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.

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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.

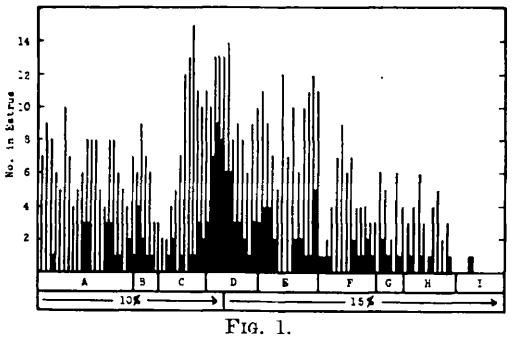

FIG. 1.

Number of mice in estrus for 97 samplings taken rotationally throughout both the control and *Lithospermum*-fed groups from the beginning of the experiment when the mice were six months old to the end of the experiment 18 months later. Each sampling comprised 25 mice. The samplings were not taken with absolute regularity throughout this period, rather Block A represents samplings taken during the 6-8 month period; B, 8-10; C, 10-12; D, 12-14; E, 14-16; F, 16-18; G, 18-20; H, 20-22; I, 22-24. Narrow lines, control mice; heavy lines, *Lithospermum*-fed mice. During the first two-fifths of the experiment the diet contained 10% *Lithospermum*; for the remainder of the experiment, 15% (see text for explanation).

At frequent intervals throughout the experimental period both the control and *Lithospermum*-fed groups were assayed for the state of their estrous condition, using standard lavage and smear methods(2). In order to obviate the simultaneous testing of all the nearly 400 mice, aliquots of 25 mice each were assayed throughout the experimental period. Such 25-mouse samplings were taken rotationally through both groups. The number of mice in each 25-mouse sampling showing estrus at the points of assay are presented in Fig. 1, with the data extending from the beginning of the experiment, when the mice were 6 months old and not yet in their normal tumor developing period, to the experiment's conclusion, about a year and a half later.

These data clearly indicate that while estrus was not wholly abolished in the *Lithospermum*-fed group, a very significant decrease in estrus frequency was sustained throughout the experimental period. Complete abolishment of estrus could easily have been achieved by merely increasing the *Lithospermum* content of the diet, but this was carefully avoided because, as known from earlier work(2), a high level of *Lithospermum* (say, 30%) would have resulted in an immediate loss in body weight. The level selected was that just great enough to induce considerable diestrus without on the other hand causing appreciable loss in body weight. This dosage level was determined to be between 10% and 15% by weight of powdered whole-plant *Lithospermum*, blended into Rockland Diet. Accordingly, the experimental group of mice was fed on a 10% *Lithospermum* diet for seven months; and then, as a slight refractoriness to the drug appeared to develop (Fig. 1), on a 15% diet for the remainder of the experiment.

That this dosage was appropriate in so far as growth was concerned is indicated from the data presented in Fig. 2. Here the body weight curves from both groups of mice, extending throughout the entire experimental period, indicate very little weight divergence from the norm on the part of the *Lithospermum*-fed group.

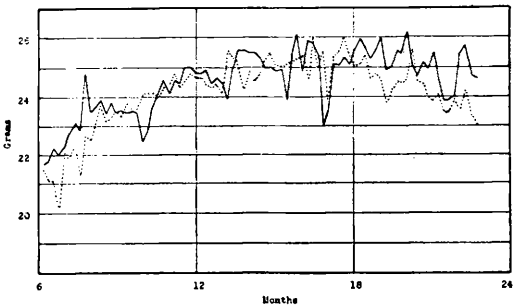

FIG. 2.

Mean whole body weight for total number of mice of both control (solid lines) and *Lithospermum*-fed (dotted line) groups, from beginning of the experiment when the mice were 6 months old to the end of the experiment 18 months later.

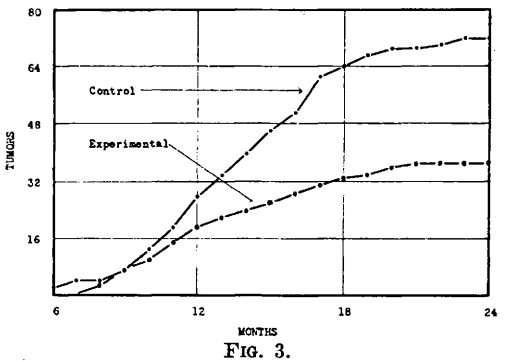

FIG. 3.

Cumulative number of tumors as of the beginning of each month throughout the experiment.

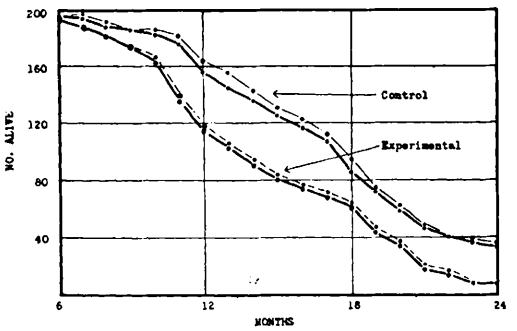

FIG. 4.

Total number of mice alive at the beginning of each month throughout the experiment. Points in heavy lines derived by subtracting deaths from all causes; points in lighter lines derived by subtracting deaths from causes other than tumors.

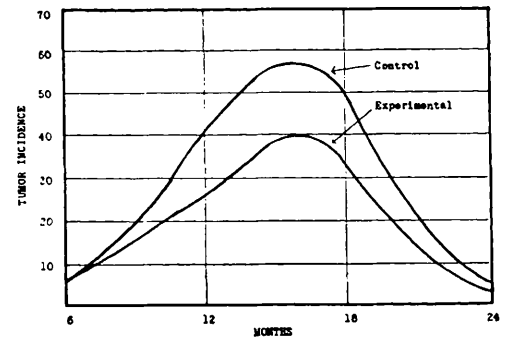

FIG. 5.

Tumor incidence, plotted as per thousand mice per month, throughout the experiment. The points from which these curves were drawn were derived from the experimental data as follows: Best fitting smooth curves were drawn by inspection through the points in Fig. 3 and 4. Points were read from these smooth curves and computed as $\Delta T/S$, where ΔT is the number of new tumors developing during each monthly period, and S is the number of mice alive at the beginning of each month. The resulting figures were plotted and the closest smooth-line curve fitted and drawn.

Fig. 3 depicts the cumulative incidence of mammary tumors from the beginning to the end of the experiment. At the end of the experiment about twice as many tumors had developed in the controls as in the *Lithospermum*-fed group: 36% in the former; 19% in the latter.

Survival curves for both control and experimental groups are presented in Fig. 4. Two sets of curves are given for each group: one showing the total number of deaths from all causes, the other showing deaths due to causes other than tumors. From a comparison of the two sets of curves it is seen that deaths among the *Lithospermum*-fed mice during the greater part of the experimental period occurred generally two to four months earlier than in the controls. Whether this was due to unidentified harmful effects of the *Lithospermum*, or to decreased resistance to infection, or to an acceleration of aging processes, cannot be said. It does mean, however, that the total number of *Lithospermum*-fed mice alive was always somewhat less than in the controls, and that the number subject to tumor development at any given time was consequently greater in the latter group. When the data are plotted as in Fig. 5—where differential monthly survival is taken into account—and the control and experimental curve areas measured and compared, the tumor incidence ratio for the two groups becomes 3 to 2.

Discussion. Two factors make it difficult to interpret the data presented here as well as those published by the Cranston group. First, there is the possible occurrence of inanition effects of the kind described by Tannenbaum(4) and by Huseby, Ball, and Visscher(5). By reducing the caloric intake these workers drastically lowered the incidence of spontaneous mouse tumors. Cranston, *et al.* showed that mice fed on a high *Lithospermum* diet secured less total nutrient than mice on normal diet; their *Lithospermum*-fed mice fell as much as 28% behind the gain in weight shown by the controls during the tumor developing period(1). In view of the work of Tannenbaum and of Huseby, *et al.*, this general growth retardation could account for the drop in tumor incidence in *Lithospermum*-fed mice reported by Cranston, *et al.*, a possibility which they themselves have pointed out. In our experiment, on the other hand, where the *Lithospermum* content of the diet was kept minimally low, and where the weight curves do not show much divergence (Fig. 2), it seems reasonable to assume that both experimental and control groups were nourished

4. Tannenbaum, A., *Approaches to Tumor Chemotherapy*, AAAS Publ., 1947, 96.

5. Huseby, R. A., Ball, Z. B., and Visscher, M. B., *Cancer Res.*, 1945, v5, 40.

at substantially similar caloric levels, and that the factor of inanition was therefore not a major one in lessening the tumor incidence in the *Lithospermum*-fed mice.

A peculiarity in our data is the fact that only 38% of the controls developed tumors, whereas the strain of C_3H mice used by us has been reported as having as high as a 97% incidence of spontaneous mammary tumors(6). Our low control incidence is probably explicable on the grounds that keeping 5-10 mice in a cage constituted crowding and competitive feeding, factors which have been shown repeatedly to lower the incidence of tumors in this strain(7). On the other hand, these factors would not seem to have vitiated the experiment, for crowding was identical in both groups. The relative rather than the absolute tumor incidence in the control and *Lithospermum*-fed groups would seem to be the figures of significance.

As to the comparative death rates in the two groups, Cranston, *et al.* unfortunately do not give survival curves for their control and *Lithospermum*-fed mice, and hence their statement that the average non-tumor death age was 483 days for both groups cannot be fully appraised. Our own finding of an earlier susceptibility of non-tumor death in the *Lithospermum*-fed mice than in the controls, as plotted in Fig. 4, is a disturbing one and suggests the need for caution in interpreting the fact that the *Lithospermum*-fed group eventually developed only about half as many tumors as the controls. Whether this decrease in tumor incidence is directly related to the *Lithospermum*-fed mice being in a state of more-or-less sustained diestrus, we are not, on the basis of the present data, prepared to say.

As pointed out by the Cranston group, experiments of this sort could lead to more decisive results if pure extracts of the *Lithospermum* anti-estrous factor were available, thus minimizing possible side reactions of unknown identity.

Summary. About 400 6-month-old virgin female C_3H mice were divided into two groups: one was maintained on a normal diet; the other on an anti-estrous diet of 10-15% *Lithospermum*. Eighteen months later the latter group had developed half as many spontaneous mammary tumors as the former; however, if the differential mortality observed throughout the experiment is taken into statistical account, the tumor incidence ratio for the total period is 3 for the control, 2 for the experimental group. This reduction in tumor incidence was possibly connected with the *Lithospermum*-diestrus, but inanition and differential survival factors remain to be more fully evaluated.

6. Shimkin, M. B., *Mammary Tumors in Mice*, AAAS Publ., 1945, 85.

7. Andervont, H. B., *J. Nat. Cancer Inst.*, 1944, v4, 597.

Received March 12, 1951. P.S.E.B.M., 1951, v77.

Neuropathology of Teschen Disease (Virus Encephalomyelitis of Swine).* (18660)

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(Introduced by Francis G. Blake)

From the 98th General Hospital, European Command, U. S. Army in Germany. Work done under auspices of Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, and the Section of Preventive Medicine, Yale University School of Medicine.

Teschen disease of swine is a virus encephalomyelitis which although its occurrence

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† Now with the Section of Preventive Medicine, Yale University School of Medicine.