proximately 60 μ moles/ml tissue water) when the level in plasma was less than 1 μ mole/ml. Supported by NSF grant GB-285 and U.S.P.H.S. grant HE 04457.

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THE INHIBITION OF SPONTANEOUS MOTILITY OF SMOOTH MUSCLE IN THE SPINY DOGFISH, <u>Squalus acanthias</u>, AND IN THE SEA ANEMONE, <u>Metridium dianthus</u> BY SULF-HYDRYL REACTANTS

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It has been established that thiol reagents can inhibit the contractility of striated muscle (Weber and Portzehl, Advanc. Protein Chem. 7:162, 1952). A similar sulfhydryl group dependence has been demonstrated for contractility and spontaneous motility of mammalian smooth muscle (Goodman and Hiatt, Biochem. Pharmacol. 13:871, 1964).

The present research involving the spiny dogfish, <u>Squalus acanthias</u>, and the sea anemone, <u>Metridium dianthus</u>, was initiated as part of a comparative study to determine whether sulfhydryl group dependence is one of the biochemical characteristics common to all smooth muscle.

Squalus acanthias. Strips of smooth muscle (5 mm by 40 mm 0.5 gm wet wt) were cut from various regions of the gastrointestinal tract, washed with sea water, then washed and mounted in Ringers solution modified to contain in meq per liter: NaCl, 256; KCl, 10; CaCl₂, 6. The tissue was maintained at 12° in the photoelectric muscle contraction device previously described (loc. cit.). Spontaneous isotonic motility characteristics were continuously recorded under constant load (0.6 gm) before and after the addition of test compounds.

Marked rhythmic spontaneous motility was demonstrated for all parts of the gastrointestinal tract. Strips of distal stomach were highly sensitive to cholinergic compounds including acetylmethylcholine. The terminal region of the spiral valve was found particularly active in this system although far less sensitive to acetylcholine. After equilibration for 30 minutes strips cut from the region of the last turn of the spiral demonstrated regular, periodic isotonic contractions for periods as long as 48 hours without extraneous stimuli or added source of energy. Normal mean frequency of contraction was 1.4 cycles per minute with linear displacement about 5% of strip length.

As in the case of mammalian small intestine, a variety of reagents which react with protein sulfhydryl groups caused marked inhibition of spontaneous motility (Table 1). However, in the dogfish, generally higher concentrations were required for inhibition. Cysteine, homocysteine, or reduced glutathione can negate the inhibitor effects of sulfhydryl group reactants. When compounds with no reactivity toward thiol groups were added, such as glycine or serine, there vas no effect on the contractile pattern.

<u>Metridium dianthus</u>. Strips of parietal muscle of the stalk (5 mm by 40 mm) were cut transversely from the body wall midway between the oral and pedal discs without anesthesia. Strips were trimmed and mounted in the above modified Ringers solution maintained at 10-12°. Spontaneous rhythmic isotonic contractions with loads of from 0.6 to 2.5 gm were recorded for as long as 48 hours on one strip with no added source of energy. The "normal" activity of <u>Metridi-</u> <u>um dianthus in the present Mg⁺⁺</u> free Ringers solution differs markedly from that of <u>Metridium</u>

	Sulfbudnul nonatant	Rate of SH uptake (µM/min)†	% inhibition in 15 minutes	
	Sunnyaryi reactant		Squalus a.	Metridium d.
1	Alloxan [*]	0.94	15	10
2	Cadmium chloride	10.	90	95
3	p-Chloromercuribenzoic [*] acid (Na salt)	10.	20	15
4	N-Ethyl maleimide	1.5	50	15
5	Fluorodinitrobenzene	1.3	90	70
6	Iodoacetamide [*]	1.5	15	5
7	Lead diacetate	10.	90	15
8	Mercuric acetate	10.	75	50
9	Mersalyl [*]	10.	10	5

Table 1

^{*}Concentration required for inhibition was 10⁻² M. In all others, concentration was 10⁻³ M. [†]Rate of SH uptake was determined by the indophenol method. (J. Am. Chem. Soc., 77:3873, 1955).

senile in Mg⁺⁺ free solution described by Ross (J. Exptl. Biol. 37:732, 1960).

Smooth sine curves were obtained with mean frequency of 12 cycles per hour and linear displacement about 8% of the length of strips. The effects of sulfhydryl reactants on muscle activity (Table 1) were quite similar to those described for the dogfish, but higher concentrations of thiol reagents were required for inhibition of spontaneous motility. In the case of compounds 1, 3, 6, and 9, inhibition was noted only at high concentrations of inhibitors. Preliminary attempts to relate inhibitor concentration with activity suggest that the copious secretion produced by the mucous gland cells of the stalk acts to interdict the reaction of inhibitors with smooth muscle cells.

Although the present study is in accord with the general conclusions of Ewer <u>et al</u>. (Doklady Akad. Nauk. SSSR 137:240, 1961) concerning inhibitory activity of thiol reagents in <u>Caliactis para</u><u>sitica</u> we were unable to confirm the reported stimulating effect of cysteine or glutathione.

A sulfhydryl dependent enzyme, β -thioglycosidase (Goodman <u>et al.</u>, Science 130:450, 1959) was found both in the intestinal mucosa of the dogfish (0.9 μ M/min/mg protein) and in the coelomic fluid of the anemone (0.2 μ M/min/mg protein) assayed at 20°C and expressed as rate of thioglucoside cleaved.

Results of the present study are in accord with the view that the integrity of the protein sulfhydryl group is one of the general chemical prerequisites for smooth muscle contraction as well as for certain types of enzyme activity. Studies of functional group dependence in smooth muscle and in enzyme activities of other species are in progress.

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