In pursuing this aspect of the stone problem further, it would be of interest to study the surface tension values along with the degree of colloid activity and to determine whether some common factor influences both of these. In the past, surface tension determinations have been difficult to perform, requiring considerable time, intricate instruments, and the solution of complicated formulas. The simple urotensiometer designed by Revici (*5*) obviates these difficulties and makes it possible to perform accurate determinations on all urine specimens as a routine procedure. Preliminary studies have indicated that urine surface tension determinations may increase our knowledge of the mechanisms involved in stone formation and may be helpful in the management of renal lithiasis (*7*).

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Production of Folinic Acid from Folic Acid by *Lactobacillus casei*[1]

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It has been reported (*1*) that naturally occurring folinic acid (citrovorum factor) appeared, on the basis of microbiological assay, to be some fifteen times as effective as folic acid in competitively reducing the toxicity of x-methyl folic (*2*) acid for *Lactobacillus casei* in a defined synthetic medium. Accumulated evidence (i.e., *3–6*) appears to place folinic acid as at least one metabolically active form of folic acid and makes it highly probable that dietary folic acid is converted to folinic acid, and that the latter active principle is the naturally occurring factor. Further support for this belief is found in noting that the methods employed in the isolaton of folic acid from natural sources are sufficient to convert folinic acid to folic acid (*7*).

In this laboratory enhanced folinic activity has been observed in the livers of normal, healthy rats growing on an adequate diet supplemented with added folic acid. In a comparative fashion, it was thought that investigation of possible biosynthesis in microorganisms would be profitable.

During experiments designed to ascertain the possible metabolic function(s) of folinic acid, the biosynthesis of this factor by each of several microorganisms was observed. *L. casei* was selected as the organism for the purpose of reporting the production of folinic acid by a microorganism growing in a defined synthetic medium.

TABLE 1

FOLINIC ACID CONTENT OF *Lactobacillus casei* MEDIA

Folic acid (μg/50 ml)	Folinic acid* (μg/50 ml)	
	Uninoculated medium	Inoculated medium
10	00	.1
30	00	1.8
100	00	23.0
300	00	28.0
1000	00	50.0
3000	00	90.0

* Amounts based upon folinic acid SF (synthetic folinic acid) as the standard.

An enriched medium, essentially that of Rogers and Shive (*8*), but modified to contain purines and varying amounts of folic acid, was prepared in double strength and diluted before sterilization with an equal volume of phosphate buffer at pH 7. A small portion of each test (10 ml) was removed from uninoculated blanks as medium controls. The remainder (40 ml) in each case was heavily seeded with 1 ml of a highly turbid 10-ml saline suspension of saline washed cells of an actively growing 24-hr culture of *L. casei*, ATCC No. 7469, carried routinely on glucose-yeast-agar in this laboratory.

After 24 hr incubation (static culture) at 37° C, the cells were removed by centrifugation, the medium was neutralized with sodium carbonate, and the relative folinic acid content in each medium, inoculated and uninoculated, was estimated microbiologically, using *Leuconostoc citrovorum*, ATCC 8081, as the test organism and synthetic folinic acid-SF[2] as the standard (Table 1). The assay medium was essentially that described by Snell *et al.* (*9*) but was modified to contain asparagine, folic acid, pyridoxine, and inositol.

All assays were incubated 16–18 hr at 37° C. Graded growth responses were measured turbidimetrically with a Klett-Summerson photoelectric colorimeter, using light filter No. 54.

At the conclusion of the incubation period it was observed that the pH of the medium containing *L. casei* was moderately acid in spite of the presence of the added phosphate buffer. Since folinic acid activity is destroyed by mild acid hydrolysis (*10*), the amount of this factor remaining in the medium (Table 1) at the conclusion of the incubation period will not necessarily present a true picture of the actual conversion of folic acid to folinic acid by this microorganism. However, these data will serve to illustrate that such a transformation is accomplished.

Samples of medium containing folinic acid activity were compared bioautographically with synthetic folinic acid, employing paper chromatograms developed

[1] Studies on possible nutritional significance of folinic acid, of which this paper is a part, are supported in part by the Williams-Waterman Fund of the Research Corporation.

[2] Supplied through the courtesy of Eli Lilly and Company, Research Laboratories, Indianapolis, Indiana.

with 2,6-lutidene. Two areas of growth were observed. One area compared favorably with synthetic material while the other area indicated a faster moving active principle.

No effort was made in this experiment to obtain maximum release of folinic acid either from the cells or from whatever bound form in which the factor may exist.

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The Life History of *Echinoparyphium flexum* (Linton 1892) (Dietz 1910) (Trematoda: Echinostomidae)

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Linton (*1*) described the trematode *Distomum flexum* found in the intestine of the black scoter, *Odemia americana,* from Yellowstone National Park. Dietz (*2*) incorporated the species in the genus *Echinoparyphium.* McCoy (*3*) obtained the adult experimentally by feeding chicks metacercariae from the snail, *Planorbis?* (= *Helisoma*) *trivolvis,* collected from Romana Lake, Missouri. Although his attempts to hatch the trematode egg were unsuccessful, he was able to obtain cercariae in 9 weeks by placing eggs in a small aquarium wth laboratory-reared *Physa integra.* Najarian (*4*) found the metacercaria in the kidneys of several species of frogs in the vicinity of Ann Arbor, Michigan.

The life cycle of the worm has been experimentally established in this laboratory, and all the stages have been studied. The chick was used as the experimental definitive host. The natural definitive host was found to be the blue-winged teal, *Anas discors.* The small intestine of two of the eight ducks shot in a woodspool area six miles west of Ann Arbor, Michigan, contained the adults of *E. flexum.*

The natural snail host in the area studied was *Lymnaea palustris.* Of the 3755 specimens collected and individually isolated, 83, or 2.2%, showed infection with the cercaria. The percentage of infected snails was small and ranged from 1.6 to 2.4 from April through October 1952. A single infected snail sheds 900–1300 cercariae within a 24-hr period. The bulk of shedding takes place between 1:00 P.M. and 4:00 P.M.

The metacercaria was found in nature both in the kidneys of frogs and tadpoles and in the kidney and heart of *Lymnaea palustris.* The cysts were found in the following species of frogs: 108 *Rana sylvatica,* 75% infected; 9 *Hyla crucifer,* 88% infected; 8 *Pseudacris migrita triseriata,* 25% infected; 7 *Rana pipiens,* 42% infected; 41 *Rana clamitans,* 14% infected.

Young adults of *R. pipiens* and *R. sylvatica,* reared in the laboratory from the egg stage, could not be infected by exposure to the cercariae. Tadpoles of the same species were easily infected. The metacercaria found in the kidneys of frogs in nature is apparently the result of the cercaria entering the tadpole and remaining in the kidney until metamorphosis is completed.

Experimentally, the cercaria encysts in the snails *Lymnaea palustris, Gyraulus parvus,* and *Physa gyrina.* In all cases the cysts are infective within 24 hr.

The feeding of the infective metacercariae to chicks was shown to give only a 1.1–1.6 yield of adult worms of *E. flexum.*

The eggs leave the uterus of the worm in an uncleaved condition. In the laboratory they were incubated in aerated tap water at room temperature from May through August 1952 and were shown to hatch in 10–14 days. The ciliated epidermal plates of the miracidium were studied by the silver nitrate technique and were found to have the formula 6–6–4–2 =18 plates. The two plates of the fourth tier are lateral and not dorso-ventral as in the genus *Echinostoma.* This feature is apparently characteristic for the genus *Echinoparyphium.*

The miracidium penetrates young specimens of *L. palustris* and within 7 hr is found within the heart of the snail. There, within 24 hr, the miracidium transforms into a sporocyst stage. Johnson (*5*) believed that the miracidium of *E. revolutum* metamorphoses directly into a redia. This study supports the results of Mathias (*6*), Rasin (*7*), and Churchill (*8*), all of whom observed sporocysts in their echinostome studies.

Mother rediae developed from the sporocysts and were first seen in the snail heart at 9 days. At 10 days they were found in the lumen of the heart and were extremely variable in shape. They were shown to be the migratory stage of the mother rediae. In no cases were daughter rediae observed in the heart of the snail.

The mother rediae migrate, apparently, via the snail's circulatory system. They were found at 10–12 days in the digestive gland and ovotestis, where they produce large numbers of daughter rediae.

The daughter rediae are avid eaters, and in both natural and experimental infections the gut was found to be filled with orange-colored material of the snail's

[1] Contribution from the Department of Zoology, University of Michigan, under the direction of Dr. A. E. Woodhead.