animals was found to be 62 percent that of the controls, suggesting that treatment with tentacle extract has a suppressive effect on splenic enlargement at the 21st day. No comparison of survival rates or study of the effect on the late development of leukemia was made, nor are separate spleen weights available.

To determine, if possible, the chemical nature of the active principle of kaunaoa extracts, tentacles were macerated with 30 percent ethanol. The mixture was agitated for 1 day under refrigeration. The cloudy suspension was then filtered and concentrated under reduced pressure at about 50°C. From this, a simple retentate (fraction A) was prepared as follows. Sixty milliliters of crude tentacle solution containing 182.8 mg of solids per milliliter (0.4 ml of solution per gram, wet weight, of tentacles) were dialyzed in a 1-inch (2.54-cm) dialysis bag with constant agitation for 72 hours at 2°C against 2 liters of water changed once. The final retentate volume was 114 ml. Solids were reduced to 8.6 mg/ml or 17.2 mg/ml of the original 60 ml basis.

A "digested" retentate (fraction B) was prepared exactly as was fraction A, with the additional step of subjecting 106 ml of the retentate to the effect of 50 mg of pronase for 1½ hours at 37°C at *p*H 8.1 (1*N* NaOH) and to a temperature of 100°C for 3 minutes, taking 8 minutes to reach 100°C. The resulting solution was reduced at 50°C under vacuum to 53 ml and redialyzed in a 1-inch dialysis tube against 2 liters of water for 45 hours at 2°C with one change of water. Eighty-four milliliters of retentate were suction-filtered with Celite and evaporated at less than 50°C and adjusted to a volume of 53 ml. Dry weight of solids was 7.3 mg/ml.

The results of bioassay of *Lanice conchilega* tentacle fractions A and B are shown in Table 1, and are statistically significant; $\chi^2 = 7.5$ and 7.3, respectively, with 1 degree of freedom.

The survival rates of mice treated with tentacle fractions suggest antitumor activity may be present in a nondialyzable component of crude tentacle extract. The persistence of the antitumor activity after pronase digestion reasonably excludes protein as the active agent.

In August 1969 a similar polychaetous worm with feeding tentacles, *Reteterebella queenslandia*, shown in Fig. 1, at bottom, was collected at Heron Island, on the Great Barrier Reef of Australia, to compare possible antitumor activity with that of *Lanice conchilega*. Preparation of body and tentacle extracts of *Reteterebella queenslandia* was done exactly as with *Lanice conchilega*, providing extracts that were comparable in final volume per wet weight of worm and tentacles.

The body extract of *Reteterebella queenslandia* appears to be somewhat toxic, causing occasional animal deaths during treatment. However, the favorable result of treatment with tentacle extract is shown in Table 1. This is statistically significant; $\chi^2 = 35.0$, with 1 degree of freedom.

Extracts of the tentacles of both *Lanice conchilega*, in Hawaii, and *Reteterebella queenslandia*, in Australia, thus act to protect mice against Erlich ascites cell tumor. Dialysis and enzyme digestion procedures suggest the activity in *Lanice conchilega* tentacles is, or is associated with, either a micelle-forming, or large molecular nonprotein component of the crude extract, based on its retention during dialysis. Although the growth of Erlich ascites cell tumor is fairly sensitive to many foreign materials, the protective effect of these relatively nontoxic sea annelid extracts, considered together with their reputed ancient use in human cancer therapy, is a striking coincidence, certainly worth further study.

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References and Notes

1. F. L. Tabrah and B. M. Eveleth, *Hawaii Med. J.* **25**, 223 (1966).
2. O. Hartman, *Occas. Pap. Bernice P. Bishop Mus.* **23**, 230 (1966).
3. We thank Mrs. Zealie Sherwood of Molokai, Hawaii, for her information about *Lanice conchilega*.
4. A. Furst, C. Klausner, W. Cutting, *Nature* **184**, 908 (1959).
5. This work was done in this department by Dr. Paul Yoder.
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Aspirin: Intestinal Damage in Rats

Abstract. *Lesions induced by aspirin in the small intestine of the rat were visualized after 4 hours by the intravenous administration of a 5 percent solution of pontamine sky blue, 6 BX dye. Dose-response curves in fasted and fed rats indicated that the fed rat was about eight times more susceptible to aspirin-induced intestinal damage than was the fasted rat, while the fasted rat was about 13 times more susceptible to aspirin-induced gastric damage than was the fed rat.*

Production of gastric mucosal damage by aspirin is well documented (*1*), but there have only been a few references to intestinal damage produced by this agent (*2*). In contrast, damage to the small intestine is a well-known side effect for certain other nonsteroid antiinflammatory agents such as indomethacin, phenylbutazone, and flufenamic acid (*3, 4*). A report that a vital aminoazo dye, pontamine sky blue 6 BX (Edward Gurr Ltd., London) was useful in assessing the damaging effect of aspirin on the gastric mucosa (*5*) suggested that this dye might be useful in the detection of intestinal lesions. We report the development of intestinal lesions after oral administration of high doses of aspirin.

Male Holtzman rats (125 to 150 g) were used. "Fasted" animals were deprived of food but allowed free access to water during the 24-hour period before and the 4-hour period after aspirin administration; "free-feeding" animals were permitted free access to both food and water up to the time of death. Aspirin was administered orally as a suspension in 1 percent methylcellulose solution over a wide dose range. Four hours after drug administration, the animals were killed with an intracardiac injection of pentobarbital. Ten minutes before death the animals were injected intravenously in the tail vein with 1 ml of a 5 percent solution of pontamine sky blue 6 BX dissolved in saline. After the animals were killed, the gastrointestinal tract was removed and opened; the gastrointestinal contents were removed by gently wiping with cotton swabs, and the mucosal surface was then examined. The presence of dark blue areas against a pale blue background in both stomach and intestine occurred when the protein-bound dye

Table 1. Comparison of the ED_{50} for aspirin-induced gastrointestinal lesions in fasted and fed rats. The ED_{50} was estimated graphically from dose response curve and is the dose at which 50 percent of the rats had gastric or small intestinal lesions.

Nutritional state	ED_{50} (mg/kg)	
	Stomach	Small intestine
Fed	300	175
Fasted	23	1600

leaked through the damaged mucosal surface. Histological examination of these intensely stained punctate foci confirmed that, by using the dye technique, it was possible to detect grossly lesions as small as 0.3 mm in diameter. In control rats ($N = 12$) no dye localization was seen in any portion of the stomach or intestine.

Gross intestinal lesions in the animals treated with aspirin were irregularly circular to oval with diameters ranging from less than 0.3 mm to 2 mm. Central depressions were visible in the large lesions. Intestinal lesions were extremely difficult to locate by means of visual examination without the use of the dye technique, because hemorrhage, which is characteristic of aspirin-induced gastric damage, is not a prominent component of aspirin-induced intestinal damage. The intestinal lesions typically occurred in the jejunum and ileum; the greatest incidence was found 60 to 85 cm distal to the pylorus. No lesions were found in the large intestine at any dose tested. Microscopically, the typical lesion was necrosis of the mucosa; both the epithelial and connective tissue components were necrotic (Fig. 1). Slight edema and hemorrhage were occasionally present, but cellular infiltration was notably absent. The necrosis frequently involved one-half to three-fourths the thickness of the mucosa, but rarely penetrated the full depth of the mucosa.

Fasted rats were more susceptible to aspirin-induced gastric lesions than fed rats (Table 1); however, the situation was reversed for lesions in the small intestine, in that fed rats were approximately eight to ten times more sensitive to the production of intestinal lesions than were fasted rats (Table 1, Fig. 2). This situation is similar to that seen with other nonsteroid antiinflammatory agents in which food deprivation markedly reduced the incidence of intestinal damage (*4*). A time response study of aspirin indicated that it was possible to produce a high incidence of gastric lesions in fasted rats in 15 minutes (64 mg/kg, orally) and a high incidence of intestinal damage in 120 minutes (1024 mg/kg, orally).

The antiinflammatory ED_{50} (effective dose; dose at which 50 percent of the rats had gastric or small intestinal lesions) for aspirin in the 4-hour carrageenin edema test in the fed rat was 85 mg/kg given orally (*6*). This is well below the ED_{50} for both gastric (300 mg/kg, orally) and intestinal (175 mg/kg, orally) damage in fed animals. This would indicate that gastrointestinal damage in fed rats would not accompany antiinflammatory doses in rats, but rather would occur in the range of toxic doses of aspirin.

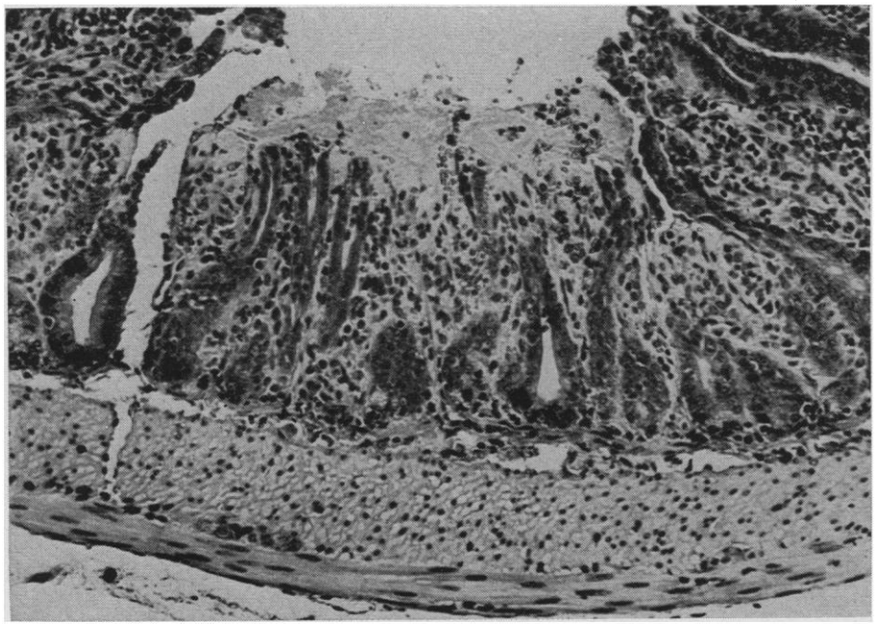

Fig. 1. Necrotic lesion (0.5 mm) found in the ileum of a male Holtzman rat 4 hours after the oral administration of 256 mg of aspirin per kilogram. Necrosis of the mucosa without cellular infiltration is typical of aspirin-induced intestinal lesions (× 300).

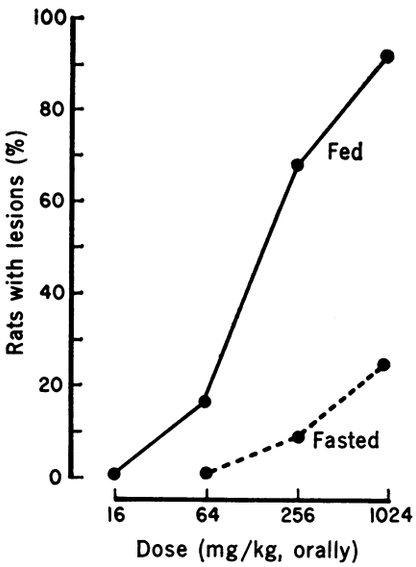

Fig. 2. Aspirin-induced lesions of the small intestine; twelve rats were used at each dose. Lesions, indicated by intense blue staining with pontamine sky blue dye, were found in the jejunum and ileum 60 to 85 cm distal to the pylorus.

The most recent theory for the mechanism of aspirin-induced gastric damage is that proposed by Davenport (*7*); aspirin alteration of gastric mucosal permeability permits rapid acid back-diffusion which damages mucosal capillaries and produces gastric hemorrhage. Since intestinal lesions can be produced by aspirin in an area that is alkaline rather than acid, some other mechanism would seem to be operating in the intestine.

The production of intestinal damage in rats by aspirin has not received wide attention, and the clinical significance of these data is not clear. The possibility exists that the occult bleeding occurring in some patients following aspirin administration may originate from the intestine as well as from the stomach. Relevant to this notion is the observation that aspirin administration with meals may not reduce fecal blood loss (*8*).

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References

1. J. B. Kirsner, *Ann. Intern. Med.* **47**, 666 (1957); H. L. Segal, *Amer. J. Med.* **29**, 780 (1960); A. Weiss, E. R. Pitman, E. C. Graham, *ibid.* **31**, 266 (1961).
2. J. E. Walter, R. M. Diener, A. E. Earl, *Toxicol. Appl. Pharmacol.* **17**, 314 (1970); L. A. Taylor and L. M. Crawford, *J. Amer. Vet. Med. Ass.* **152**, 617 (1968).
3. D. H. Kaump. *Ann. Phys. Med. Suppl.* **17**, 16 (1966); A. Somogyi and K. Kovacks, *J. Pharm. Pharmacol.* **21**, 122 (1969); T. H. Kent and R. M. Cardelli, *Amer. J. Pathol.* **54**, 237 (1969).
4. D. A. Brodie, P. G. Cook, B. G. Bauer, G. E. Dagle, *Toxicol. Appl. Pharmacol.*, in press.
5. K. Takagi and K. Kawashima, *Jap. J. Pharmacol.* **19**, 431 (1969).
6. C. G. Van Arman, unpublished observations.
7. H. W. Davenport, *N. Engl. J. Med.* **276**, 1307 (1967).
8. F. O. Stephens, E. J. Milverton, C. K. Hambly, E. K. Van der Ven, *Digestion* **1**, 267 (1968).

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Strain C3H-A^{vy}fB Mice: Ninety Percent Incidence of Mammary Tumors Transmitted by Either Parent

Abstract. *Mammary tumorigenesis in C3H-A^{vy}fB female mice is not due to milk-borne mammary tumor virus but to factors transmitted at conception. Prominent among these is the* A^{vy} *gene which may increase the virulence or transmissibility of a variant of mammary tumor virus vertically transmitted by either parent, or may increase the tumorigenic response of the mammary tissue. These factors together with the influence of hormones of pregnancy resulted in the high incidence observed.*

In 1968 we (*1*) reported that mammary gland tumors occur at high incidence in our strain C3H-A^{vy}fB female mice in the absence of the mammary tumor virus (MTV) that is normally transmitted by the milk. Strain C3H-A^{vy}fB originated in 1964 from a C3H-A^{vy} litter delivered by cesarean section and nursed upon a strain C57BL female to prevent infection with MTV. Now, after 20 generations of inbreeding, mammary tumors continue to occur in approximately 90 percent of breeding females, appearing when the mice average 15 months of age. Tumors appear in 20 percent of the virgin females at an average age of 15 months.

The viable yellow mutation (*A^{vy}*), which characterizes strain C3H-A^{vy}fB, was discovered by Dickie (*2*) in a litter of mice born to C3H/HeJ parents. From a litter with the viable yellow mutation, sent to us by Dickie in 1961, we developed strain C3H-A^{vy} which is highly susceptible to mammary tumors and in which MTV is transmitted to the offspring by nursing mothers. The strain was introduced into our colony by a C3H-A^{vy} litter born by cesarean section and nursed upon a C3H/He female, which introduced MTV from our colony of C3H. Mammary tumors occur in 100 percent of breeding and virgin females of strain C3H-A^{vy} at approximately 6.5 months of age. In our strain C3H mice mammary tumors occur in 100 percent of breeding and virgin females, but at a later age (approximately 8 and 10 months, respectively). Strain C3HfB (*3*), derived from a litter of strain C3H born by cesarean section and nursed on strain C57BL, has an incidence of mammary tumors of 40 percent in breeding females; the tumors occur at an average age of 19 months. The incidence in virgin females is 2 percent at 22 months of age.

The higher susceptibility to mammary tumors in strain C3H-A^{vy}fB females, compared with that in C3HfB, appears to be determined by the *A^{vy}* gene, which also produces the yellow coat color and increases body weight. In addition to genetic susceptibility, the presence of type B particles [presumably nodule-inducing virus (NIV)] (*4*) and the hormonal stimulation of pregnancy represent the other factors which interact to produce the high incidence of mammary tumors in strain C3H-A^{vy}fB.

When we found that C3H-A^{vy}fB had such an unexpectedly high incidence of mammary tumors, it seemed worthwhile to examine further the transmission of this susceptibility. To determine whether it was transmitted in the milk as MTV is, through some other avenue of maternal transmission, or by the male parent as readily as by the female parent, we carried out foster nursing and hybridization experiments with strain BALB/c mice. This strain normally has a low incidence of mammary tumors, but it is genetically very susceptible to the tumorigenic influence of MTV and it has a high incidence of tumors after infection with MTV.

Reciprocal matings were made between the yellow strain C3H-A^{vy}fB (*A^{vy}A^{vy}BBCC*) and albino strain BALB/c (*AAbbcc*) to produce the F_1's that were genotypically *A^{vy}ABbCc*, and phenotypically large and yellow. Fifty-seven (C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 females and 54 (BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 females were set up as breeders, each being mated to an F_1 male of the same hybrid type. Most of these females were allowed to have only two litters, with a very few having either one or more than two

Table 1. Occurrence of mammary gland tumors, hepatomas, and cholangiomas in breeding strain C3H-A^{vy}fB females. MT, mammary tumors.

Generation	Females (No.)	Incidence of MT (%)	Average age MT appeared (mo)	Average age died without MT (mo)	With hepatomas (No.)	With cholangiomas (No.)
F_1	1	100	15		1	
F_2	1	100	14		1	
F_3	2	100	17.5		2	
F_4	6	83.3	14.8	21.0	6	
F_5	17	76.5	16.5	18.3	16	1
F_6	15	93.3	15.3	19.0	15	
F_7	20	100	15.8		19	1
F_8	21	90.5	16.2	19.5	21	2
F_9	15	81.3	13.3	12.0	14	
F_{10}	13	84.6	15.6	18.0	13	2
F_{11}	24	83.3	15.4	17.8	23	1
F_{12}	17	94.1	14.4	13.0	16	1
F_{13}	5	100	14.0		5	1
F_{14}	13	92.3	14.0	20.0	12	1
F_{15}	21	90.5	15.7	15.5	21	
F_{16}	11	100	14.1		10	
F_{17}	8	100	12.6		6	
F_{18}	14	85.7	12.5	10.5	11	
F_{19}	19	94.7	13.9	18.0	19	1
F_{20}	15	73.0	14.2	16.3	13	
F_{1-20}	258	89.5	14.8	16.7	244	11