fect, this action of morphine upon the gut must be mediated through the central nervous system. Similar actions have also been observed in rats and guinea pigs. 2. Pharmacological inhibition or blockade of this morphine action upon the gut could not be demonstrated with a wide variety of autonomic blocking agents or central nervous system depressants. Concurrent, surgical interruption of the sympathetic and parasympathetic outflows from the central nervous system to the gastrointestinal tract also failed to block the activity. 3. The findings suggest that morphine may be causing inhibition of gut propulsive action by initiating a neurohumoral discharge from the central nervous system into all the circulating blood of the body.

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Influence of Tumor Fractions on Incidence of Spontaneous Mammary Tumors in C3H/HeJ Mice.* (28027)

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Relatively few experiments have been reported on attempts to decrease the incidence of spontaneous tumors in mice, in part, no doubt, because of the considerable duration of such experiments. Isojima *et al.*(1) reported that injection of a homogenate of spontaneous tumors from C3H mice lowered the incidence of spontaneous tumors in such mice. Hirsch and Iversen(2), on the other hand, found that pretreatment with C3H tumor tissue had no significant effect in lowering tumor incidence and, indeed, significantly reduced age of tumor onset and survival time. These experiments were attempts to immunize. Our previous partial success in inducing resistance to transplantable tumors by use of an alcohol-soluble, protein-free tumor fraction(3,4) which seems unlikely to act as an antigen, led us to conduct a pilot experiment to determine the effects of a similar fraction from spontaneous tumors on the incidence of spontaneous tumor.

Materials and methods. Virgin female C3H/HeJ mice, originally obtained from the Roscoe B. Jackson Memorial Laboratory, were used as the experimental animals and as sources of spontaneous mammary tumors. The dbrB adenocarcinoma from DBA/1 mice

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was the source of one tumor fraction. Pooled tumors (spontaneous or dbrB) were lyophilized and extracted with absolute ethanol 3 times for 15 minutes each in the ratio of 85 mg of tissue to 10 ml of ethanol(3). After centrifugation for 15 minutes at 2400 rpm, the alcohol was removed from the supernate by distillation to approximately ¼ volume under reduced pressure. The residual extract was added to Tyrode's solution in the ratio of 8 ml of extract to 5 ml of Tyrode's solution, followed by further reduction to 5 ml by vacuum distillation. Complete removal of alcohol was insured by adding sterile distilled water and again reducing the volume to 5 ml *in vacuo*. The dry weight was approximately 4.0 mg per ml, or each injected dose contained approximately 0.2 mg; different preparations gave some variation in yield. The fractions were free of protein (biuret) but gave a positive ninhydrin reaction. The fraction from the spontaneous tumor contained 1.94% nitrogen, due mostly to amino acids of which the following were identified by paper chromatography: alanine, proline, leucine, valine, taurine, glutamic acid, aspartic acid, histidine, arginine, and α-aminobutyric acid. No purines or pyrimidines were found after hydrolysis. The Molisch test was positive, but the carbohydrate concentration was only 0.25% as ribose and no carbohydrates were identified. Total phosphorus was 1.61%, with 0.42% as inorganic phosphorus. Cholesterol was present to the extent of 1.5%. Lactate, chloride, an unidentified anion, and sodium were detected.

Virgin C3H female mice 225 days old were divided into 5 groups of 20 mice each. Group I (control) received 6 intravenous injections of 0.05 ml of Tyrode's solution at 2-week intervals. Group II received 6 intravenous injections of 0.05 ml of the fraction of dbrB adenocarcinoma, and Group III 6 intravenous injections of the fraction of spontaneous C3H mammary tumor. Group IV received 6 injections of spontaneous C3H tumor fraction intraperitoneally. Group V received a single intravenous injection containing 10 times the quantity of the individual dose of spontaneous tumor extract. Observations were recorded weekly until all mice, both tumorous and non-tumorous, were dead. The last observation was made on a mouse (in Group II) at the age of 813 days. Preliminary experiments had shown that the fractions at the levels administered had no observable toxic effects (including weight effects) and therefore weights were not recorded throughout the experiment.

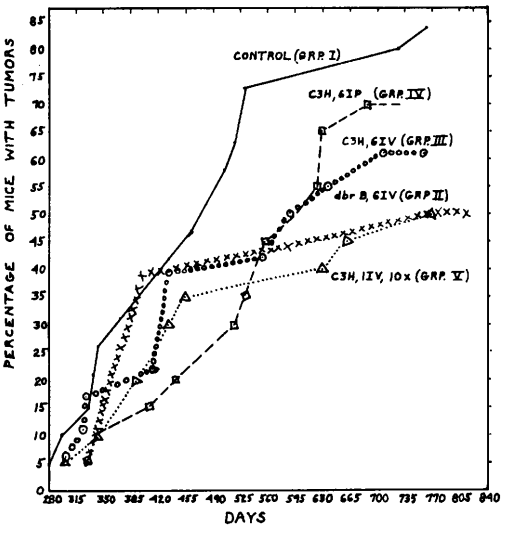

FIG. 1. Influence of injection of tumor fractions on incidence of spontaneous tumors in female C3H/HeJ mice.

Results. Results on tumor incidence (number of mice which developed tumors) by day 813 (at which time all mice were dead) and on mean age at tumor appearance, mean survival time after tumor onset, and mean total survival time of tumorous animals, non-tumorous animals and all animals are given in Table I. A X^2 test on a comparison between the proportion of tumors in the control group (I) *vs* the combined experimental groups (II, III, IV and V) gives $X^2 = 4.52$, $.01 < P < .05$. A standard error of the proportions test on these data gives $t = 2.2$, $.02 < P < .05$. No significant differences were found in mean tumor age or mean survival time, although mean survival time after tumor onset approaches significance ($.05 < P < .1$).

Fig. 1 shows the percentage of mice with tumors as a function of time. Extensions of the curves beyond the last point represent the age of the last surviving mouse in each

TABLE I. Effects of Tumor Fractions on Incidence of Spontaneous Tumors.

Group	I(control)	II(6 iv.inj) dbrB extract	III(6 iv.inj) C3H extract	IV(6 ip.inj) C3H extract	V(1 iv.10xinj) C3H extract	Significance
Total No. mice* (tumor and nontumor)	19	18	18	20	20	
No. mice with tumors at day 813	16	9	11	14	10	$X^2 = 4.5$
% mice with tumors at day 813	84	50	61	70	50	$.01<P<.05$
Mean age at tumor appearance†	454 ± 32	433 ± 48	466 ± 39	524 ± 28	479 ± 46	$F<1$ N.S.
Mean survival time after tumor onset†	57 ± 6	69 ± 12	81 ± 7	53 ± 3	63 ± 10	$F = 2.1$ $.05<P<.1$
Mean survival time, tumorous animals†	511 ± 29	502 ± 46	546 ± 38	570 ± 27	542 ± 44	$F<1$ N.S.
Mean survival time, nontumorous animals†	547 ± 64	619 ± 62	646 ± 15	635 ± 25	540 ± 47	$F<1$ N.S.
Mean survival time, all animals†	517 ± 28	561 ± 43	585 ± 33	594 ± 22	541 ± 33	$F<1$ N.S.

* All groups began with 20 mice. Eliminated from tabulation are: Group I, 1 mouse died on 242nd day without tumor and before treatment completed in experimental groups; Groups II and III, 2 mice died without tumors during treatment period.

† Days ± S.E.

group. Percentage rather than absolute numbers is used because of the different numbers of mice in the various groups. The tumor fractions had no significant effect on tumor incidence as compared with the control in the early part of the experiment. From the 505th day to the end of the experiment a comparison of the proportions of tumors in the control group with the combined experimental groups showed a significant lowering in the latter. However, these curves suggest that the intraperitoneal route may be more effective in lowering the early incidence of tumors. This possibility will be investigated.

Discussion. The results of Isojima *et al.* (1) and of Hirsch and Iversen(2) are not in agreement as to the effectiveness of C3H spontaneous tumor material in reducing spontaneous tumor incidence. In fact, Hirsch obtained earlier tumor appearance and a reduction in survival time of treated animals. Both investigators seem to have assumed an immunological mechanism and attempted to increase antigenic efficacy by the use of Freund's adjuvant. The disparity between Isojima's and Hirsch's results is reminiscent of our experience(5,6) that either increased resistance or susceptibility could be obtained for the transplantable dbrB mammary adenocarcinoma in DBA/1 mice, depending upon the manner of preparation of the "immunizing" material. Fractions similar to those of the current experiment were found to be effective when given subsequent to or simultaneously with the challenge dbrB tumor, whereas viable cell suspensions produced resistance only when administered 2 to 3 weeks in advance of the challenge tumor. These results, together with the non-protein nature of our fractions, cast doubt upon an immunologic mechanism, but further discussion of mechanism is best deferred until specific study of the possible antigenicity of the fractions has been made. Current work in our laboratory(7) suggests that additional fractionation procedures can further separate non-protein inhibiting fractions from enhancing fractions containing nucleoprotein in transplantable tumors.

Summary. One to 6 injections of alcohol-soluble, protein-free fractions from a transplantable (dbrB adenocarcinoma in DBA/1 mice) and a spontaneous tumor (from C3H mice) significantly lowered the incidence of spontaneous tumors in virgin female C3H mice, but did not cause significant differences in mean age at which tumors appeared, mean survival time after tumor onset, or mean total survival time.

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Technique for Temporary or Chronic Vascular Occlusion or Narrowing In Intact Animals.* (28028)

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A snare constructed from polyethylene tubing has been developed and successfully used during the past several years to produce both temporary and permanent coronary arterial occlusion in intact dogs. It may be useful in other situations where control of arterial or venous flow is desired.

Materials and methods. The snares are constructed from tubing purchased from Clay-Adams, Inc. A ¼-inch length of P.E. 280 tubing is slipped over the end of a length of P.E. 205. The resulting cuff is enlarged by a ¼-inch length of P.E. 330 tubing (Fig. 1, A). The end is heated over a cautery tip until the softened tubing molds itself into a flange which measures 6 to 7 mm in diameter. Three holes are melted through the flange with a hot wire or ordinary straight pin to a size which will admit P.E. 50 tubing (Fig. 1, B).

Approximately 1 cm of one end of a length of P.E. 50 tubing is melted to form a cup-shaped expansion. The opposite end is passed through one of the holes in the flange and pulled so that the expanded end is tight against the flange. Excess plastic is trimmed from the opposite rim of the flange. A length of No. 1 surgical silk is passed through the smaller tubing and anchored in place with a knot pulled into the expanded end (Fig. 1, B). The thread is cut to project several inches beyond the free end of the small tubing.

This device has been successfully used to produce both temporary and chronic occlusion of the left circumflex coronary artery of dogs. With the chest open, the artery is dissected free for a length of about 5 mm. The free end of the small tube along with the projecting thread is passed beneath the artery toward the cardiac apex and then through the center hole in the flange to form a loose snare about the artery. The pointed end of a hollow trocar is forced through the chest wall from inside to outside near the sternum between the sixth and seventh or seventh and eighth ribs while the skin is drawn toward the incision. The free end of the snare is passed into the hollow end of the trocar and both are pulled through the chest wall. Threads through the remaining holes in the flange are sutured to available tissue near the artery to prevent rotation of the snare. The outside tube is cut to extend about 2 inches beyond the skin allowing some slack within the chest. A source of heat is used to produce a slight flare on the end of the larger tubing.

An air seal is constructed from a pull-over type rubber serum bottle stopper with a plug

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