fowl ticks collected at a farm where a proved fatal case of Western equine encephalitis in a child had occurred[6] were likewise negative. The above procedures were repeated in each case. Tests for neutralizing antibodies in the serum of the chickens on the various farms have not been completed.

These studies are being extended and will be repeated during the summer and fall months when the common chicken mite is more prevalent. Meanwhile, studies are in progress to determine whether, under experimental conditions, hereditary transmission of the Western equine virus in chicken mites can be effected.[12]

SUMMARY

The Western type of equine encephalomyelitis virus has been isolated from chicken mites (*Dermanyssus gallinae*) in nature during an outbreak of the equine disease in the Southwest.

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THE ANTIBACTERIAL PROPERTIES OF DICUMAROL

DESPITE the considerable amount of work done on dicumarol, it has not been recognized up to now that this compound has marked antibacterial properties. The growth inhibitory effect of dicumarol was studied against fourteen bacterial species and the results are represented in Table 1.

TABLE 1

	Growth inhibitory concentrations of dicumarol
Staphylococcus aureus	1:100,000
Streptococcus pyogenes	1:100,000
Streptococcus viridans	1:100,000
Streptococcus fecalis	Not inhibited at 1:25,000
Bacillus anthracis	1:100,000
Bacillus subtilis	Not inhibited at 1:25,000
Corynebacterium diphtheriae	1:25,000
Clostridium welchii	Not inhibited at 1:25,000
Brucella abortus	1:25,000
Eberthella typhosa	Not inhibited at 1:25,000
Escherichia coli	Not inhibited at 1:25,000
Salmonella paratyphi A	Not inhibited at 1:25,000
Proteus vulgaris	Not inhibited at 1:25,000
Pseudomonas pyocyanea	Not inhibited at 1:25,000

Since naphthoquinones having Vitamin K activity are the physiological antagonists of dicumarol, it was of interest to determine whether naphthoquinones would antagonize the antibacterial properties of this compound. This possibility was tested in the case of *Staphylococcus aureus*. It was found that methyl-1,-4-naphthoquinone failed to antagonize the growth inhibition caused by dicumarol.

[12] S. E. Sulkin and C. L. Wisseman, Jr., to be published.

[13] With the technical assistance of George C. Patterson.

Dicumarol was first isolated by Link and associates[1] from spoiled sweet clover. One may be allowed to speculate on the possibility that if it could be shown that the spoilage of sweet clover was due to the action of microorganisms, dicumarol could be considered a naturally occurring antibiotic.

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THE MECHANISM OF GROWTH INHIBITION BY HEXENOLACTONE

A POTENT growth-inhibitor, supposedly parasorbic acid, occurs in a variety of natural sources, notably malt, yeast, orange peels[1] and the ripe berries of the mountain ash, Sorbus.[2] The inhibitor suppresses the germination of seeds and pollen as well as the growth of certain microorganisms and animal tissues.[1, 3] Among the bacteria are some (lactic acid bacteria, *Streptobacterium plantarum*) which, like animal epithelia (chick epithelia, Ehrlich carcinoma), are relatively unresponsive; and others (*Staphylococcus aureus*) which, like fibroblasts and mesenchymal cells, are very sensitive to the inhibitor.[1, 3, 4] Kuhn and Jerchel[2] have recently identified parasorbic acid (I) in extracts of Sorbus berries and established proof for its structural configuration. Synthesis was reported independently by two groups of investigators.[1, 2]

In the form of a relatively simple and comparatively stable molecule, *i.e.*, an unsaturated delta-hexenolactone, a potential tool is thus provided for the elucidation of the mechanism of an antibiotic activity.

Because of structural resemblance between hexenolactone (parasorbic acid) and pantolactone, which reacts with beta-alanine to form pantothenic acid, Medawar, Robinson and Robinson[1] suggested a possible interference of the inhibitor with pantothenic acid metabolism. In experiments designed to test this hypothesis[3, 5] pantothenic acid did not weaken the activity of hexenolactone. On the other hand, both alpha-alanine and beta-alanine as well as glutathione were shown by Hauschka[5] to inactivate the inhibitor, while glycine, iso-leucine and glutamic acid had no such effect. Further experiments, to be summarized here, have established cysteine, but not cystine, as antagonistic to hexenolactone. This antagonism was demonstrated by bio-assay and its mechanism elucidated in part by colorimetric and spectrophotometric methods.

[1] M. S. Stahmann, C. F. Huebner and K. P. Link, *Jour. Biol. Chem.*, 138: 513, 1941.

[1] Medawar, Robinson and Robinson, *Nature*, 151: 195, 1943.

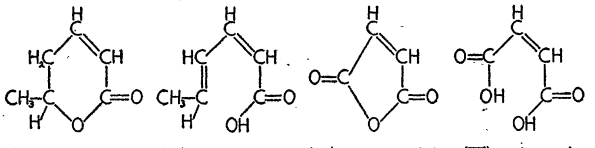
[2] Kuhn and Jerchel, *Ber. Chem. Ges.*, 76B: 413, 1943.

[3] Kuhn, Jerchel, Moewus, Moller and Lettre, *Naturwiss.*, 31: 468, 1943.

[4] De Lor and Means, *Rev. Gastroenterol.*, 11: 319, 1944.

[5] Hauschka, *Nature*, 154: 769, 1944.

When the planarian *Dugesia tigrina* is exposed to 5×10^{-5} molar hexenolactone for 12 to 20 hours, a characteristic three-partite lesion develops in the dorsal body-wall of the animal. The lesion pattern resembles an inverted "Y" and always corresponds in extent to the underlying triclad intestine. Cross-sections reveal that the inhibitor takes effect from within the animal, producing first a partial evisceration, secondly disintegration of the three branches of the gut and of the surrounding mesenchyme, and lastly the triclad wound in the dorsal body-wall. There is no indication of an axially graded susceptibility to hexenolactone. Disintegration is tissue-differential, mesenchyme being most and dermal epithelium least affected. The above sequence of events is apparently very specific, as it can not be induced by a large variety of inorganic and organic toxic substances in various concentrations, including the structurally similar isomere sorbic acid (II), maleic anhydride (III) and maleic acid (IV).



(I) hexenolactone (II) sorbic acid (III) maleic anhydride, (IV) maleic acid

Quantitative evaluation of the degree of antagonism between various amino-acids and hexenolactone was based on measurements of lesion area, extent of repair-growth and survival. The planarians were individually matched as to size and pigmentation, and comparable lots of from 10 to 20 animals were exposed simultaneously to test medium (amino-acid plus 5×10^{-5} M hexenolactone at pH 6.7–7.0) and control solution (5×10^{-5} M hexenolactone, same pH as test).

TABLE 1

ACTIVITY OF 5×10^{-5} M HEXENOLACTONE (= 100%) OVER A PERIOD OF 20 HOURS, AS INFLUENCED BY THE ADDITION OF VARIOUS AMINO ACIDS AND A TRI-PEPTIDE

Effect	% activity	Test substance in $n \times 10^{-5}$ moles per liter added to 5×10^{-5} M hexenolactone	n	
	0	Cysteine	25.0	
I	35.3	Glutathione	25.0	
	49.0	Cysteine	2.5	
Significant	62.2	Glutathione	2.0	
Inactivation	67.5	Beta-alanine	5.0	
	68.2	Beta-alanine	2.5	
	69.9	Glutathione	5.0	
	96.5	Beta-alanine	20.0	
II	100.0	Hexenolactone	Control	
Within range of	101.4	Cystine	12.5	
standard control	106.6	Iso-leucine	5.0	
variability	111.6	Iso-leucine	2.0	
III	122.3	Glycine	1.0	
Activation	124.5	Cystine	2.5	
or	125.1	Glycine	2.5	
double toxicity	132.5	Glutamic acid	2.0	pH adjusted
	160.5	Glutamic acid	2.0	pH not adjusted

The experimental results, based on lesion measurements in 81 separate tests performed on 2,146 matched animals, are condensed in Table 1.

Both cysteine and glutathione were most effective in the higher concentrations, while beta-alanine 20×10^{-5} M was consistently without effect. Control solutions of the amino acids alone in corresponding concentrations were not detectably deleterious.

Mendez[6] has recently reported that the cardiac activity of the angelicalactones (which are structurally related to the gamma-lactone rings in the side-chains of certain aglycones of the digitalis group) may be directly ascribed to peroxides formed in aqueous solutions in the presence of metallic impurities. This peroxide effect was counteracted by cysteine, glutathione and other SH compounds, the sulfhydryl presumably preventing the oxidation by forming complexes with the metallic catalysts.

Analysis of delta-hexenolactone solutions, such as those used in our Dugesia experiments as well as much higher concentrations, gave no positive tests for peroxide, even after prolonged aeration in the presence or absence of either copper or animal tissues. Our findings for delta-hexenolactone are not to be taken as contradicting Mendez' results with gamma-angelicalactone. Attempts to reproduce the hexenolactone lesion pattern in Dugesia with inorganic peroxide or with the organic tertiary-butyl-hydroperoxide[7] were unsuccessful. The inactivation of hexenolactone by SH compounds is, therefore, not explainable by the inhibition of metal catalysis or by a reaction between sulfhydryl and peroxide. The inactivating mechanism is actually a direct and reversible interaction between R–SH and the lactone, as shown by colorimetric and spectrophotometric studies.

Cysteine (1×10^{-2} and 1×10^{-3} M) was added to equimolar solutions, buffered at pH 7.0, of (A) freshly dissolved hexenolactone, (B) freshly dissolved hexenolactone and beta-alanine, (C) hexenolactone dissolved 48 hours earlier, (D) hexenolactone having stood for 48 hours with beta-alanine. Buffered cysteine (E) and cysteine-beta-alanine solutions (F) served as controls. The reactions were studied, at constant temperature (18° C ± 1° C), under aerobic conditions as well as under nitrogen, by the colorimetric Folin-Shinohara method.[8]

While in the control solutions (E, F) sulfhydryl and disulfide together accounted for 100 per cent. of the original SH contents, the test solutions A, B, C, D all showed a rapid reaction (84–87 per cent.) be-

[6] Mendez, *Jour. Pharmacol. and Exp. Therap.*, 81: 151, 1944.

[7] Milas and Harris, *Jour. Am. Chem. Soc.*, 60: 2434, 1938.

[8] Shinohara, *Jour. Biol. Chem.*, 112: 709, 1936.

tween hexenolactone and cysteine. The presence of beta-alanine or the age of the hexenolactone solution did not alter the nature or significantly influence the kinetics of this process, as given in Fig. 1. Alanine,

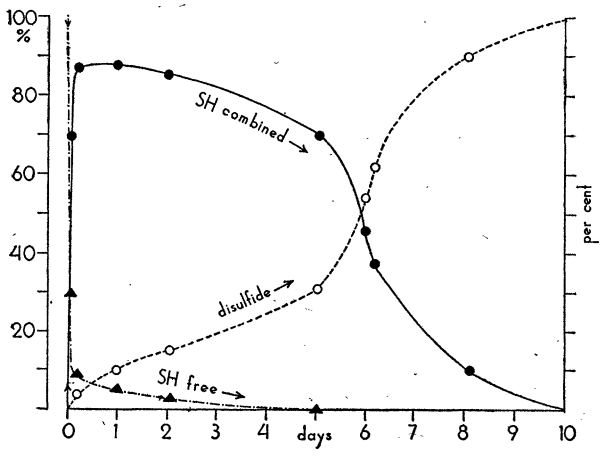


FIG. 1. Interaction between hexenolactone and cysteine (under relatively anaerobic conditions).

therefore, appears to counteract the biological effect of hexenolactone through a mechanism distinct from inactivation by SH, as borne out also by the concentration optimum of beta-alanine (Table 1).

The hexenolactone-cysteine addition compound is gradually decomposed again, yielding disulfide in sufficient quantity to account for all the cysteine. Decomposition under relatively anaerobic conditions is five to seven times slower than in the presence of O_2.

The ultraviolet absorption peak, characteristic for the conjugated double bond in the hexenolactone molecule (I), is markedly lowered during the height of the reaction with cysteine, but 9 days later has approximately returned to pre-reaction shape and magnitude. At no time does the spectrum resemble the typical absorption curve for sorbic acid (II). The spectral changes are consistent with the colorimetric data and suggest regeneration of hexenolactone. Furthermore, the complete inactivation of the hexenolactone (5×10^{-5} M) effect on Dugesia by cysteine (25×10^{-5} M), shown in Table 1, lasts for only 20 to 24 hours. After this period triclad lesions gradually appear in the test animals. However, the molecule which emerges from the reaction with cysteine is only about one third to one half as active as a hexenolactone control. This raises the problem of a structural change, now under investigation.

Hammett[9] has demonstrated the rôle of the sulfhydryl group in cell proliferation. The activity of the many enzymes with SH groups in the protein portions of their molecules appears dependent on the maintenance of SH in the reduced state which, according to Barron and Singer,[10] is insured by the presence of glutathione. Inactivation by SH of the antibiotic properties of penicillin and several widely different bacteriostatic substances was recently reported by Cavallito and Bailey.[11] In view of our similar findings for hexenolactone it is suggested that some of the most effective antibiotics interfere with cellular proliferation mainly through their reactivity with SH groups essential to enzyme function.

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HYDROLYSIS OF CHOLINE ESTERS BY LIVER

IN a recent paper Mendel, Mundell and Rudney[1] have shown that acetyl-beta-methylcholine (Merck's mecholyl) is a specific substrate for true cholinesterase (ChE) and benzoylcholine, a specific substrate for pseudo-ChE. Both of these enzymes hydrolyze acetylcholine (ACh) more efficiently than their specific substrates, and when a mixture of the enzymes occurs, both take part in the total ACh hydrolysis.

While investigating the true ChE and pseudo-ChE content of various organs by a microchemical titrimetric method[2] it was noted that the above conditions did not always hold in the case of rodent livers. The results (Table 1) are expressed in terms of Q_{ChE} (mgms ACh hydrolyzed by 100 mgms tissue per hour) and for the sake of comparing enzyme activities on a molecular basis, the data for mecholyl and benzoylcholine have been calculated in terms equivalent to that of ACh.

In the cat and rat livers ACh hydrolysis appears to be dependent on the enzymes hydrolyzing mecholyl and benzoylcholine, principally pseudo-ChE. But in the rabbit and especially in the guinea pig livers, ACh hydrolysis seems to be purely a function of true ChE; the relatively high means and low standard errors in the appropriate "percentage of ACh" column testify to this relationship. Benzoylcholine hydrolysis by rabbit and guinea pig livers, however, show no constant ratio to ACh hydrolysis. In fact, the guinea pig liver splits benzoylcholine 5 to 11 times as rapidly as ACh.

The guinea pig pancreas and submaxillary salivary gland, whose ability to split benzoylcholine is of the

[9] Hammett and Hammett, *Protoplasma*, 15: 59, 1932.

[10] Barron and Singer, SCIENCE, 97: 356, 1943.

[11] Cavallito and Bailey, SCIENCE, 100: 390, 1944.

[1] B. Mendel, D. B. Mundell and H. Rudney, *Biochem. Jour.*, 37: 473, 1943.

[2] C. H. Sawyer, *Jour. Exp. Zool.*, 92: 1, 1943.