sisted mainly of drug nucleotides. Near 0°C, phosphorylation was inhibited, and drug accumulation was similar in both cell lines. In other studies, we found that uptake of cytosine arabinoside, during the initial 30 seconds of incubations, was similar in all 14 cell lines examined. Under these conditions, less than 10 percent of the cellular radioactivity accumulated at 37°C represented drug nucleotides.

The accumulation of cytosine arabinoside nucleotides by cell lines examined was increased by raising the drug concentration in the medium, thereby increasing the rate of drug uptake. This suggests that variations in the rate of cytosine arabinoside uptake, although apparently not a factor here, could affect responsiveness to the drug in other cell lines.

Uptake of cytosine arabinoside can be stimulated by pyrimidine and certain purine nucleosides (*19*). This structural specificity suggests interaction between transport systems (*20*). Our attempts to show an energy requirement for transport of cytosine arabinoside in L1210 or L1210/CA were unsuccessful. Jacquez (*21*) found evidence to suggest mediated transport of three pyrimidine nucleosides—thymidine, uridine, and fluorouridine—by Ehrlich ascites tumor cells.

We conclude that cytosine arabinoside does not diffuse freely into leukemia cells of mice. There is evidence to suggest that uptake of the drug is mediated; however, uptake was not impaired in drug-resistant cell lines. Variation in cellular capacity for phosphorylation of the intracellular drug provides the basis for responsiveness to cytosine arabinoside in the cell lines that we examined. Similar correlations between capacity for drug phosphorylation in vitro and drug response in vivo were found in human leukemias (*22*).

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11. TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] was obtained from Calbiochem Corp. This buffer contained 62 m*M* TES, *p*H 7.2; 65 m*M* NaCl; 15 m*M* KCl; and 8 m*M* $CaCl_2$.
12. Tritiated cytosine arabinoside (2 c/mmole) was purchased from Schwarz BioResearch Corporation. Other labeled nucleosides were purchased from New England Nuclear Corporation.
13. The scintillator solution was 10 ml per vial of 600 ml of toluene, 400 ml of methyl cellosolve, 60 g of naphthalene, and 4 g of 2,5-bis-[2(5-*tert*-butylbenzoxazolyl)] thiophene (BBOT, Packard Instrument Co.). A Nuclear-Chicago Mark I liquid scintillation counter was used.
14. Whatman No. 1 paper was used for descending chromatography. Solvent systems used were: 94.6 ml of 85 percent *n*-butanol plus 5.4 ml of concentrated ammonium hydroxide (*8*), and 100 ml of isobutyric acid plus 60 ml of 1*M* ammonium hydroxide adjusted to *p*H 6 [H. A. Krebs and R. Hems, *Biochim. Biophys. Acta* **12**, 172 (1952)]. In cell extracts, triphosphates of nucleosides predominated; lesser amounts of monophosphates and traces of diphosphates were also found.
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17. Conversion of labeled cytosine arabinoside, deoxycytidine, cytidine, adenosine, and uridine to nucleotides by P815 cells proceeded at 10 to 20 percent of the corresponding rates observed in L1210 cells. These studies were performed by incubating cells in medium containing the labeled nucleosides (0.1 m*M*) and then by determining accumulation of nondiffusible nucleotides. The identity of the latter compounds was verified by paper chromatography of cell extracts.
18. I. Wodinsky and C. J. Kensler, *Cancer Chemotherap. Rep.* **43**, 1 (1964). L1210/CA cells showed a specific enzymatic impairment; cytosine arabinoside and deoxycytidine were phosphorylated (*17*) at <1 percent and 10 percent, respectively, of the rates found in L1210. Phosphorylation of uridine, adenosine, deoxyadenosine, and cytidine proceeded at similar rates in L1210 and L1210/CA.
19. These included deoxycytidine, thymidine, thymine riboside, uridine, cytidine, deoxyadenosine, and 5-fluorouridine, but not guanosine, adenosine, cytosine, uracil, or arabinose. Addition of a 50-fold molar excess of any of the first group of compounds increased the ratio of distribution of cytosine arabinoside in L1210/CA cells from 0.2 to 0.4 (Fig. 2).
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23. We thank Susan Blakely for technical assistance. Supported by Cancer Chemotherapy National Service Center contracts PH 43-62-169, PH 43-66-541, and PH 43-65-61, and by NCI grant C-6516.

28 March 1967

Cell Division: Direct Measurement of Maximum Tension Exerted by Furrow of Echinoderm Eggs

Abstract. *Tensions exerted by cleavage furrows in isometric contraction were measured by means of flexible glass needles whose characteristics of bending had already been determined. The tension of* Astriclypeus manni *furrows in second division is* $3.04 \times 10^{-3} \pm 0.95 \times 10^{-3}$ *dyne; that for* Pseudocentrotus depressus *eggs in first division is* $2.00 \times 10^{-3} \pm 0.43 \times 10^{-3}$ *dyne. The tension required for cleavage probably does not exceed* 1.5×10^{-3} *dyne. According to existing morphological evidence, these values can be accounted for by a substance whose capacity for exerting tension does not exceed that of an actomyosin thread.*

Since echinoderm eggs divide despite removal, replacement, or drastic rearrangement of the endoplasm and subsurface cytoplasm (*1*), the mechanism of division must lie in or very close to the cell surface. The observation that the surface of the base of the furrow can bend a glass needle placed in its path at right angles to the cleavage plane (*2*) indicated that direct measurement of the maximum tension generated by the furrow should be possible.

Thin glass needles were drawn with an incandescent filament and micromanipulators; they were calibrated with a reference needle (*3*). Immediately after the furrow appeared, the calibrated needle was inserted through one polar surface, and a stouter holding needle was thrust in the opposite direction through the other polar surface. Both needles were positioned so that they passed through the cleavage plane and lay in approximately the same plane of focus (Fig. 1). Opera-

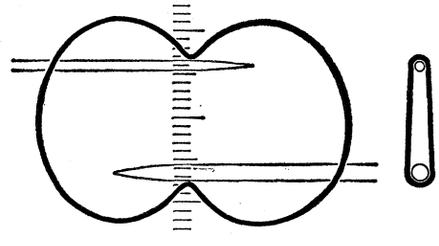

Fig. 1. (Left) Arrangement of cell and needles for determination. Upper, calibrated needle is deflected downward during cleavage. Lower, holding needle does not move. (Right) Schematic section through and parallel to the cleavage plane during isometric contraction. Diameter of the needles is exaggerated.

tions and measurements were made at a magnification of × 400 with Peterfi micromanipulators and an inverted microscope. Before the furrow tip touched the calibrated needle, the position of both needles was measured with an eyepiece micrometer. As the furrow deepened, both needles touched the surface. The holding needle did not move, but the calibrated needle was bent inward until the needle's resistance to deflection equaled the force exerted by the furrow. At that time, the position of both needles was remeasured. Only data from cells in which the holding needle remained fixed were used. Using calibrated needles of different diameters and inserted at different points in the polar area, I found that possible surface movements in that region did not affect the values achieved. When necessary, correction for the refractive index of cytoplasm was made (*4*). Four calibrated needles of different thickness, length, and sensitivity were used. In isometric contraction, the connection between the blastomeres usually persists as a flattened tube whose dimensions are determined by the diameters of the needles and the distance between them (Fig. 1) (*2*).

Because the large size of the uncleaved egg made manipulation difficult, measurements on *Astriclypeus manni*, the Japanese perforated sand dollar, were made on blastomeres in the second division. Eggs were mechanically denuded after fertilization and were transferred with thorough rinsing to synthetic seawater free of calcium, where the measurements were subsequently made. The calibrated needle was inserted under the animal pole, and the first contact between the needle and the furrow tip took place when the cell diameter in the furrow was about 90 percent of the value at interphase (*5*). *Pseudocentrotus depressus* (sea urchin) eggs were divested of membranes and the hyaline layer by treatment with 1*M* urea and then returned to normal seawater. Measurements were made during the first cleavage. The first contact between the furrow tip and the needle took place when the cell diameter at the furrow was about 50 percent of the value at interphase.

In *Astriclypeus manni* the maximum tension exerted by the furrow in isometric contraction is $3.04 \times 10^{-3} \pm 0.95 \times 10^{-3}$ dyne (42 measurements). For *Pseudocentrotus depressus*, the value is $2.00 \times 10^{-3} \pm 0.43 \times 10^{-3}$ dyne (24 measurements). The extreme values for *Astriclypeus* were 5.25×10^{-3} dyne and 1.62×10^{-3} dyne. The extremes for *Pseudocentrotus* were 3.56×10^{-3} and 1.30×10^{-3} dyne. In all cases, the furrow immediately resumed activity after the needles were removed.

The "average" echinoderm cleavage furrow may be assumed to exert a maximum tension of 2.5×10^{-3} dyne in isometric contraction. This capacity for exerting tension must originate in regional differences in the physical properties of the cell surface or of the cytoplasm immediately beneath it. Electron-microscopic studies and experiments with microdissection have both demonstrated differences between the polar and furrow regions, but the relation between these observations and the cleavage mechanism is unresolved. The most compact structure observed is an electron-opaque layer which appears under the entire surface of the egg at cleavage and achieves greatest development in the furrow. Mercer and Wolpert (*6*) in their original description stated, "The distribution of this material is so similar to that of the contractile gel or ring postulated by Marsland as to lend strong support to his views." The layer is about 0.1 μ thick in the furrow. It was also observed in the cleavage furrow of *Arbacia* blastomeres and subjected to more detailed geometrical analysis (*7*). Older evidence from microdissection suggested that cleavage was associated with a gelated cortical region lying immediately under the cell surface. During division the cortex is best developed in the furrow region where a thickness of 4 μ has been reported (*4*). Wolpert (*8*) assumes that the contracting portion of the furrow region is 5 μ wide, and measurements of living cells in division indicate that the figure is reasonable. If this value is used in calculations, the total cross-sectional area of the dense layer which would act upon the needle is 1.0 μ^2 since the region operates as two nearly parallel strips in isometric contraction (Fig. 1). If the gelated cortical region is 3 μ thick, the total cross-sectional area acting on the needle will be 30 μ^2. To exert the forces reported here, a contractile zone of the dimensions of the dense layer must exert a tension of 2.5×10^5 dyne/cm^2. The other possible mechanism of cortical material would have to exert a tension of 8.33×10^3 dyne/cm^2.

Since there are some biochemical resemblances between the cleavage mechanism and muscular contraction (*9*) and since contractile protein has been isolated from dividing sea urchin eggs (*10*), comparison between the tensions exerted by cleavage furrows and by muscle fibers and those exerted by proteins is appropriate. The maximum tension exerted by frog sartorius muscle is about 1.5 kg/cm^2 (*11*), or 1.47×10^6 dyne/cm^2. This is about six times the contractile capacity of the electron-opaque layer. Actomyosin threads, on the other hand, exert a maximum tension of about 250 g/cm^2 (*12*) or 2.45×10^5 dyne/cm^2, which is close to the value calculated for the capacity of the electron-opaque layer. Therefore, according to existing morphological evidence, the tension exerted by the cleavage furrow reported here could be accounted for by the presence of a substance whose contractile capacity approximates that of an actomyosin thread. If the cross-sectional area of the contractile mechanism eventually proves to be greater than that of the electron-opaque zone, then the maximum tension per unit cross-sectional area would be appropriately reduced.

These measurements indicate the maximum tension the furrow can exert. They do not reveal the amount of force actually used for division, but they do establish an upper limit. A strip of contractile material 10 μ wide exerting a maximum tension of 2.5×10^{-3} dyne would cause a tension at the surface of 2.5 dyne/cm. But since furrows which exert tensions of about 1.5 dyne/cm (1.5×10^{-3} dyne) completed division when the needles were removed, the tension required for division need not exceed that value. On the other hand, tension in the furrow must exceed the overall tension at the surface which was recently found to be 0.2 dyne/cm in fertilized *Hemicentrotus pulcherrimus* eggs shortly before division (*13*).

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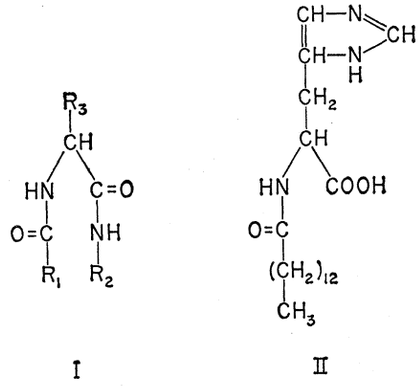
12 April 1967

Catalysis of Ester Hydrolysis by Mixed Micelles containing N-α-Myristoyl-L-Histidine

Abstract. *Model compounds are described for the study of the properties of amino acid side chains in the surface of micelles. Mixed micelles of* N-α-*myristoyl*-L-*histidine and cetyltrimethylammonium bromide catalyze the hydrolyses of* p-*nitrophenyl acetate and* p-*nitrophenyl caprylate at much higher rates than imidazole or histidine do. The reaction shows a kinetic behavior similar to that of surface-catalyzed reactions.*

Kauzmann (*1*) has emphasized the importance of hydrophobic bonding in stabilizing the native conformation of proteins. This postulate has received support of Kendrew's (*2*) and Perutz's (*3*) x-ray data which implies that in many regions the orientation of the side chains in proteins is similar to that occurring in micelles of amphipathic molecules (*1*, *3*, *4*). That is, the nonpolar amino acid side chains are directed away from the water in close van der Waals contact, whereas the polar side chains are directed so that they have maximum contact with water. This orientation would place many functional side-chain groups adjacent to or within hydrophobic regions, giving them properties which might differ from those expected if these same groups were in an aqueous environment (*5*).

We report an initial example of a model system (*6*) which permits study of the properties of amino acid side chains when they are present at the lipid-water interphase. For this purpose, compounds with the general formula (I) may be synthesized; R_1 and R_2 are hydrocarbon chains whose length may be varied depending on the hydrophilic nature of the amino acid functional group R_3. In this manner, the proper hydrophilic-to-hydrophobic ratio could be maintained to yield the desired solubility in water and the desired micelle-forming character (*4*). These micelles would have the hydrocarbon chains directed away from the water, while the amino acid side chain would lie in the micellar surface at the lipid-water interphase. Both R_1 and R_2 need not be present simultaneously, because only one hydrocarbon chain of proper length is necessary for micelle formation.



I II

The imidazole group of histidine is apparently involved in the active center of enzymes (*7*). Also, histidine-containing peptides (*8*) and imidazole *per se* (*9*, *10*) can catalyze the hydrolysis of esters. We therefore have made tests to ascertain whether histidine, when located in a micellar surface, can catalyze the hydrolysis either of *p*-nitrophenyl acetate (NPA) or of *p*-nitrophenyl caprylate (NPC). For this purpose *N*-α-myristoyl-L-histidine (II) was synthesized (*11*). Initial findings indicated that aqueous solutions of (II) at *p*H 7.2 did not accelerate the hydrolysis of NPA. These solutions, although clear when initially prepared, became opalescent on standing and showed strong flow birefringence, indicating that marked association occurred. Also, it seemed likely that the micelles formed by (II) would have the ionized carboxyl groups on the surface, decreasing any possible interaction of the ester with the imidazole group. In order to overcome these difficulties, mixed micelles of II and cetyltrimethylammonium bromide (III) were studied. The rate of NPA hydrolysis increased markedly as the ratio of III to II was increased, a maximum rate being attained at a ratio of 20:1. In the case of NPC, the maximum rate of hydrolysis occurred at a ratio of 2:1 and decreased sharply at higher ratios. All solutions at ratios of (III) to (II) greater than one were clear and showed no opalescence on standing.

When the rate of formation of *p*-nitrophenolate ion was studied under conditions where the concentration of (II) was greater than that of the ester, satisfactory *pseudo*-first-order kinetics were observed. In view of the variation in the extinction coefficient of the *p*-nitrophenol in the presence of varying concentrations of detergent, the specific rate constants were calculated from the amount of *p*-nitrophenol liberated at infinite time (20 to 40 hours) determined for each experimental point. Typical curves from which the specific *pseudo*-first-order rate constants, k_{obs}, were obtained are shown in Fig. 1. These rate constants were corrected by subtracting the rate constants obtained in the presence of (III) alone (less than 3 percent in all cases studied) (*12*). At low concentrations of

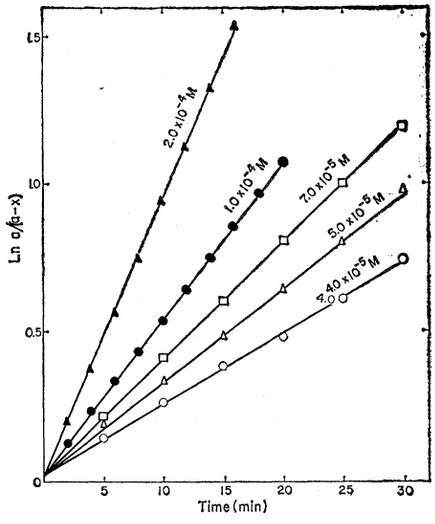


Fig. 1. *Pseudo*-first-order rate constants for the hydrolysis of *p*-nitrophenyl acetate by mixed micelles of *N*-α-myristoyl-L-histidine (II)-cetyltrimethylammonium bromide (III). In addition to the concentrations of II shown in the figure, the assay system contained a 20-fold greater concentration of III, both dissolved in 50 m*M* tris-HCl buffer, *p*H 7.2. To this was added 10 μl of the NPA (3.45 to 6.91 × 10^{-6}*M*) dissolved in acetonitrile. The rate of *p*-nitrophenol liberation was measured by following the change in optical density at 400 mμ at 25 ± 0.5°C in a Cary 15 recording spectrophotometer. *a* is the optical density of the *p*-nitrophenol liberated at infinite time. *x* is the optical density of the *p*-nitrophenol liberated at the times indicated.