertical bars show standard deviations.
the motive force, was almost the same at either temperature. This was further confirmed by measuring motive forces per unit volume of many slugs at various temperatures. As shown in Fig. 7, no significant changes in motive force were observed within the temperature range of 13.5 to 26 °C.

Effects of slug age

The migrating velocity of a slug is known to decline progressively as it continues to migrate (Bonner et al. 1953). To examine the validity of our prediction that this decline is due to a decrease in the motive force (Inouye & Takeuchi, 1979), motive forces of slugs which had migrated on agar for various periods were determined. As shown in Fig. 8, motive forces per unit volume decreased exponentially during migration. All the differences were statistically significant ($P < 0.05$).

![Fig. 8. Changes during migration in motive force per unit volume of slugs. Slugs which had migrated for several hours, 2 days or 4 days on agar surface were introduced into agar capillaries and their motive forces determined. Vertical bars show standard deviations.](image)

Motive forces of anterior and posterior isolates

To examine whether or not motive forces per unit volume are the same throughout a slug, they were determined using anterior and posterior isolates of slugs. The motive forces of anterior isolates were determined around 1 h after dissection and found to be almost twice as much as those of intact slugs (Table 1).

Posterior isolates similarly cut out of a slug on agar usually culminated on the spot, as Raper noticed (1940). When the isolate was incubated on an inverted plate, it rounded up initially but several hours later was reorganized into a slug to resume migration (Sakai, 1973). However, it was found that if the dissection was made in an agar capillary, as described in the Methods section, the isolate continued to migrate...
without interruption. Motive forces of such posterior isolates (two thirds of the length of original slugs) were determined around 1 h after the dissection and found to be much lower than those of intact slugs (Table 1). These results indicate that about two thirds of the motive force of a slug is generated by the cells occupying the anterior one third of the slug.

To examine whether or not such a difference in motive force between both the isolates

Table 1. Motive forces per unit volume of anterior and posterior isolates of slugs, in comparison with those of intact slugs

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<th>Motive forces, $\times 10^6$ dyne/cm³</th>
<th>No. of expts.</th>
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<tr>
<td>Anterior isolates*</td>
<td>11.44 ± 2.08</td>
<td>4</td>
</tr>
<tr>
<td>Posterior isolates*</td>
<td>3.60 ± 0.48</td>
<td>4</td>
</tr>
<tr>
<td>Intact slugs†</td>
<td>5.85 ± 1.45</td>
<td>11</td>
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</tbody>
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* Determined about 1 h after isolation.
† Calculated from the data shown in Fig. 5.
‡ Note; $10^6$ dyne/cm³ = 10 N cm⁻³.

Fig. 9. Changes in motive force per unit volume of anterior isolates after isolation. Slugs were dissected at one-third slug length from the tips. After the anterior isolates were allowed to migrate on agar at 21 °C, they were introduced into agar capillaries about 1 h before the measurements. Shaded zone indicates the range of standard deviation for motive forces per unit volume of intact slugs.
was maintained, motive forces of anterior isolates were measured after various times of migration on agar. As shown in Fig. 9, the motive forces remained high up to 3 h after dissection, but fell to the normal level by 6 h. Such changes in motive force were, however, blocked in the isolates kept at a low temperature (data not shown). Similar changes in motive force were also observed in posterior isolates which had been dissected on agar at two thirds length from the rear and incubated on inverted plates. After they were reorganized into slugs, they showed motive forces comparable to those of intact slugs after 6 or 9 h of isolation (data not shown).

DISCUSSION

No information has hitherto been available on the motive force of a migrating slug of the cellular slime moulds, with the exception of a brief report by Yamamoto & Kamiya (1971). Using essentially the same method as in the present study, they measured the balance pressures with 5 slugs, which amounted to from 8 to 27 cm Hg (11 – 36 kN m$^{-2}$). We have obtained balance pressures of the same order and found that the motive force of a whole slug is proportional to its volume, that is the number of cells constituting the slug. The motive force thus obtained per unit volume of migrating slugs, however, seems notably high as compared with other organisms. For example, the motive force per unit volume of *Chaos* *chaos*, estimated from the data given by Kamiya (1964), is lower by nearly two orders of magnitude.

Although the exact reason for such a difference is unknown, it was realized that the motive force expressed per unit area of cell surface is nearly the same in both the cases. Assuming the average diameter of slug cells to be 8 $\mu$m (compare Bonner & Frascella, 1953), the motive force of $5.85 \times 10^8$ dyne/cm$^3$ (58.5 N cm$^{-3}$) (see Results) is converted to $8 \times 10^8$ dyne/cm$^3$ ($8 \times 10^{-5}$ N cm$^{-3}$): this is comparable to the motive force similarly estimated for a cell of *Chaos* *chaos* and is about one tenth as much as that of protoplasmic streaming in the plasmodium of *Physarum polycephalum* (Kamiya, 1953). Comparison of motive forces on the basis of cell surface area is not merely for the sake of convenience, but is in agreement with the idea that motive forces in many cell systems are generated in the proximity of the plasma membrane. It is known that microfilaments which are regarded as responsible for cytoplasmic movement are located near the plasma membrane in *Dictyostelium discoideum* (Clarke, Schatten, Mazia & Spudich, 1975; Eckert, Warren & Rubin, 1977) as well as in *Chaos* *chaos* (Komnick & Wohlfarth-Bottermann, 1965), *Physarum polycephalum* (Wohlfarth-Bottermann, 1962; Nagai & Kamiya, 1966) and *Nitella axillaris* (Nagai & Rebhun, 1966).

The present study showed that the motive force of a slug is independent of temperature. This is also the case with other motile systems, such as visceral smooth muscle of guinea pig (Gabella, 1976), myxomycete plasmodium (Kamiya, 1953) and inter-nodal cells of *Chara* (Hayashi, 1960) and *Nitella* (Tazawa, 1968). It is probable that motive forces are dependent on the absolute temperature, but that temperature changes in usual experimental conditions are too small to affect the motive forces. It was previously shown that the migrating velocity of a slug decreases at a low temperature.
In view of the independence of the motive force on temperature, it was concluded that the decrease is due to an increase in resistance of the slime sheath at the tip (Inouye & Takeuchi, 1979).

The fact that the motive force of a slug is generated by all the constituent cells does not mean that motive force of each cell is the same throughout the slug. The measurements of motive forces of the anterior and posterior isolates of slugs revealed that motive forces per unit volume of the former are much greater than those of the latter. This is in good agreement with the previous conclusion derived from analyses of migrating velocities of anterior isolates of various proportions (Inouye & Takeuchi, 1979). Nevertheless, the motive force of either isolate was not maintained, but regulated after a period of migration to reach the normal value of an intact slug. It is known that a slug consists of anterior prestalk and posterior prespore cells and that both types of cells are converted into each other in either isolate to achieve the normal proportion between them (Bonner, Chiquoine & Kolderie, 1955; Miné & Takeuchi, 1967; Sakai, 1973). It was found that the time needed for regulation of the motive force in the isolates is about the same as that for cell-type conversion as observed by Sakai (1973). This suggests that motive force on each cell in a slug is determined by its cell type.

It has previously been suggested that cells in a slug are initially arranged in order of their motive forces by sorting out according to a variation present among preaggregation cells (Inouye & Takeuchi, 1979). If this is the case, the above fact indicates that motive force of each cell is further amplified or modified as the cells differentiate into prestalk or prespore cells. Prestalk and prespore cells, when isolated and mixed, were shown to be sorted out either in a re-formed slug (Takeuchi, 1969) or in an immovable cell agglutinate (Tasaka & Takeuchi, 1979). As was discussed in the previous paper, such sorting out will result from a difference in motive force between both the types of cell. If motive force of a cell in a cell mass is related to its chemotactic parameters, the present finding that prestalk and prespore cells differ in motive force is consistent with the notion that the sorting out occurs via chemotaxis, as recently proposed by Matsukuma & Durston (1979).

This work was in part supported by grants-in-aid to I. T. from the Ministry of Education of Japan (No. 384038) and the Takeda Science Foundation.

REFERENCES


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(Received 26 June 1979)