and Foster(4) may well be due to the lecithin. Wynne and Foster employed vegetable lecithin, whereas in studies reported here animal lecithin was used exclusively.

Summary. Sodium oleate in a concentration of 90 γ of sodium oleate per ml of medium inhibits the growth of *Streptobacillus moniliformis*. This inhibitory effect was reversed by addition of adequate amounts of bovine serum. Animal lecithin in concentrations of 100 and 200 γ per ml of medium failed to neutralize the inhibitory effect of sodium oleate.

Received February 10, 1951. P.S.E.B.M., 1951, v77.

Effect of Derivatives of L-Cysteine on the Growth of *Leuconostoc mesenteroides* P-60.* (18658)

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Sulfur containing substances play an important role in cellular metabolism. Some microorganisms require organic sulfur compounds for growth while others can utilize inorganic forms of sulfur(1-4). In a nutritional study of *Escherichia coli,* Bargoni(5) reported that alliin (S-allyl-L-cysteine sulfoxide), methionine, and cystine promoted growth of this organism. Work in this laboratory has shown that alliin also stimulates the growth of *Leuconostoc mesenteroides* but to a lesser degree than L-cystine. Since L-cystine is an essential amino acid for the growth of *Leuconostoc mesenteroides*(6,7), the stimulation observed for alliin may be exerted indirectly by participating in a biological synthesis of L-cystine. Because of the close relationship of alliin, L-cysteine and L-cystine and the possible participation of L-cysteine derivatives in the biological synthesis of L-cystine, it was of interest to determine the effect of various L-cysteine derivatives on the growth of *Leuconostoc mesenteroides.*

Experimental. Preparation of derivatives of L-cysteine. S-methyl-L-cysteine, S-ethyl-L-cysteine, S-allyl-L-cysteine, S-propyl-L-cysteine, S-ethyl-L-cysteine sulfoxide, S-propyl-L-cysteine sulfoxide, S-allyl-L-cysteine sulfoxide (alliin) and L-cystine disulfoxide were prepared by previously described methods of synthesis(8-11). All compounds were recrystallized from the appropriate solvent until acceptable nitrogen values and melting points were obtained. The properties of these compounds are summarized in Table I.

Method of assay. Leuconostoc mesenteroides P-60 was used as the test organism throughout the experiment. Riesen *et al.*(7) found that this organism responded to cystine in a consistent and reproducible manner. The derivatives of L-cysteine were assayed for

* Journal Article No. 1213 from the Mich. Agri. Exp. Station, East Lansing. This research was supported in part by the Horace H. Rackham Research Endowment of Michigan State College.

1. Fildes, P., and Richardson, G. M., *Brit. J. Exp. Path.,* 1937, v18, 292.

2. Meyers, F. P., and Porter, J. R., *J. Bact.,* 1945, v50, 323.

3. Wood, H. G., Geiger, C., and Werkman, C. H., *Iowa State Coll. J. Sci.,* 1940, v14, 367.

4. Hutchings, B. L., and Peterson, W. H., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 36.

5. Bargoni, N., *Boll. soc. ital. biol. sper.,* 1950, v26, 161; *Chem. Abst.,* 1950, v44, 10035.

6. Dunn, M. S., Shankman, S., Camien, M. N., Frankl, W., and Rockland, L. B., *J. Biol. Chem.,* 1944, v156, 703.

7. Riesen, W. H., Spengler, H. H., Robblee, A. R., Hanks, L. V., and Elvehjem, C. A., *J. Biol. Chem.,* 1947, v171, 731.

8. Clark, H. T., and Inouye, J. M., *J. Biol. Chem.,* 1931, v94, 541.

9. Stoll, A., and Seebeck, E., *Helv. Chim. Acta,* 1948, v31, 189.

10. Stoll, A., and Seebeck, E., *Helv. Chim. Acta,* 1949, v32, 866.

11. Toennies, G., and Lavine, F., Jr., *J. Biol. Chem.,* 1936, v113, 571.

TABLE I. Properties of L-Cysteine Derivatives.

Compound	Formula	m.p. °C (decomp.)	% nitrogen Found	% nitrogen Calc'd
S-Methyl-L-cysteine	$CH_3SCH_2CH(NH_2)COOH$	240-243	10.6	10.4
S-Ethyl- ''	$C_2H_5SCH_2CH(NH_2)COOH$	234-236	9.4	9.4
S-Propyl- ''	$C_3H_7SCH_2CH(NH_2)COOH$	228-233	8.4	8.6
S-Allyl- ''	$C_3H_5SCH_2CH(NH_2)COOH$	214-216	8.7	8.7
S-Ethyl-L-cysteine sulfoxide	$C_2H_7SOCH_2CH(NH_2)COOH$	156-158	8.4	8.5
S-Propyl- '' ''	$C_3H_7SOCH_2CH(NH_2)COOH$	151-154	7.8	7.8
S-Allyl- '' ''	$C_3H_5SOCH_2CH(NH_2)COOH$	163-165	8.1	7.9
L-Cystine disulfoxide	$SOCH_2CH(NH_2)COOH$ | $SOCH_2CH(NH_2)COOH$	180-183	10.4	10.3
L-Cystine (control)	$SCH_2CH(NH_2)COOH$ | $SCH_2CH(NH_2)COOH$	254-259	11.8	11.7

TABLE II. Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* P-60.

Compound	ml of 0.05 N NaOH; μg of compound per tube: 0	5	10	15	20
S-Methyl-L-cysteine	1.02	1.02	0.98	1.01	1.03
S-Ethyl- ''	0.99	1.02	0.96	1.04	1.04
S-Propyl- ''	1.10	1.06	1.14	1.12	1.08
S-Allyl- ''	1.07	1.05	1.13	1.08	1.10
S-Ethyl-L-cysteine sulfoxide	1.05	1.05	1.12	1.08	1.14
S-Propyl- '' ''	1.12	1.41	1.42	1.65	1.98
S-Allyl- '' ''	1.06	2.15	3.05	4.12	4.84
L-Cystine disulfoxide	1.02	2.10	2.93	3.42	4.01
L-Cystine (control)	1.05	4.63	7.46	8.13	8.21

growth response by substituting them for L-cystine using the peroxide-treated peptone basal medium as described by Riesen *et al.*(7). The hydrogen peroxide-treated peptone was prepared according to the method described by Lyman *et al.*(12). Riesen *et al.*(7) found that from 44 to 48% of the cystine activity for *Leuconostoc mesenteroides* was lost by autoclaving the complete media in the usual manner. In view of this, the method employed successfully by Camien and Dunn(13) was used in assaying L-cystine and the L-cysteine derivatives. This method differs from the usual microbiological procedure in that sterile glucose solution is added aseptically to each tube of previously sterilized media containing all of the essential ingredients except glucose.

Results and discussion. From Table II, it is noted that only S-allyl-L-cysteine sulfoxide (alliin) and L-cystine disulfoxide stimulated the growth of *Leuconostoc mesenteroides* to any marked degree. Substitution of the sulfur hydrogen of L-cysteine by methyl, ethyl, allyl and propyl groups did not produce any observable response. A very slight but consistent growth response was noted in the case of S-propyl-L-cysteine sulfoxide. Since S-propyl-L-cysteine was found to have no activity, it would appear that the presence of the sulfoxide group in S-propyl-L-cysteine sulfoxide was responsible for this activity. It is interesting to note that S-ethyl-L-cysteine sulfoxide and S-propyl-L-cysteine did not promote growth of this organism while S-allyl-L-cysteine sulfoxide stimulated growth. This indicates that the activity of the latter compound may be due to both the presence of the sulfoxide group and the unsaturation in the allyl radical.

The possibility always exists that the activity of these various derivatives for *Leuconostoc mesenteroides* may have been modified by the use of the peroxide-treated peptone

12. Lyman, C. M., Moseley, Olive, Wood, Suzanne, and Hale, Fred, *Arch. Biochem.*, 1946, v10, 427.

13. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1950, v183, 561.

basal medium. Camien and Dunn(14) have found that the addition of oxidized casein hydrolysate to an otherwise synthetic basal medium, markedly suppressed the response of *Lactobacillus arabinosus* to D-methionine and DL-methionine sulfoxide.

Summary. 1. Alliin and L-cystine disulfoxide stimulated the growth of *Leuconostoc mesenteroides* but to a lesser degree than L-cystine. 2. Substitution of the sulfur hydrogen of L-cysteine by alkyl and alkene radicals did not produce any observable response. The sulfoxide of S-ethyl-L-cysteine was also inactive; however, S-propyl-L-cysteine sulfoxide was found to have a very slight activity. 3. The activity of alliin may be due to both the presence of the sulfoxide group and the unsaturation in the allyl radical.

14. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1950, v187, 365.

Received February 14, 1951. P.S.E.B.M., 1951, v77.

Incidence of Spontaneous Mammary Tumors in Mice with *Lithospermum*-Induced Diestrus.* (18659)

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Cranston, Kucera, and Bittner(1) reported that C_3H mice fed on a diet containing *Lithospermum* exhibited a decreased incidence of spontaneous mammary tumors: from the 58.1% norm to 2.8%. Setting out with the same endocrinological premises as the above workers, a program, begun by us in 1946, was likewise aimed at investigating the effect of *Lithospermum*-induced diestrus on the development of spontaneous mammary tumors in C_3H mice; and this program was in its concluding phases when the above-cited paper appeared. In view of potentialities in the pharmacological approach to tumor prophylaxis, we feel that our methods and results should be recorded for comparison with those of the Cranston group.

The rationale underlying this type of experimentation has been presented adequately in the paper by Cranston, *et al.* It suffices to say here that through the induction of sustained diestrus in C_3H mice by the administration of *Lithospermum* in the diet, it was hoped to impair the mammary tissue substrate on whose normal functioning the development of mammary carcinoma in C_3H mice is known to depend. We have reported earlier that the mammary tissue of *Lithospermum*-treated C_3H mice is rudimentary (2); and the endocrinic mechanism of *Lithospermum* action has recently been discussed by Drasher(3).

Procedure and results. About 400 virgin C_3H female mice† were divided into two groups: one control, the other experimental. All were 6 months old at the beginning of the experiment. Stainless steel cages, 11″ x 11″ x 6″, were used to house the mice; between 5 and 10 mice were maintained together in each cage. Coarse-wire hampers were kept filled with dietary pellets for *ad lib.* feeding. The control group was supplied with pellets of regular Rockland Mouse Diet; the experimental group with pellets of Rockland Diet containing *Lithospermum*. Methods for preparing such pellets have been described previously(2).

* The work reported in this paper was supported in part by grants from the U. S. Public Health Service on recommendation of the National Advisory Cancer Council. Grateful acknowledgement is made to Dr. F. S. Cooper for advice as to presentation of the data in graphic form.

1. Cranston, E. M., Kucera, G. R., and Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 779.

2. Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 405.

3. Drasher, M. L., *Endocrinology*, 1950, v47, 399.

† Purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.