Abstract—The apparent diffusion coefficient of Ca ions in the extracellular space of guinea pig taenia coli was estimated from model experiments to be $3.2 \times 10^{-6}$ cm$^2$ sec$^{-1}$, while the relationship between Ca concentration in the medium and tonic tension in 40 mM K-contracture was measured in the same muscle both in the presence and absence of dextran 10. On the basis of these experiments and certain assumptions, the time course of tension decline by Ca withdrawal during K-contracture was calculated. Under all dextran concentrations tested (0–15%) the calculated time course of tension decline was in good agreement with one actually observed except in a short period of time immediately after Ca withdrawal. The results suggested that Ca distribution in the extracellular space during the loss of Ca is in good agreement with the diffusion theory in cylinder, and that each muscle fiber shows its tension without delay in response to the change in Ca concentration in the vicinity of the fiber according to the tonic tension-Ca relationship mentioned above. The discrepancy between calculation and observation was partially explained on an experimental basis.

High concentration of potassium (K) induces a tonic contracture in guinea pig taenia coli following the initial phasic contraction, and the tonic tension remains almost unchanged over a period of time (1, 2). When the environmental calcium (Ca) concentration is lowered in the course of the tonic contracture, the developed tension gradually declines to an equilibrium level which is a function of Ca concentration (1). In the present study, the relationship between the equilibrium tension level and Ca concentration was determined in the media of different viscosities, while the “apparent” diffusion coefficient of Ca in the extracellular space (ECS) was estimated using some models of the muscle and alizarin yellow as a substitute for Ca ions. On the basis of these experiments, as well as the diffusion theory and certain assumptions, the time course of tension decline by Ca withdrawal during K-contracture was calculated. Except for a short period of time immediately after Ca withdrawal, the calculated time course of tension decline was in good agreement with the observed time course, under all viscosities examined. Our results suggested that the distribution of Ca in ECS during the loss of Ca is in agreement with the diffusion theory in cylinder, and that each muscle fiber shows its tension without delay in response to the change in Ca concentration in the vicinity of the fiber according to the above-mentioned relationship between Ca concentration and equilibrium tension. The discrepancy between calculation and observation immediately after Ca withdrawal was partially explained on an experimental basis.
MATERIALS AND METHODS

Model of muscle (M-cylinder)

Resin columns or metal wires representing muscle fibers were bundled into a cylindrical form to form a model of muscle tissue. The columns made of acrylic resin, each 12 mm in diameter, were arranged in parallel with a definite inter-columnar distance so that the columns formed a cylinder as a whole (Fig. 1, A, right), and that the inter-columnar space was of 22% of the cylinder volume. The volume of 22% is one reported for the ECS of guinea pig taenia coli (3). Cross-sectional ends of the cylinder were covered with the resin disks (Fig. 1, A, right). The inter-columnar space between the disks was filled with 2% agar containing

![Diagram of cylinders with labels: ag: agar, cn: column, dk: disk, sp: supporting plate, L: axial cylinder length, R: cylinder radius, X, Y, and Z: different position of cylinder axis in relation to column array, sw: supporting wire, th: thread, wr: wire, rg: ring. The materials used are as follows: cn, dk, sp and rg: acrylic resin, sw: stainless steel, th: cotton, wr: silver.]

**TABLE 1. Parameters of cylinders**

| Cylinder | Cylinder | Column or Wire |  \( R/r \) | Inter-columnar or inter-wire space (\%) | Cylinder axis* |
|----------|----------|----------------|----------|---------------------------------------|---------------|
| No.      | Radius (\( R \) mm) | Length (\( L \) mm) | Material | Number | Radius (\( r \) mm) |                      |                          |
| I        | 3        | 120            | Ag       | 240    | 0.15             | 20.0            | 40                |
| II       | 4        | 120            |          | 418    | 0.15             | 26.7            | 42                |
| III      | 17       | 50             |          | 7      | 6.0              | 2.83            | 23                | Z                |
| IV       | 17       | 50             | Acrylic resin | 12    | 6.0              | 2.83            | 23                | Y                |
| V        | 30       | 50             |          | 27     | 6.0              | 5.00            | 22                | Y                |
| VI       | 50       | 50             |          | 64     | 6.0              | 8.33            | 21                | X                |
| VII      | 14       | 200            | P-cylinder |       |                  |                  |                   |
| VIII     | —        | 200            | Triangular prism, Cross-sectional sides = 38 mm each |       |                  |                  |                   |

* See Fig. 1.
0.05% alizarin yellow. In another type of model, a definite number of silver wire pieces, each 0.3 mm in diameter, were bundled into cylindrical form (Fig. 1, B, right), and the inter-wire space was filled with the same agar as above. In this type of cylinder, the wire pieces could not be arranged in a perfect parallel. It was, therefore, impossible to make an inter-wire space of 22% but with a larger value (Table 1). The cylinders of both types described above are hereafter termed "M-cylinder". Parameters of M-cylinders, such as radius, length, etc. are shown in Table 1. The inter-columnar or inter-wire space of each cylinder was preliminarily determined both by weighing the cylinder and by determining the alizarin yellow content in agar in the space.

Control cylinder (P-cylinder)

A plain cylinder, with neither column nor wire, made of only 2% agar containing 0.05% alizarin yellow was made of the same size as each M-cylinder by filling the space between two end-disks with agar (Fig. 1, A, left and B, left). A thin resin plate or a piece of wire supporting the end-disks were not included, and the cylinder was regarded as one made of agar only.

In all experiments, each of M- and P-cylinders was weighed to detect the accidental incomplete filling with agar perhaps as the result of air bubbles. No defect was found throughout all experiments. For this detection, the specific gravity of agar was determined by weighing the pipet containing agar. In some experiments a prism made of agar was used, the structure being the same as that of the P-cylinder, but the cross-section was an equilateral triangle having the same area as one of the P-cylinders (Table 1).

Diffusion coefficient of alizarin yellow in agar

An M-cylinder with acrylic resin columns and its control, P-cylinder, were installed on the bearings placed in a photographic print washer, and the cylinders were washed with running tap water for 2.5-22 days, during which period each cylinder was rotated by a water jet against the wings fixed to the end-disks. An M-cylinder with silver wires and its control, P-cylinder, were hung in a jar, and washed in running tap water for 1-2 hr. Throughout all experiments, the water temperature ranged from 14.1-22.4°C, and the temperature change during each washing was 0.0-4.0°C. After a definite washing time, agar of each cylinder was dissolved in water at 90°C, and alizarin yellow in the solution was determined with a spectrophotometer at 500 nm. The concentration of agar in the final solution was 0.04% and such was confirmed not to interfere with the determination of alizarin yellow.

The diffusion theory in cylinder gives the concentration distribution curves of the diffusing substance along the cylinder radius at different $Dt/R^2$ (Fig. 2, A1) where $D$ is diffusion coefficient, $t$ is time from the start of diffusion, and $R$ is the cylinder radius (4). The volume of the solid obtained by revolving about the cylinder axis the area bounded by the concentration distribution curve, axis, and radius of the cylinder represents the amount of the diffusing substance remaining in the cylinder at a particular $Dt/R^2$ (Fig. 2, A2). Thus the relationship between $Dt/R^2$ and the amount of diffusing substance ($y$) in the cylinder was obtained and is shown in Fig. 2, A3. The relationship shown in Fig. 2, A3 was analyzed.
graphically on the tentative assumption that the relationship can be approximated to a multiple-exponential function. The analysis gave a triple-exponential function shown below in the range of $Dt/R^2=0-0.4$. The accurate values of the six constants in the function were obtained by a trial and error method with a computer starting with the values obtained graphically. The errors of graphical analysis and of rounding in computation were so small that the constants obtained were sufficiently accurate for practical purposes.

$$y = 0.129 e^{-241.0(Dt/R^2)} - 0.168 e^{-41.57(Dt/R^2)} + 0.703 e^{-5.848(Dt/R^2)}$$

The equation gives $D$ in P-cylinder when $R$, $t$, and $y$ are known. The equation, or Fig. 2, $A_3$, was applied also to M-cylinder assuming that the distribution of alizarin yellow in the inter-columnar or inter-wire space follows the diffusion theory in cylinder. Diffusion coefficient thus obtained in M-cylinder is expected to be smaller than the true value in agar, and is termed here an "apparent" diffusion coefficient.

**Mechanography**

A strip of taenia coli, approx. 2.5 cm long in situ, was isolated from a male guinea pig weighing 400-600 g, and was suspended in the organ bath described by one of the authors (1). In this bath the medium could be changed within 4 sec. An end of the muscle strip was connected to an isometric mechano-electric transducer (Nihon Koden SB-1T), and the tension was recorded with an ink-writing oscillograph (Nihon Koden WI-130M) through the amplifiers (Nihon Koden RP-3 and AD3-2). Normal medium used was of the following composition: NaCl 135, KCl 5.00, CaCl$_2$ 2.50, MgCl$_2$ 0.100, Na$_2$HPO$_4$ 1.00, NaHCO$_3$ 10.0, dextrose 12.0 mM, bubbled with a gas mixture (95% O$_2$ and 5% CO$_2$), 37°C, pH 7.2. The
medium containing high K (40 mM) was prepared by adding KCl to normal medium without compensation for hypertonicity which has been shown to exert no effect on the K-contracture (1, 5). The medium containing dextran was prepared by adding dextran 10 (Dextran T 10, Pharmacia Fine Chemicals). High K-induced tonic tension in the presence of normal Ca was measured 15 min after introduction of high K. After the measurement, Ca was withdrawn from the medium successively, in descending order, in the presence of high K, and the equilibrium tension was measured at each Ca concentration. When dextran-medium was used, the tissue was allowed to contact with dextran-medium at least 60 min prior to the experiment.

Determination of dextran in tissue

The tissue was placed in the medium containing 15% dextran 10 for different periods of time, and dextran in the tissue was determined as follows: the tissue was decomposed into solution as described by Ross and Mokotoff (6) except that phenolphthalein was used as an indicator, and that the solution was deproteinized with trichloroacetic acid according to Roe's suggestion (7). One milliliter of the solution thus obtained was added to 8 ml of anthrone solution (20 mg/100 ml of 80% $\text{H}_2\text{SO}_4$), heated in a boiling water bath for 15 min, and the developed color was determined at 630 nm.

Tissue geometry

1) Tissue radius was estimated from its weight, mean density, and length. The tissue was weighed after blotting its surface (1). The tissue density was estimated by a sink and float test in sucrose solutions of different densities. The tissue length was measured when the preparation was suspended in a bath as described in “mechanography”. 2) When the tissue was removed from the medium, some of the medium of course adheres to the tissue surface. The thickness of the liquid layer on the tissue surface was estimated as follows: the tissue was weighed immediately after taken out of the medium, and re-weighed after trailed on a rubber sheet until no trail was observed. The thickness was calculated from these weights, as well as the densities of tissue and medium, and tissue length. The medium density was determined with a specific gravity flask to be 1.0005, and 1.00 was used in calculation. The tissue length was measured out of the medium, and change in length was prevented by using a Ca-free medium.

Miscellaneous

Absolute viscosity of the medium was obtained by multiplying the kinematic viscosity by the medium density. The medium density and the kinematic viscosity were measured at 37.00±0.01°C with a specific gravity flask and an Ubbelohde viscosimeter, respectively. The additional pressure was not applied to the viscosimeter. Oxygen content in the medium was determined with an oxygen electrode (Yellow Spring Instrument, 4004) at 37.0°C. To determine the ion content, dextran was ashed at 600°C for 2-3 hr, and the ash was dissolved in water. The solution was subjected to atomic absorption spectrophotometry at 422.7 nm for Ca (Hitachi, 207), and to flame photometry at 589.0 and 766.5 nm for Na and K, respectively (Tokyo Koden, ANA-10A).
**RESULTS**

**Diffusion coefficient**

Each M-cylinder was washed along with its control, P-cylinder, under the same condition. The apparent diffusion coefficient of alizarin yellow in M-cylinder (Dm), the diffusion coefficient in P-cylinder (Dp), and a ratio (Dm/Dp) were obtained. The ratio is graphed as a function of R/r, where R is cylinder radius and r is columnar or wire radius (Fig. 3). At an R/r value of 2.83, two different pairs of M- and P-cylinder (Table 1) gave different Dm/Dp ratios (Fig. 3) probably because the areas of side opening of M-cylinders are considerably different when R/r is small owing to the difference in relative position of cylinder axis to the column array (Y or Z in Fig. 1 and Table 1). For this reason, Dm/Dp ratios at R/r=2.83 and 5.00 were discarded. The remaining ratios obtained in a range of higher R/r were consistent. The largest R/r examined here was 26.7, and is far smaller than R/r=150 in taenia coli estimated from V/A=1.5 μm (8) and R=0.046 cm obtained here. Since it was technically impossible to make M-cylinder having R/r larger than 26.7, a mean Dm/Dp of 0.32 obtained in an R/r range of 8.33–26.7 was used for Dm/Dp of taenia coli.

The diffusion coefficient of Ca ions in the medium multiplied by Dm/Dp of 0.32 gives an apparent diffusion coefficient of Ca ions in ECS of taenia coli. The diffusion coefficient of Ca ions in the medium was not experimentally determined in the present study, but an approximate value was presumed to be 1×10⁻⁵ cm² sec⁻¹ on the basis of the diffusion coefficient of CaCl₂ in water (9). Thus the apparent diffusion coefficient of Ca ions in ECS of taenia coli was estimated to be 3.2×10⁻⁶ cm² sec⁻¹ in normal medium.

**Ca concentration and K-induced tonic tension**

High K (40 mM) induces a long sustained tonic tension, and the tonic tension level is
a function of environmental Ca concentration almost irrespective of whether Ca concentration is changed before or after high K is introduced (1). In the present study the relationship was determined between Ca concentration and 40 mM K-induced tonic tension at an equilibrium state in the media of different viscosities, and such is illustrated in Fig. 4.

Calculation of tension decline curve

When the environmental Ca is totally removed, the high K-induced tonic tension declines approximately to zero. The time course of the tension decline was calculated on the following assumptions: (a) taenia coli was tentatively assumed to be cylindrical and the cylinder radius \( R \approx 0.046 \) cm, (b) distribution of Ca ions in ECS of taenia coli follows the diffusion theory in cylinder during the loss of Ca after Ca removal, (c) the apparent diffusion coefficient of Ca \( D = 3.2 \times 10^{-6} \) cm\(^2\) sec\(^{-1}\) in normal medium. In the medium containing dextran, this value was corrected on the basis that the diffusion coefficient is inversely proportional to the absolute viscosity of the medium (13). (d) K-induced tonic tension of each muscle fiber changes without delay in response to the change in Ca concentration in the vicinity of the fiber according to the Ca-tension relationship shown in Fig. 4.

Calcium distribution curve given by diffusion theory (Fig. 2, A,) was transformed to a tension distribution curve (Fig. 2, B,) using Fig. 4, and the tension distribution curve was revolved around the axis to obtain a bell-shaped solid as in Fig. 2, B, the volume of which represents the total tension of all muscle fibers at a particular \( Dt/R^2 \). The volume, or tension, was plotted on a logarithmic scale against \( Dt/R^2 \) to draw a tension decline curve (Fig. 2, B). The curves thus calculated, as well as the curves actually observed, are shown in Fig. 5. During the initial 1–2 min after Ca removal, the calculation did not agree with the observation, but after that the observed and the calculated curve were nearly linear and
paralleled each other in a semi-logarithmic plot.

Loss of alizarin yellow from a triangular prism

A P-cylinder was washed along with a triangular prism, of which cross-section was an equilateral triangle having the same area as of the P-cylinder. The time course of the decrease in alizarin yellow content in agar was observed as illustrated in Fig. 6. In the early stage of washing, alizarin yellow was lost at a higher rate from the prism than from the cylinder, and afterward the wash-out rate from the prism was much the same as that from the cylinder.
Miscellaneous

1) The radius of the tissue assumed to be cylindrical was estimated to be 0.046 ± 0.001 cm (S.E.) (n=25), and tissue density to be 1.045. The thickness of the liquid layer on the tissue surface was 0.060 mm immediately after the tissue was removed from the normal medium, 0.052 mm with 5% dextran-medium, and 0.070 mm with 15% dextran-medium. Different media revealed no detectable differences.

2) Calcium, Na, and K found in dextran 10 were determined, and the contents are presented below in mM in a solution containing 15% dextran: Ca=less than 0.002 mM, Na = 6.2 mM, and K=0.062 mM. The values were perfectly negligible in the present study.

3) Tissue contents of dextran were 38.8 g/kg wet tissue at 15 min contact with 15% dextran-medium, 43.1 at 45 min, 45.1 at 60 min, and 42.7 at 90 min, indicating that 60 min preincubation in dextran-medium was sufficient for ECS to be filled with dextran.

4) Oxygen content in the medium was determined to be 98.6% of the normal medium in a 7.5% dextran-medium, and 95.2% in a 15% dextran-medium.

DISCUSSION

The calculated tension decline curve after Ca removal was not in agreement with the observed curve in the initial period of 1-2 min, yet after this period, the calculated and the observed curve paralleled each other (Fig. 5). The initial disagreement will be discussed later. The parallelism indicates that the initial phenomena, in which the shift of the observed curve from the calculation originates, do not take place in the stage of the parallelism. And it is highly probable that in this stage the assumptions which the calculation are based upon are correct or nearly applicable to taenia coli, at least under the present conditions. If D value smaller than the present one were used in calculation, the calculated tension decline rate would be lower than in Fig. 5, while the rate would be higher if a smaller R value were used. Therefore, the probability cannot be ruled out that the present D and R values are incorrect but do show a parallelism between calculation and observation, as a result of accidental counterbalance. A similar discussion is also tenable for the other assumptions used. Such an offset is, however, much less probable than the case that all assumptions are correct because the offset requires the simultaneous occurrence of two or more accidental phenomena which are completely counterbalanced in a quantitative aspect.

The present calculation of the apparent D of Ca in ECS is based on model experiments, and assumes the absence of interaction between the ions and extracellular structure. In the stage of parallelism in Fig. 5, therefore, the interaction, such as adsorption, is presumably negligible or does not take place.

In normal medium the observed tension decline is of a higher rate in the initial stage than the calculated rate (Fig. 5). The diffusion theory in cylinder was applied to the present calculation, whereas the cross-section of taenia coli was observed not to be circular. The Ca ions are lost from the projecting part presumably at a higher rate than the theoretical rate. Afterward, however, isoconcentration lines of Ca become close to circle so that the diffusion theory in cylinder is nearly applicable to the loss of Ca, and that the calculated and
the observed curve parallel each other. To ascertain these assumptions, the wash-out of alizarin yellow was studied in a triangular prism, in which the cross-sectional area was the same as that of one of P-cylinders. The results shown in Fig. 6 indicate that the initial disagreement between calculation and observation in normal medium can be attributed to the geometry of taenia coli as mentioned above, and that the diffusion theory in cylinder is acceptable except in the initial 1–2 min of Ca loss.

In dextran-medium, however, the initial tension decline rate is lower in observation than in calculation. When the normal Ca-medium is changed to a Ca-free one, the tissue surface is initially covered with a layer of normal Ca-medium of unnegligible thickness as compared with the tissue radius (Results), whereas the diffusion theory assumes that the Ca concentration is always zero at the tissue surface. The more viscous the medium, the more slowly Ca around the tissue is removed under Ca-free condition, and the more slowly ECS Ca is lost until the diffusion theory is acceptable. In dextran-medium, this viscosity effect probably overcomes the above-mentioned effect of tissue shape, while the viscosity effect is so small in the absence of dextran that the effect of tissue shape is dominant. The discussion is supported by the fact that the correlation coefficient was 0.829 between absolute viscosity of the medium and the initial shift of observed tension from the calculated one (vertical distance between two curves in Fig. 5, the sign being noted). The value is close to the statistically significant value, 0.878, suggesting that the viscosity effect described above is reasonably accurate.

One of the problems in the present study is that the diffusion coefficient of Ca ions in the medium was not determined experimentally because of difficulties in the determination techniques. The $D$ value of $1 \times 10^{-8}$ cm$^2$ sec$^{-1}$ used here is presumed to be not far from the true value since the presence of counter-ions, such as $\text{PO}_4^{3-}$ and $\text{CO}_3^{2-}$, decreases the $D$ value and is considered to overcome the temperature effect (9). The following facts also support the reliability of the value used here for the apparent $D$ of Ca in ECS. A ratio of $D_{\text{m}}/D_{\text{p}}=0.32$ is applicable to the other substances under the same conditions unless different factors such as chemical reactions, are introduced in the diffusion process. On the other hand, the diffusion coefficient of NaCl is of the same order of magnitude as of CaCl$_2$ in water (9), suggesting that the apparent $D$’s of Na and Ca ions in ECS are close to each other. The apparent $D$ of Na ions has been reported to be 2.6 (10) and $3.1 \times 10^{-6}$ cm$^2$ sec$^{-1}$ (11) in skeletal muscle, and of Ca ions to be $2 \times 10^{-6}$ cm$^2$ sec$^{-1}$ (12). Although the muscles used were different, these values, as well as the present value, are all in good agreement despite the fact that the methods applied in determination of $D$ were entirely different.

The theory of the random walk gives the equation, $d^2 = n\ell$, where $d$ is a distance from the starting point after $n$ time turns with a mean square of the free-path $\ell$ (14). The theory also gives the equation, $d^2 = 6Dt$, where $D$ is diffusion coefficient and $t$ is time from the start of the random walk (15). On the other hand, the kinetics of gas molecules give the number of molecules ($N$) which collide during a period of time $\Delta t$ with a surface, of which area is $\Delta s$; $N = (1/4)Mc\Delta s\Delta t$, where $M$ is a number of whole molecules and $c$ is a mean velocity. From these three equations, an equation is obtained; $N = k_1D$ or $N = k_2\frac{1}{\eta}$, where $\eta$ is abso-
lute viscosity of the medium, and \( k_1 \) and \( k_2 \) are constants. The last equation gives the relative number of Ca ions which collide with the cell surface in a dextran-medium. In 15% dextran-medium, for instance, the number of colliding Ca ions is 27.8% of that in the normal medium.

On the basis of this value only, the Ca-tension relationship in 15% dextran-medium would be slightly convex upward in contrast to the actual relationship shown in Fig. 4, suggesting that the other factors are not negligible, such as a decreased oxygen supply.

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