Table 1. The occurrence in the bile of man of ornithocholanic acids and small, soft stones consisting of cholesterol and pigment, compared with the infections and terminal bacteremias found in the same patients. The numbers in parentheses indicate the number of patients. H-B indicates heart blood.

K. pneumoniae (15)			*E. coli* (6)			*E. freundii* (1)	*Proteus* (1)	*Pseudomonas* (3)	Staph. (7)	Strep. (3)	Negative culture (26)	
H-B	Lung	Kidney	H-B	Lung	Other	H-B	H-B	H-B	H-B	H-B	H-B	Other
Cultural results												
3*	10	2	4†	3	1	1	1	3	7	3	26‡	26
Small stones												
1*	9	2	0	2	0	0	0	0	0	0	0	1
Ornithocholanic acids												
1*	9	2	0	2	0	0	0	0	0	0	0	0

*, †, and ‡ indicate, respectively, 8, 2, and 26 identical cultural results in lung or other tissue.

The exact mechanisms through which infections with *K. pneumoniae*, *E. coli*, and perhaps other microorganisms lead to the precipitation of bile constituents has not been proven. A reasonable hypothesis, based upon present information, would be that a metabolic derangement in the liver, perhaps in the ornithine-arginine-urea cycle (Krebs-Henseleit) results in conjugation of L-ornithine to the free bile acids. The SO_3H radical of taurine has been thought to be important in micelle formation in bile (*7*). The ornithine conjugates have a free NH_2 and COOH rather than SO_3H group. Thus, precipitation of cholesterol and pigment could follow changes in the micellar suspension of bile acids due to the presence of the abnormal ornithocholanic acids, especially during concentration of bile in the gall bladder. The precipitate could serve as a nucleus for the further accumulation of cholesterol and pigment.

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Muscle: Volume Changes in Isolated Single Fibers

Abstract. *Volumetric experiments on single fibers isolated from semitendinosus muscles of frogs, some performed in correlation with measurements of membrane potential, confirm the data obtained on whole muscles, but only for the specific range of conditions in which most of the latter experiments have been done. These conditions are restricted to media in which the anion (Cl usually) is permanent and the K is 10 to 12.5 meq/liter, or four to five times above the normal level in Ringer's solution. When other ionic conditions are employed, phenomena are disclosed which have not previously been described. The findings throw doubt upon the validity of some generally accepted views regarding the permeability properties of the membrane of frog muscle fibers and regarding the nature of the mechanisms which regulate their volume.*

The osmotic behavior of whole frog muscles has provided the basis for a number of important conclusions regarding the properties of cell membranes and of the processes of ionic distributions between cells and their environment (*1*). We now report on osmotic studies of single fibers isolated from semitendinosus muscles of frog. The ionic conditions of the experiments were basically of four classes, depending on: (i) whether the anion of the medium was Cl or an impermeant anion—the latter was usually propionate, but similar data were also obtained by substituting SO_4 for Cl; (ii) whether the solutions had an elevated concentration of K (to 12.5 meq/liter) or were free of K. Bicarbonate was used to buffer the solutions to *p*H 7.4 to 7.6. The experiments involved about 300 single fibers and were carried out between October 1962 and June 1963—that is, on "winter" frogs.

After dissection, the single fiber was mounted in a chamber which permitted photomicrographs to be made at chosen intervals while the fiber was subjected to various osmotic or ionic changes, or to both. The fibers are essentially cylindrical. Measurements of their diameters from enlargements of the photomicrographs gave the relative volumes of the fibers during various stages in the experiments. The volume could be observed within 1 or 2 minutes after a change in the medium.

Under similar conditions data on single fibers are similar to those reported on whole muscle. Figure 1 shows measurements on fibers which were initially equilibrated in the Cl-saline control medium containing 12.5 mmole of KCl per liter. The osmotic pressure was changed by adding or removing NaCl. The relation between relative osmotic pressure (abscissa) and relative volume (ordinate) was linear for the range up to halving the NaCl. It then flattened, as is also the case in experiments on whole muscles (*2*). The intercept of the straight line on the volume axis was 0.35, also nearly that (0.345) obtained in experiments on whole muscle (*3*).

The latter intercept has been ascribed (*3*) to the sum of the fractions of osmotically inactive solids of the fibers (0.20) and of the fluid spaces between the fibers of the muscle (0.13). In measurements on single fibers, however, the latter space is absent, but it is also unlikely that the solid matter of the muscle fiber constitutes as much as 35 percent of the total volume. Thus the data on single fibers raise some questions regarding the interpretation of the intercept, and of the similar intercept (0.4) found in osmometric experiments on single muscle fibers of crayfish (*4*).

The limitation on the swelling of whole muscle in hyposmotic media has been interpreted (*2*) as indicating some mechanical constraint by the fiber structures. However, the data representative of 12 types of experiments which are shown in Fig. 2 disclose that the volumes of frog muscle fibers are complexly regulated and that they can rise higher than the limit observed in Fig. 1.

The 12 fibers of the experiments shown in Fig. 2 were initially equilibrated in one of the four control solutions described above. In six of the experiments the fiber was then exposed for a time to a solution made hyposmotic by diminishing the concentration of the Na salt by 60 meq/liter (*A*, *D*, *E*). In four experiments the fibers were exposed to media made hyperosmotic by adding 100 or 120 meq of the Na

salt per liter (*C*, *F*). Two other experiments are shown in which fibers that had been initially equilibrated in media containing 12.5 meq of K per liter were then exposed to isosmotic media in which 80 meq/liter of the Na salt was replaced stoichiometrically with K salt (*B*).

Fibers which were equilibrated in the Cl-saline control medium containing 12.5 mmole of KCl (Fig. 2, left) swelled promptly in the hyposmotic medium (broken line, *A*), and at first usually considerably above the steady-state level. The latter was attained only after several hours exposure to the hyposmotic medium, the return from the greater swelling taking an oscillatory course. When the control medium was restored the fiber returned promptly to the initial volume.

When the Cl was replaced with an impermeant anion the fiber did not respond with a stepwise volume change to the hyposmotic condition (solid line, *A*). If the Na removed exceeded about 20 meq/liter there could be an initial swelling, increasing in magnitude with the degree of the hyposmotic condition, or there could be only a shrinkage (when only 20 to 40 meq/liter of the Na salt was removed). In all cases, however, there was a decrease in the volume with more or less shrinkage. The membrane potential showed only a stepwise depolarization. Thus the shrinkage was due to a loss of salts with an osmotic activity of 80 to 100 mosmols. When the fibers were returned to the control medium all promptly shrank still more, while the membrane potential returned to its initial value. Both facts confirm that the fibers had lost about 80 to 100 mosmols of osmotic components. The cation lost must have been mainly K. The amount of anion lost is too great for the Cl content of the fibers, and it may be, in part, PO_4.

In the isosmotic media in which KCl was substituted for NaCl (*B*, broken line), fibers swelled to about the same level as they did in the hyposmotic Cl-saline media, but more slowly, presumably reflecting the slow entry of KCl into the cells. Fibers in the isosmotic media with impermeant anions shrank rather rapidly (*B*, solid line), again to a level which corresponds to a loss of 80 to 100 mosmols of intracellular constituents. The shrinkage also occurred when the medium was maintained isosmotic by addition of sucrose to compensate for the removal of the Na salt. The return to

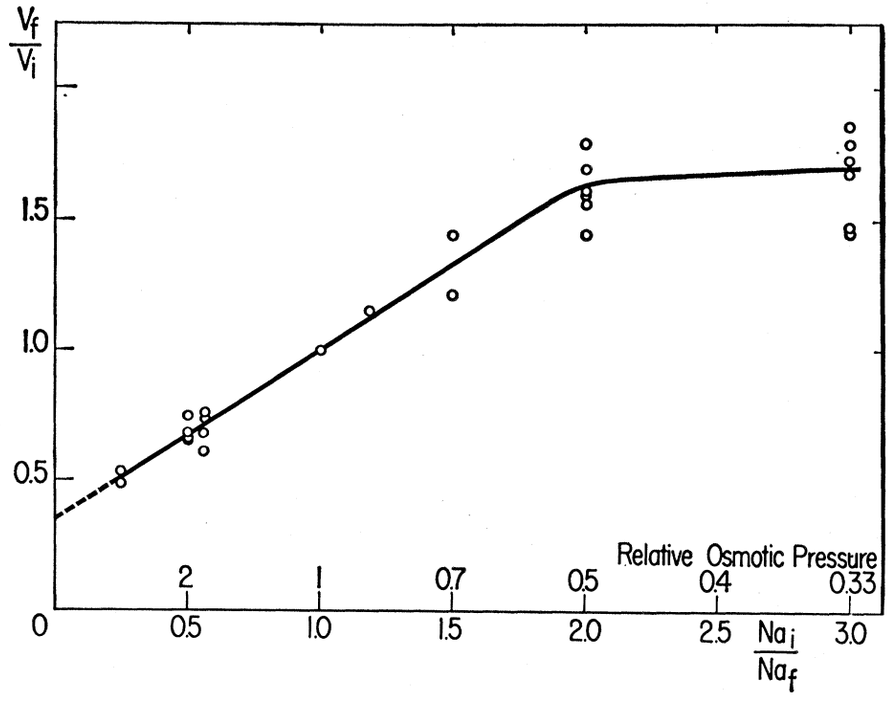

Fig. 1. Changes in volume of single muscle fibers with changes in osmotic pressure. *Ordinate,* Ratio of new volume (V_f) to initial volume (V_i). *Abscissa,* Ratio of initial concentration of Na (Na_i) to the new concentration (Na_f), and the relative osmotic pressures resulting therefrom. All experiments done in Cl-salines, with 12.5 mmole of KCl per liter present.

the control media, with decrease of K, was accompanied by a complex secondary increase in volume in both types of experiments (*B*). This swelling probably represents "anomalous" electroosmotic effects like those that have been observed in crayfish muscle fibers when the latter are repolarized (*4*).

In the presence of 12.5 meq of K per liter, the fibers shrank promptly and remained shrunken in hyperosmotic media until the control medium was replaced (*C*). This step-like response was independent of the presence or absence of Cl in the media.

In K-free media the fibers responded to osmotic changes in still other ways (Fig. 2, right). Exposure to hyposmotic Cl-saline caused only a small swelling in fibers which had been initially equilibrated for a few hours at room temperature (*D*, broken line). The swelling decreased gradually, and when the fiber was returned to the control medium there was a slight shrinkage below the control volume. The fibers in Cl-free media, when treated similarly, showed only a large stepwise swelling (*D*, solid line). When the fibers were first equilibrated for 16 hours or more in the cold (2° to 4°C) the response to a hyposmotic Cl-free medium (*E*, filled circles) was essentially the same as in the previous experiment (*D*). The fiber in the Cl-saline (*E*, broken line) now swelled markedly, the volume change having two phases. There was an initial swelling, to about the same level as in the K-enriched medium (*A*). Then followed a slow increase which was usually to a level considerably above the limiting value shown in Fig. 1.

The resting potentials increased from about 90 mv, in the presence of 2.5 meq of K per liter, to about 110 mv when fibers were placed in K-free media (*D*). The fibers in the Cl-free medium remained at 110 to 115 mv after long equilibration in the cold, whereas fibers in the Cl-saline (*E*) always depolarized to between 50 and 85 mv. The depolarization reflects the exchange of Na for intracellular K (*1*). Thus the exchange can occur only as movements of the salts.

When the fibers were returned to their respective control media there was always a prompt return to, or slightly below, the control volume (*D*, *E*). In the absence of Cl, and also in the fibers equilibrated in the cold in the Cl-saline media, there followed a secondary slow rise in volume.

Fibers subjected to hyperosmotic solutions (*F*) also responded differently from those which had been equilibrated in 12.5 meq of K per liter. In Cl-free media (solid line) the shrinkage was essentially step-like. The volume re-

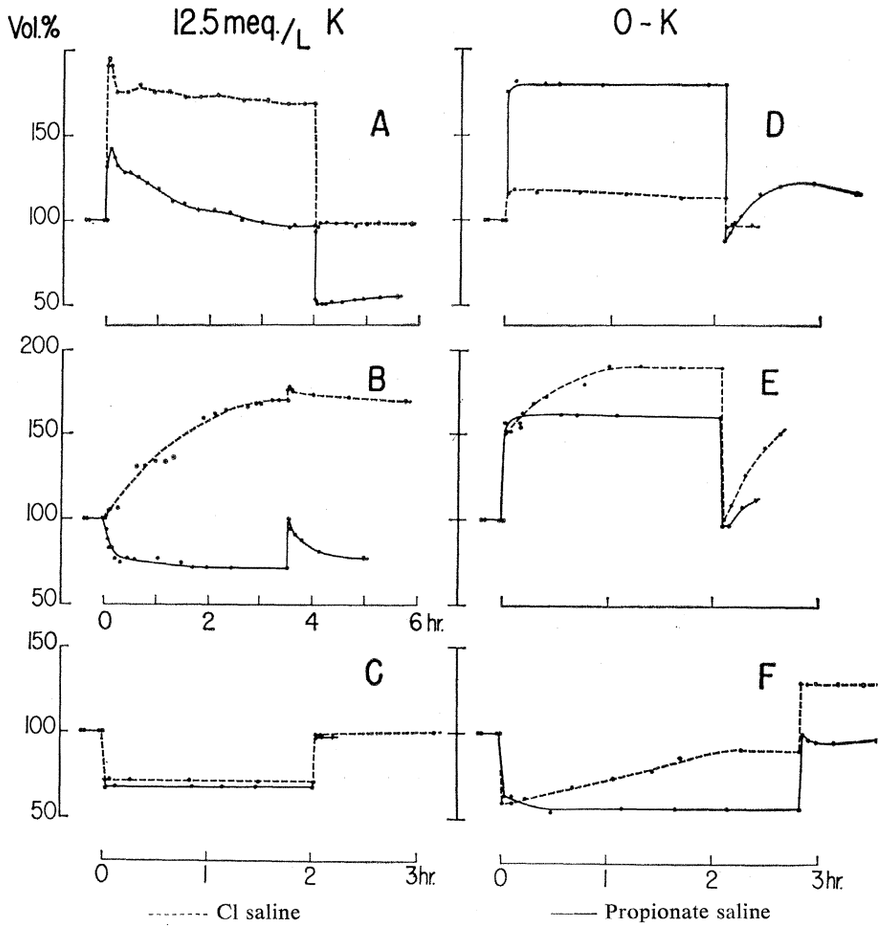

Fig. 2. Volume changes induced in individual muscle fibers under different experimental conditions: *A–C*, in the presence of 12.5 meq of K per liter; *D–F*, in K-free media. Circles on the broken line refer to experiments done in Cl-saline media; circles on the solid line, experiments in propionate media. *Ordinate*, Change in volume. *Abscissa*, Time in hours after the initial change in the medium. *A*, Responses to removal of 60 meq of Na salt per liter (hyposmotic media) and subsequent return to the respective control media. *B*, Removal of 80 meq/liter of the Na salt was compensated by addition of 80 meq/liter of the K salt (isosmotic condition). *C*, Media were made hyperosmotic by addition of 100 meq of the Na salt per liter. *D*, Media were made hyposmotic as in *A*, 2 hours after the fibers had been placed in the K-free control salines. *E*, Same as *D*, except that the fibers were equilibrated in the K-free control media for 16 hours at 2° to 4°C. *F*, K-free media were made hyperosmotic by addition of 120 meq of the Na salt per liter.

turned promptly toward the initial level when the control solution was reintroduced, indicating that the osmotically active constituents of the cells had not been altered significantly. Fibers in the K-free Cl-salines (broken line), however, always underwent a slow secondary swelling after the initial shrinkage. When they were returned to the control medium they promptly swelled further, remaining at the new volume for a long time. Thus, the fibers must have gained NaCl when exposed to hypertonic NaCl media in the absence of external K.

It appears, therefore, that the volume of the fiber is complexly determined by the interplay of several factors. The final volume attained by a fiber also depends on the temperature, *p*H, Ca, and the nature of the buffering agent (*5*). Dependence on the mechanisms of active transport is also suggested by the actions of various metabolic inhibitors and by experiments in which LiCl was substituted for NaCl. The intracellular accumulation of Li slows the efflux of both Na and Li (*6*). When fibers soaked in control media made with LiCl substituted for NaCl were subjected to solutions which were rendered hyposmotic by decreasing the LiCl, they always swelled considerably more than did fibers in comparable NaCl media.

A qualitative description of the foregoing data is as follows: The permeability of Na is dependent on the external K, increasing when K is absent from the medium. Thus, there is a change in the membrane, denoted by a decrease in the reflection coefficient for Na. However, Na can enter the cell only with a permeant anion, not by exchange for K. In the presence of 10 to 12.5 meq of K per liter, a reduction of Na by 20 meq/liter or more triggers an efflux of intracellular constituents which probably reflects an effect of decreased Na on metabolic processes of the cell. This loss is compensated by the entry of salts from the medium when the anion of the latter is permeant. However, if the anion is impermeant the loss cannot be compensated.

The totality of the data thus leads to the conclusion that the cell volume is not a function of a simple Donnan distribution in which the cell contains a fixed amount of an impermeant anion. Under given osmotic drive the final volume is determined by a steady-state condition of inward and outward fluxes of ions as well as by the initial change of the activity gradient of water across the cell membrane. Futhermore, as the external medium and the cell interior both become more concentrated, a volume of water equal to that of the cell solids is probably bound to them.

In view of the complexities exhibited by frog muscle fibers, it may be necessary to reevaluate some of the conclusions regarding ionic permeability of the cell membrane which have been formulated on the basis of data obtained with amphibian striated muscle (*7*).

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