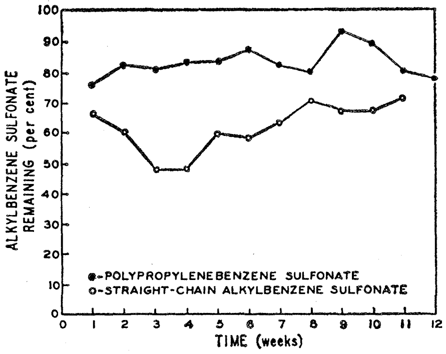

Fig. 1. Removal of alkylbenzene sulfonates in a simulated septic tank (no drain field).

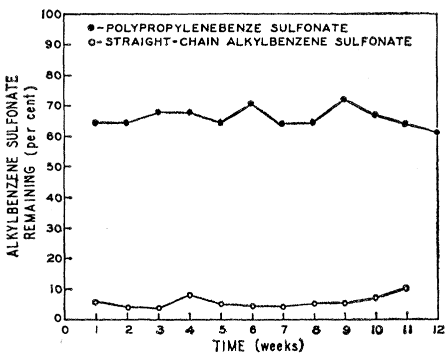

Fig. 2. Overall removal of alkylbenzene sulfonates in a simulated septic tank and drain field.

effluent containing 6 to 14 parts of detergent per million. Replacement with straight-chain alkylbenzene sulfonates could lower this concentration to 0.5 to 2.0 ppm. These concentrations are further reduced in the ground water by subseqeunt dilution with water from other sources, such as rain and agricultural or horticultural water (*4*).

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14 August 1963

Ornithocholanic Acids and Cholelithiasis in Man

Abstract. *Ornithocholanic acids and small soft gall stones were found in the bile of patients from which* Klebsiella pneumoniae *was also isolated. Cholelithiasis may be initiated by metabolic disturbances in the conjugation of bile acids in liver tissue and the subsequent precipitation of cholesterol and bile pigment.*

In the guinea pig, suppurative lesions due to *Klebsiella pneumoniae,* as well as the parenteral injection of toxic products of this microorganism, led to the excretion in bile of ornitholanic acids and the rapid formation of cholesterol and pigment precipitates in the gall bladder (*1*). The specificity of the klebsiella organism or its products in the induction of this process was not evaluated. However, ornithine was a metabolic byproduct of these microorganisms. When C^{14}-labeled ornithine was injected into the guinea pig, some of it was found to conjugate with the three cholanic acids in the bile (*2*). Studies in vitro also indicated that ornithine conjugates with cholanic acids in the liver tissue of the guinea pig and rat.

The correlation between ornithocholanic acids, recent gall stones, and bacterial infections, other than in the gall bladder, has now been studied in man. Autopsies were performed on 62 unselected patients, and bacterial cultures were made from cardiac blood and areas of grossly discernible exudative inflammation. The presence of small soft stones, measuring 1 to 3 mm in diameter, was noted, and the presence of conjugated bile acids in fluid bile was determined by column and paper chromatography (*2*). The soft biliary stones, when present, contained 78.6 ± 6.8 percent cholesterol, 16.3 ± 4.7 percent bile pigment and no bile acid, thus being very similar in composition to the material found in the guinea pig (*3*).

The bacterial cultures, from 26 of the patients, were negative; these patients had no significant inflammatory lesions, and neither ornithocholanic acids nor soft stones (with one exception) were found (Table 1). *Klebsiella pneumoniae* was found in cultures from pulmonary tissue from ten patients which had suffered from pneumonia; cultures from the blood of eight of these patients were also positive for *K. pneumoniae.* Small soft gall stones and ornithocholanic acids were found in all ten patients which had pneumonia, and also in two patients which had pyelonephritis due to *K. pneumoniae.* The incidence of Klebsiella infections in our patients is higher than reported elsewhere, but has been confirmed by type-specific antisera in doubtful cases. No gall stones or ornithocholanic acids were found in two out of three patients which had positive post-mortem blood cultures but showed no signs of disease due to this organism. Except for two patients which had pneumonia due to *Escherichia coli,* the other organisms recovered from tissue and blood were unassociated with soft biliary stones or ornithocholanic acids (Table 1). It is of interest that Bacmeister in 1908 produced biliary precipitates upon addition of bile to the culture media of gram-negative bacteria (*4*).

The average relative amount of ornitho- as compared to tauro- and glycocholanic acids in human bile at autopsy is indicated in Fig. 1. However, Wiggins and Wootton (*5*) and Sjövall (*6*) have reported that human bile contains more glycine than taurine conjugates. Although L-ornithine, unlike taurine and glycine, is a diamino acid, the correlated results of acidometric and gravimetric determinations of isolated ornithocholanic acids suggest that the ornithine is linked by a single amino group to the cholanic acids. Furthermore, a positive ninhydrin reaction is given by ornithocholanic acids from the bile of guinea pig, rat or man, and from the liver incubated in vitro.

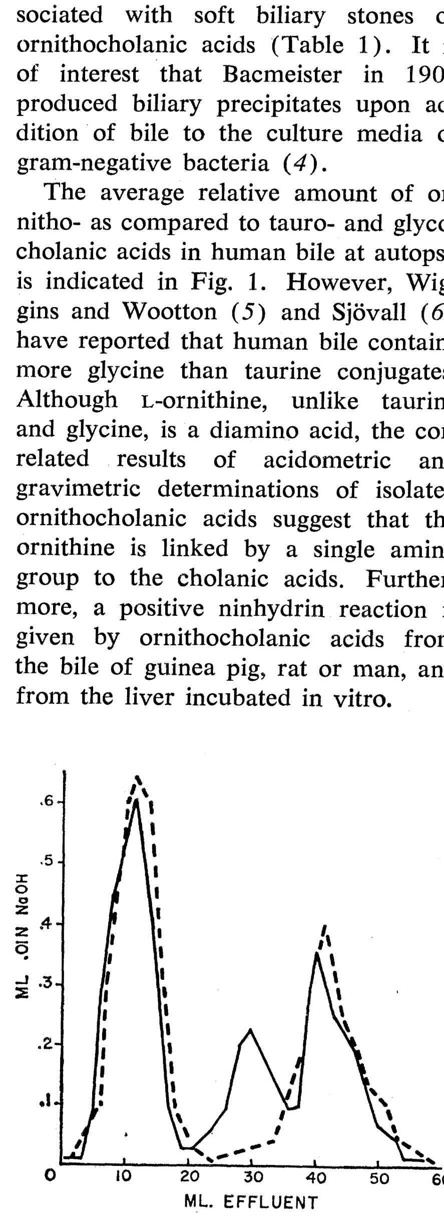

Fig. 1. When conjugated bile acids from patients with klebsiella infections are analysed by column chromatography, ornithocholanic acids appear between bile acids conjugated with taurine (left) and with glycine (right). The dotted line indicates the presence of only taurine and glycine conjugates in normal human bile.

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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25 July 1963

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