mechanism whereby phagocytes could be either beneficial or harmful with respect to neoplasia. Products of phagocytic oxidative metabolism, such as superoxide anion, hydrogen peroxide, and hydroxyl radical can be lethal to tumor cells (*7*). Furthermore, lipids can be peroxidized by these reactive oxygen species, producing cytotoxic metabolites such as malonyldialdehyde (*8*). However, these products are also capable of interacting with DNA and producing mutations (*9*). To test our hypothesis further, we compared the mutagenicity of neutrophils from a patient with chronic granulomatous disease (CGD) with that of neutrophils from normal individuals. In CGD, neutrophils have a defect in the NAD(P)H oxidase-superoxide–generating system and are thus unable to generate superoxide and hydrogen peroxide. In other respects, they are normal phagocytes (*7*). As shown in Table 3, the mutagenicity of cells from the CGD patient was markedly diminished compared to that of normal cells.

These data indicate a major role for oxygen metabolites in the mutagenic process. Therefore, as in the case of ionizing radiation, it is likely that the seemingly antithetical effects of tumor cell toxicity and carcinogenesis are produced by the same basic mechanism. In particular, efforts to study the role of oxygen in mutagenesis produced by phagocytes should be pursued.

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4 December 1980

Transformation of Paralytic Shellfish Toxins as Demonstrated in Scallop Homogenates

Abstract. *Toxins in shellfish, which are responsible for paralytic poisonings, undergo reductive transformation when incubated with the homogenate of various portions of the scallop,* Placopecten magellanicus. *The transformation includes the reductive elimination of* O*-sulfate groups, a change that is most evident in the locomotor tissue homogenates. The commercially important adductor muscles can also inactivate the toxins.*

Paralytic poisons in shellfish present serious health and economic problems. They are also a spectacular manifestation of metabolite transfers prevalent among many marine organisms through the food chain.

Off the west coast of the United States, the toxic dinoflagellate, *Gonyaulax catenella*, is ingested by shellfish, and the toxic component, saxitoxin (**1**) accumulates in certain tissues of the shellfish (*1–4*). In other parts of the world, shellfish poisoning is more complex, involving several other toxins (*5, 6*). In 1978, we reported on the toxins found in toxic sea scallops from the Bay of Fundy, New Brunswick, Canada (*7*), and later another report gave a considerably different toxin profile on the scallops obtained from almost the same location (*8*). These conflicting results indicated the possibility of bioconversions of the toxins in the scallops.

Scallops, *Placopecten magellanicus*, collected in the Bay of Fundy in October 1977, were toxic. The adductor muscles

Table 1. Toxin contents in the homogenate of toxic scallops before and after incubation and in the toxin mixtures incubated with different body regions (gill, foot, visceral mass, and muscle) of nontoxic scallops in acetate buffer (*p*H 5.8) for 3 days. Recovery of toxicity was calculated as 100 times the total toxicity after incubation divided by the total toxicity before incubation. Toxicity was determined by the method of the Association of Official Analytical Chemists (*18*); 1 MU is equivalent to 0.18 μg of saxitoxin dihydrochloride.

Toxin	Percent toxicity in scallops		Percent toxicity in toxin extracts incubated with			
	Before incubation	After incubation	Gill	Foot	Visceral mass	Adductor muscle
Gonyautoxin-IV + gonyautoxin-V	3.2	0	3.5	0	2.2	3.6
Gonyautoxin-I + gonyautoxin-III	30.0	13.8	29.0	13.9	22.3	15.3
Gonyautoxin-II	25.0	9.7	24.5	8.3	29.6	33.3
Neosaxitoxin	32.0	13.8	30.9	12.5	23.7	5.0
Saxitoxin	6.4	62.7	6.6	65.3	22.2	42.8
Recovery of toxicity		100	100	100	100	46.8

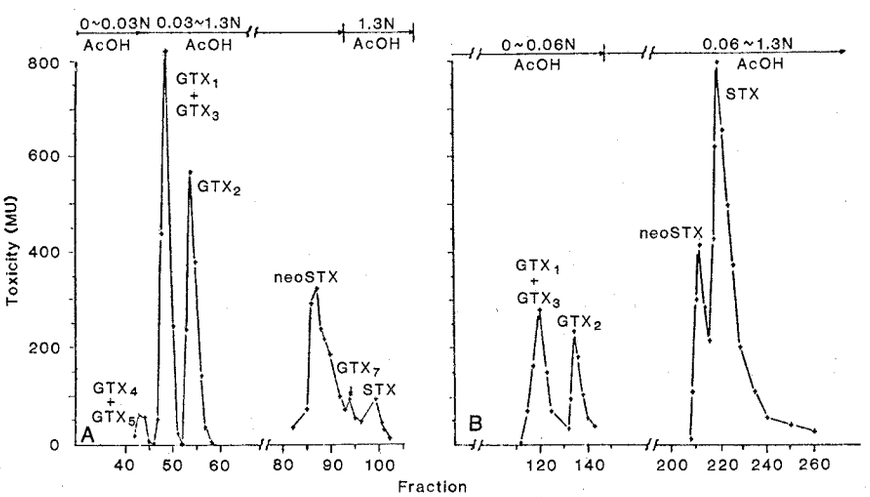

Fig. 1. Comparison of toxin patterns of toxic scallop homogenates (A) not incubated and (B) incubated. Fractions (7.5 ml each) were collected from Bio-Rex (1.5 by 110 cm) with use of the indicated buffer solutions (*7*). Toxicity level was expressed in mouse units (*MU*) (*18*). *GTX*$_1$ to *GTX*$_7$, gonyautoxin-I to gonyautoxin-VII; *STX*, saxitoxin; *neoSTX*, neosaxitoxin.

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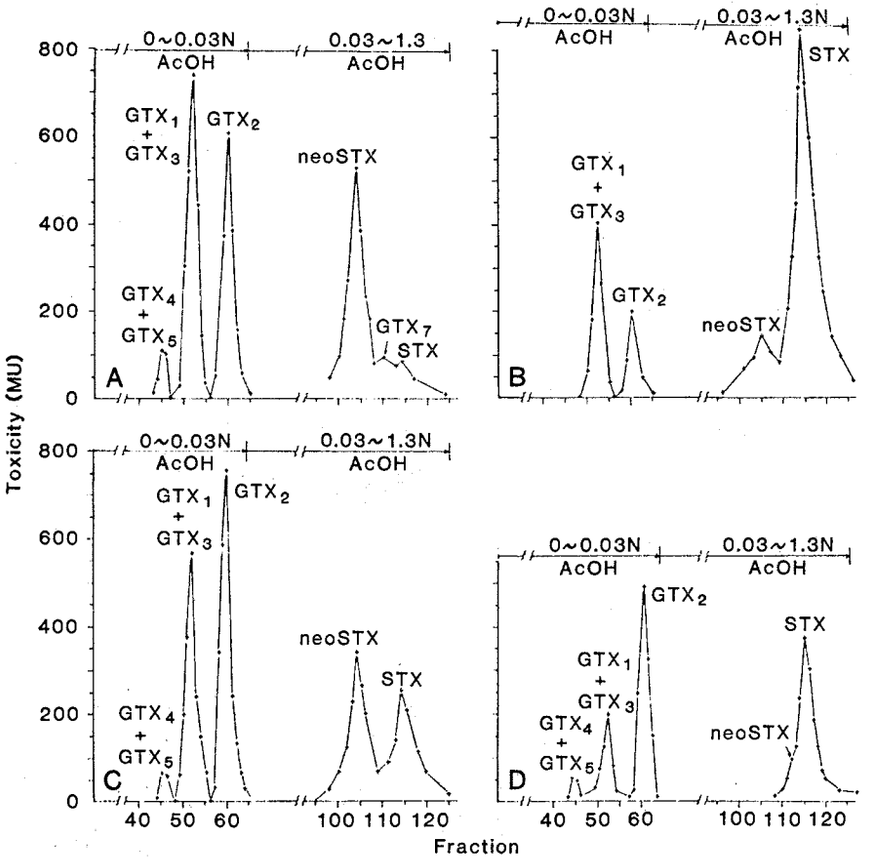

Fig. 2. Toxin profiles of the toxin mixtures after incubation with different regions of nontoxic scallops: (A) gill, (B) foot, (C) visceral mass, and (D) adductor muscle. Fractions (7.5 ml each) were collected from a Bio-Rex 70 column (1.5 by 110 cm) (*7*) with use of the indicated buffer solutions. Toxicity level was expressed in mouse units (*18*). Abbreviations as in Fig. 1.

were devoid of the toxicity [less than 40 mouse units (MU) per 100 g] and were removed. One batch of the remaining tissue was homogenized and immediately processed and anlayzed according to the previously established method (*9, 10*). Another batch of the same toxic tissue was homogenzied with 0.5*N* acetate buffer (*p*H 5.8), kept in a dark place at an ambient temperature for 3 days, and then processed for anlaysis (Fig. 1).

In a separate experiment, nontoxic scallops collected in the Bay of Fundy in October 1978 were dissected; gill (20 g), foot (20 g), visceral mass (20 g), and adductor muscle (21 g) were then homogenized individually with 25 ml of 0.5*M* acetate buffer (*p*H 5.8). To the homogenates were added toxin mixture solutions (1 ml, 7800 MU each) isolated from the toxic scallop by Bio-Gel P-2 column chromatography (*9*). The mixtures were kept at room temperature (23°C) for 64 hours and then processed for analysis (Fig. 2).

The total toxicity of the homogenized scallops without adductor muscles remained almost unchanged after the 3-day incubation. The toxin profiles, however, differed drastically before and after incubation; saxitoxin increased by 56.3 percent, while other toxins, gonyautoxin-I, gonyautoxin-II, gonyautoxin-III, and neosaxitoxin decreased by 12.2, 15.3, 4.1, and 18.2 percent, respectively (Table 1). The results suggest that the toxin undergoes transformation, with saxitoxin the final product.

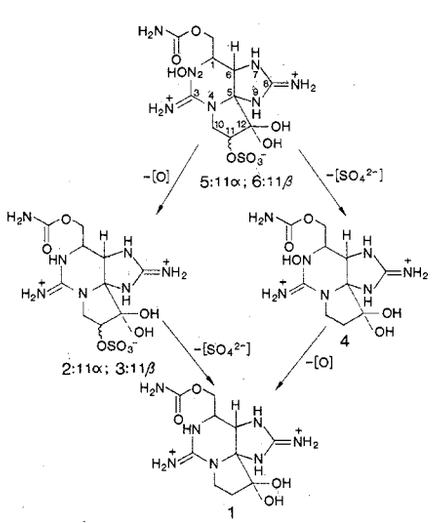

Fig. 3. Structures and biochemical relationship of paralytic shellfish toxins, gonyautoxin-I (**5**), gonyautoxin-II (**2**), gonyautoxin-III (**3**), gonyautoxin-IV (**6**), neosaxitoxin (**4**), and saxitoxin (**1**). The reductive removal of the *N*-hydroxyl group is indicated by $-[O]$, and the reductive cleavage of the *O*-sulfate group is indicated by $-[SO_4^{2-}]$.

Since the structures (Fig. 3) of gonyautoxin-II (**2**) and gonyautoxin-III (**3**) were established as epimeric 11-hydroxysaxitoxin sulfates (*11, 12*), neosaxitoxin as 1-hydroxysaxitoxin (**4**) (*13*), and gonyautoxin-I (**5**) and gonyautoxin-IV (**6**) as epimeric 11-hydroxyneosaxitoxin sulfates (*14*), the conversion has to involve a reductive process: the reduction of the *N*-oxide, and the reductive cleavage of the *O*-sulfate group. The reductive elimination of *N*-oxides is a common reaction, but the hydrogenolysis of *O*-sulfate groups may be unprecedented in biological systems. In fact, the normal metabolic process follows the reverse pathway, namely, hydroxylation and subsequent sulfate conjugation. It is feasible, however, that a good leaving group, such as *O*-sulfate, which is enhanced by the presence of the α-keto group, can be easily hydrogenolyzed. In fact, a mild reduction (zinc dust and HCl) of gonyautoxin-I yields a mixture of gonyautoxin-II and neosaxitoxin, which are eventually reduced to saxitoxin (*13, 14*). An analogy of such reduction is the well-known reductive cleavage of *O*-mesylate or *O*-tosylate groups. Similar mechanisms involving pyrophosphates as leaving groups are also universal in biochemical reactions.

The toxin profiles after incubation with various tissue homogenates from nontoxic scallops are summarized in Table 1. The gill tissue has no effect on either the total toxicity or the toxin profile, whereas foot and visceral mass were responsible for changes of the toxin patterns, but not for total toxicity. In the homogenate of foot tissue, saxitoxin increased by 59 percent, and gonyautoxin-I, gonyautoxin-II, gonyautoxin-III, and neosaxitoxin decreased by 11.8, 17.7, 4.4, and 19.5 percent, respectively; these changes are similar to those observed with the homogenate of the toxic scallop without adductor muscle. Thus the possibility is ruled out that the initially observed change in toxin composition was due to the release, as a result of incubation, of cryptic saxitoxin bound with the tissues.

In the adductor muscle homogenate, the toxicity decreased by 53 percent during the 3-day incubation period. Saxitoxin increased by 36.4 percent, but the major toxins, gonyautoxin-I and neosaxitoxin, decreased by 16.6 and 27 percent, respectively. The adductor muscles, which are the commercially important part of the scallops, have very low toxicity and are sent to market regardless of

the toxicity of the other parts (*15*). Foot and muscle, which have a locomotor function, change the toxin profile conspicuously. Such locomotor tissues have large amounts of the enzymes that participate in the redox reactions.

The toxic shellfish samples analyzed for the toxin contents have shown various ratios of saxitoxin to other toxins (*5, 6*). In some cases, such as a *Mytilus* sp. from Haines, Alaska, saxitoxin was almost absent, whereas other specimens from nearby waters contained significant amounts of saxitoxin (*16, 17*). Our findings, although limited to experiments in vitro, can at least partly explain these discrepancies.

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Tritiated Thymidine Incorporation Does Not Measure DNA Synthesis in Ribavirin-Treated Human Cells

Abstract. *When the incorporation of tritiated thymidine into acid insoluble material was measured, ribavirin appeared to be a potent inhibitor of DNA synthesis in KB cells and human lymphocytes. Inhibition was nearly 100-fold less, however, when DNA synthesis was measured by incorporation of phosphorus-32–labeled phosphate or by DNA fluorescence. The potent inhibition detected by incorporation of tritiated thymidine into DNA actually was the result of a potent effect on the labeling of deoxythymidine triphosphate, not on the synthesis of DNA.*

The mode of action of the antiviral drug ribavirin (*1, 2*) involves inhibition of nucleic acid biosynthesis (*3–7*). The effect of ribavirin on DNA synthesis in uninfected mammalian cells appears to be especially potent when measured by incorporation of [^{3}H]thymidine into acid precipitable material. As little as 2 μM ribavirin produces 50 percent inhibition in primary (*1, 3*) and established (*5, 6*) cell lines from various species. It is difficult to explain such potent inhibition of DNA synthesis, however, on the basis of inhibition of the enzymes (*8*) known to be affected by ribavirin. Furthermore, potent inhibition of DNA synthesis is not consistent with the low cytotoxicity of the drug in cell culture (*1, 2, 9*) and with the low toxicity observed in animals and humans (*1, 2*). To resolve this inconsistency, we have reexamined the effects of ribavirin on both DNA synthesis and [^{3}H]thymidine metabolism in KB cells.

We incubated monolayer cultures of KB cells with selected concentrations of ribavirin or vidarabine (ara-A)—a known inhibitor of DNA synthesis (*5*). Both [^{3}H]thymidine and [^{32}P]phosphate were used as precursors to measure DNA synthesis. Figure 1 shows that ara-A had nearly the same effect on the incorporation of [^{3}H]thymidine and [^{32}P]phosphate into DNA. In contrast, the effect of ribavirin was much more potent on [^{3}H]thymidine incorporation than it was on [^{32}P]phosphate incorporation, suggesting the two precursors measured different events. We also examined the effect of ribavirin on incorporation of [^{3}H]thymidine and [^{32}P]phosphate into concanavalin A–stimulated lymphocytes. The experimental protocol was similar to that described in the legend to Fig. 1; other techniques have been detailed by Lopatin *et al.* (*10*). Results obtained with lymphocytes were virtually identical to those obtained with KB cells (data not shown). The concentrations of ribavirin that inhibited [^{3}H]thymidine and [^{32}P]phosphate incorporation by 50 percent (I_{50}) were 5 μM and 225 μM, respectively. These concentrations correlate well with the I_{50} concentrations of 3 μM and 230 μM interpolated from the data for KB cells in Fig. 1.

To explore why ribavirin produced such different effects on [^{3}H]thymidine and [^{32}P]phosphate incorporation, we examined the metabolism of [^{3}H]thymidine in ribavirin-treated KB cells. Several concentrations of ribavirin were incubated with duplicate monolayer cultures of KB cells. To each flask we added [^{3}H]thymidine at selected times; then we extracted the radioactive intracellular nucleotides and characterized them chromatographically as described in the legend to Fig. 2. Ribavirin (131 μM) caused a protracted decline in the labeling of deoxythymidine triphosphate

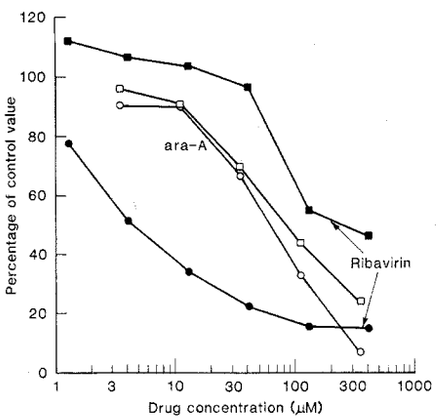

Fig. 1. Effect of ribavirin and vidarabine (*ara-A*) on the incorporation of labeled precursors into cellular DNA. Monolayer cultures of KB cells in 25 cm^2 plastic tissue culture flasks were incubated at 37°C for 14 hours in the presence of selected concentrations of ribavirin (closed symbols) or vidarabine (open symbols) plus [Me-^{3}H]thymidine (2 μCi/ml; 5 μM) (○, ●) and carrier-free [^{32}P]orthophosphate (5 μCi/ml) (□, ■). After the incubation period, the cells were harvested, and cell pellets were dissolved in 1 ml of 0.3*N* KOH and incubated at room temperature for 72 hours to hydrolyze RNA. Portions (100 μl) of the KOH solutions were spotted in triplicate onto filter paper disks which were processed as described previously (*14*) for determination of label incorporated into DNA. For other experimental details, see (*5*). In the absence of drugs, the radioactivity incorporated per 100 μl of cell solution was as follows: 67,600 count/min for [^{3}H]thymidine and 78,900 count/min for [^{32}P]phosphate. All data points represent arithmetic means of results from duplicate cultures.

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