are such that one would not likely encounter its counterpart in either human cases or other experiments. To produce it, the anoxia must be of long duration—so long, in fact, that the victim dies of circulatory failure and never lives to show morphologically the anoxic insult to the adrenal cortex. This may account for the absence of reported pathologic changes even in uncomplicated acute anoxic death, such as reported by Kritzler(13). Life must persist in the dog for at least 6 hours for these lesions to develop. Animals surviving longer than 48 hours after this period of anoxia are not likely to show any more than mild cytological changes in the epithelial cells of the adrenal cortex.

It was concluded in a previous report by one of us (H.G.S.) that an irreversible cerebral insult was produced in dogs by 6½ minutes of fulminating anoxia(9). The present study shows that this estimate is a little too short, for 4 out of 24 (17%) made apparently complete recovery from this length of exposure. At 8 minutes, however, all dogs either died or would have succumbed. These figures are very similar to those of Kabat, Dennis and Baker(14). Undoubtedly an irreversible cerebral insult takes place in both cats and dogs after approximately seven minutes of fulminating anoxia.

Summary. Dogs were given pure nitrogen to breathe and then resuscitated by lung insufflation and cardiac massage about 6½ minutes after the onset of anoxia. This was at a time when breathing and circulation had failed. These animals were sacrificed at various intervals thereafter. The earliest morphological changes that occur in the adrenal glands are pyknosis of the nucleus and coagulation of the cytoplasm of epithelial cells in the zona fasciculata and zona reticularis. Subsequently, polymorphonuclear leucocytes infiltrate these areas of the cortex. A few petechiae occur. Clinical manifestations in such anoxic dogs appear to be the result of cerebral injury and not due to adrenal insufficiency.

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Antagonism of Mercurial Poisoning in Growing Cells Investigated with Onion Root Tips. (20039)

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Growing root tips of the bulbs of onions (*Allium cepa L.*) are highly sensitive to the organic mercurials phenylmercuric hydroxide (PMOH) and basic phenylmercuric nitrate (PMN), and the gross reactions provide delicate indicators for a range of very low concentrations(7,8,10). Aqueous solutions of alcoholic extracts of beef spleen and of yeast protected roots during short exposure to lethal concentrations of PMN(9), but did not pre-

TABLE I. Antagonism Efficiency of Cellular Extracts, Cysteine and Glutathione with PMOH Shown by Gross Reactions of Allium Roots after One Hour Exposure.

PMOH, ppm	Antagonist, %	Reaction grade in days		PMOH ppm equivalent	Relative % efficiency	Original root length, cm	MLD –100 PMOH, ppm
10	—	IV	½	—		3– 8	2.3
10	1 yeast	II	2–4	1	90	3– 7	"
10	1 "	III	3	1.5	85	6–10	"
10	1 spleen	IV	3	2.8	72	4– 7	"
10	.01 cys.	III	3	1.5	85	7–12	"
10	.5 glut.	II	4	1	90	2– 4	"
10	.5 "	III	3	1.5	85	5–10	"
10	1 yeast	II–III	2	2	80	3– 6	4
5		IV	3	—	—	5– 6	"
5	.5 "	II	3	2	60	6– 7	"
5	.5 spleen	II	5	2.5	50	5– 7	"
5	.01 glut.	II	1	.5	95	4– 6	"
	1 yeast	II	2	—		3– 6	
	.5 spleen	III	5	—		4– 6	
	.01 cys.	I	2	—		7–12	
	.01 glut.	I	4	—		6– 7	

I = Recovery, no c-tumor. II = Arrest and recovery after c-tumor. III = Permanent arrest. IV = Death. ppm = Parts per million in tap water.

vent temporary growth arrest nor the formation of terminal swellings—the colchicine-like reaction or c-tumor. It is not clear how these extracts protect the respiratory enzymes(2,3) and we have now compared the antagonism of the mercurials by the thiols cysteine hydrochloride and glutathione utilizing the gross reactions of these tissues.

Method. Bulbs of the yellow globe type of onion were germinated in the dark on glass jars over tap water. Tests were run on roots 3.5-7 cm long, as described previously(9). The test solutions were made up with the local tap water in which the roots were cultured. For convenience in tabulation and as a scale for comparison, the gross reactions have been classified in 4 grades: Grade I—slight torsion; sometimes accelerated growth rate. II—arrest and c-tumors with eventual recovery of normal growth rate. III—as II with or without recovery of slow growth in clubbed tips. IV—death, soon or later and evident first in older elongating tissue of second 5 mm of tip. Treatment for an hour in Cincinnati tap water resulted in Grade II after PMOH 0.5-1.5 ppm (2-5.2 $\times$ 10^{-6} M); III occurred in surviving organs up to the 100% lethal threshold 2.3 ppm (8 $\times$ 10^{-6} M = LD 100). In Palm Beach water, the reactions were induced by concentrations around twice these values(7).

Results. a. *Short treatment.* Table I shows the relative efficiency of the antagonists against PMOH 10 ppm in an hour's exposure as evaluated by the concentration of the mercurial alone which produced a similar gross reaction under the same conditions. With 1% beef spleen extract, death was only delayed and became apparent in a restricted zone as after an hour in PMOH 2.8 ppm. Table II shows the effects of different concentrations of the thiols and extracts with lethal amounts of PMOH and alone. Best protection was obtained in Palm Beach water with glutathione 0.01% at 18.8 times in excess and cysteine 47 times in excess. Glutathione 0.01% in Palm Beach also antagonized PMN 10 ppm with mixed II and III reactions. Glutathione 0.001% antagonized PMN 10 ppm (1.6 $\times$ 10^{-5} M) or half its molar strength with a Grade III reaction as induced by PMN 5 ppm indicating a 50% antagonism. The c-tumor effect was eliminated in short treatment only when sub-lethal (Grade III) PMOH 5 ppm was antagonized by glutathione 0.01% in bottled Deep Rock mineral spring water, and there was a reduced growth rate for a few hours. The absence of gross effects did not, however, prevent cytological abnormalities, as will be reported later.

Effective concentrations of all antagonizing agents alone in an hour's treatment produced

TABLE II. Reactions of Onion Roots to Antagonism of Lethal Doses of Organic Mercurials by Cellular Extracts and Thiols after an Hour's Exposure. Gross reactions I–IV and time in days to reach equilibrium after exposure.

Antagonist	Conc., %	Antagonist alone		PMOH 10 ppm, 3.4×10^{-5}M		PMOH 5 ppm, 1.7×10^{-5}M	
0				IV	½ day	IV	2–3 days
AYE	1	I–II	1–2 days	II–III	2–4 days		
	.5	I–II	1 "			II	2–3 "
ASE	1	III	2–4 "	IV	3 "		
	.5	III	2–4 "			II	5–6 "
CSH							
8×10^{-2}M	1	IV	1 hr	IV	¼ hr		
3.2×10^{-2}M	.4	IV	2 "				
3.2×10^{-3}M	.04	II	3–5 days				
8×10^{-4}M	.01	I	2 "	III	3 days	II	2 "
GSH							
1.6×10^{-2}M	.5	II	2–4 "	II–III	3–4 "		
3.2×10^{-4}M	.01	II	1–2 "			II	1–2 "

AYE—Alcoholic yeast extract; ASE—Alcoholic beef spleen extract; CSH—Cysteine Hydrochloride; GSH—Glutathione.
Underscored—Cincinnati tap water; Others—Palm Beach tap water.

some growth arrest (see Tables). Cysteine 1% with PMOH 10 ppm given for an hour caused death about as rapidly as PMOH 4.5 $\times$ 10^{-5} M alone due, no doubt, to the excess thiol. Concentrations of cysteine alone between 10^{-3} M and 10^{-2} M had a delayed lethal effect on the elongating tissue and above 0.25% it killed them within an hour's exposure, partly because of the low pH. Glutathione 0.5% (1.6×10^{-2} M) caused a Grade II reaction by itself.* There was a slightly greater arresting effect in the presence of PMOH (Table II) or of PMN at 10 ppm.

Considerable excess of the yeast extract was required for successful antagonism. With PMOH 10 ppm 1% of the extract gave 85-90% antagonism in Cincinnati (Table I), but with PMOH 13 ppm it failed to protect and the tissue died within 4 days. In Palm Beach PMN 20 ppm was antagonized by 1% of the extract, while 0.5% and 0.1% merely delayed the appearance of death. In Cincinnati PMN 10 ppm was antagonized by 1% and 0.8% extract to give mixed Grades II and III after 3 days, an efficiency of about 60%. Cook and Kreke(2) found that 1% yeast extract antagonized the depressant effect of PMN 10 ppm on the growth of yeast cells by 50% but had little effect in the presence of higher concentrations of the poison.

Prior immersion of the roots in the antagonists for 30 minutes failed to protect them against subsequent exposure to lethal PMOH 10 ppm.

b. *Continuous exposure.* PMN 0.2 ppm (0.32×10^{-6} M), which alone was LD 100 in 2-4 days in Cincinnati, was antagonized for 3 days continuously by cysteine 0.01% and by glutathione 0.01%. Growth was inhibited after 36 hours by the mixtures and by either thiol alone,† but all roots remained straight, rigid and normal in form throughout the treat-

* A day after an hour in glutathione 1% and 0.5%, a girdle-like lesion, 1 mm high, of atrophied cells appeared immediately behind the meristem in both waters. The same injury occurred after an hour in PMOH 10 ppm and GSH 0.5% and some tips fractured here. Those that remained intact recovered normal growth in a few days.

† Upon return to water after 3 days in GSH 0.01%, the roots immediately attained a daily increment slightly above the controls in sturdy normal tips. Cysteine 0.01% arrested growth of immersed roots sooner. When returned to water after 3 days, they gradually recovered only from 50 to 75% of normal growth rate in the next 3 days, and the new growth was slightly tortuous. CSH 0.005% (4×10^{-4}M) in continuous treatment had no inhibitory effect on roots; rather it stimulated growth somewhat the first day.

ments, and when returned to tap water, they recovered normal growth without c-tumor formation. High dilutions of various amino acids depress the growth of roots of cress seedlings(1).

Discussion. These mercurials are known to depress enzymes of the respiratory chain, partly by combination with the –SH groups, and this can be prevented by cysteine and glutathione(4). The inhibition of the cytochrome oxidase is probably not entirely due to sulfhydryl combination(6,11). It is a question, therefore, whether the successful antagonism of the depressant effects on living cells by cellular extracts is due chiefly to the presence of –SH groups in them, or whether the inactivated enzymes are side-stepped(2,3). It has been found that cysteine and glutathione in slight excess at 7×10^{-4} M will protect catalase and antagonize its depression by PMOH and PMN at 1.6×10^{-4} M(13). In the presence of air the thiols interfere with a factor which links succinic dehydrogenase and cytochrome oxidase(12). Cysteine at 13×10^{-6} M increased the inhibition of invertase by mercuric chloride 0.13×10^{-6}M(5). Macfarlane(7) suggested that growth by elongation can be recovered by onion roots when depression of the succinoxidase system has not been below 50% for short treatment as produced by concentrations of the mercurials up to approximately 10^{-6} M. The antagonists seem to reduce the effective internal concentration of the poison 50 to 95% and in some cases to an equivalent of 10^{-6} M or below. The active principle of the cellular extracts is probably a relatively small fraction of the crude extract because the latter was required in considerable excess to afford protection.

Summary. The fatal effects of PMOH and PMN at near 10^{-5} M on enlarging cells of onion root tips were successfully antagonized in an hour's exposure with cysteine and glutathione at around 10^{-4} M and by extracts of yeast (0.5-1%) and of beef spleen (0.5%). Gross reactions provided a delicate indicator for estimating the efficiency of the antagonists and showed a reduction in toxicity of 50% to 95%. Cysteine and glutathione at 0.01% also protected roots against PMN 0.2 ppm for 3 days continuously. Effective concentrations of all antagonists by themselves caused some growth arrest. The enzymic systems involved and the possible mechanism of antagonism are discussed.

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