the supplemented controls. This difference obtains whether the L.C.F. supplement is administered from hatching or only as a curative measure during the last ten days preceding autopsy.

In the curative experiments there was considerable variability in the degree of therapeutic effect as evidenced by increase in body weight. The stilbestrol response varied accordingly, thus reducing the mean oviduct weight for the group as a whole. Nevertheless, the restorative effect of the administered L.C.F. on the oviduct response is apparent.

The pantothenic acid deficient chicks showed a growth failure quite comparable to that observed in the L.C.F. deficient animals. Despite this marked effect, they exhibited a good response to stilbestrol, indicating that debility and limitation of growth are not sufficient, *per se*, to effect the reduction in oviduct response observed in the L.C.F.-deficient chicks.

The normal response to stilbestrol stimulation is characterized by a substantial increase in the thickness of the muscular and mucosal layers of the oviduct. Gross examination of the oviduct of the stilbestrol treated L.C.F.-deficient chick reveals that the small weight increase over the normal may be attributed to accumulation of fluid in the lumen and that the walls of the oviduct remain thin and translucent.

The data indicate that an adequate intake of *L. casei* factor is essential for the normal metabolism of stilbestrol in the chick. This finding is an interesting example of the interdependence of a dietary trace substance and a hormone-like factor and further emphasizes the importance of adequate nutrition for normal endocrine function.

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INHIBITION OF METAL CATALYSIS AS A FUNGISTATIC MECHANISM

MECHANISMS of action for chemicals that inhibit growth of or are lethal to microorganisms are little understood. Knowledge of positional effects of various atomic groupings is extensive, and general mechanisms are frequently cited, including protein precipitation, enzyme inactivation, inhibition of respiration. However, specific cases are rare for which the *modus operandi* of inhibitory or lethal action is known. Possible exceptions are the sulfonamide antagonism,[1,2] the blocking of enzyme surfaces by adsorption of heavy metals to the active areas,[3] and the inhibition of the -SH group by $HgCl_2$.[4]

The fact that microorganisms require traces of several metal elements indicates that these elements are involved in catalytic processes in the cells; *i.e.*, that the metals function as part of one or more enzyme systems.[5] Hence a chemical capable of precipitating the metals in such enzyme systems should prevent growth of microorganisms by inhibiting metal catalysis, particularly if metals are precipitated in the form of chelate inner complex salts which are non-electrolytes. 8-hydroxyquinoline forms such chelate salts with many metals,[6] and is also an efficient fungistatic and bacteriostatic compound.[7,8] It is suggested that the latter property is the result of the former. This theory for the mechanism of fungistatic action of 8-hydroxyquinoline was tested biologically in the following ways:

(1) At lower pH values (in general, below pH 3.5) complex formation does not take place between 8-hydroxyquinoline and certain metals,[9,10] including several considered essential for growth of fungi: copper, manganese, iron and zinc. Hence the chemical should not be fungistatic at lower pH values as essential metal ions should be available. Flasks of Tochinai nutrient solution (1.5 g. KH_2PO_4, 0.5 g. $MgSO_4$, 10 g. Bacto Peptone, 20 g. C.P. Maltose, 1,000 ml distilled water), in pH series, were inoculated with three test fungi (*Fusarium oxysporum* f. *lycopersici*, *Ceratostomella ulmi* and *Penicillium* sp.). Normal growth was found to occur at low pH values in the presence of as much as 100 mg of 8-hydroxyquinoline per liter (Table 1), a dose far in excess of that causing complete inhibition at higher pH levels.

TABLE 1

EFFECT OF pH ON FUNGISTATIC ACTION OF 8-HYDROXYQUINOLINE

	Average weight (mg) of Penicillium mat at pH:					
	2.2	2.5	3.2	3.8	4.5	5.7
Nutrient plus 100 mg of 8-hydroxyquinoline per liter	378	260	111	0	0	0
Nutrient alone	390	401	347	352	311	184

The effect of increasing acidity is not merely to neutralize the weak basic properties of 8-hydroxyquinoline. Neither is the compound destroyed at low pH values.

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(2) Zinc was found to be the most important essential trace element for the vascular pathogens (*F. oxysporum lycopersici, C. ulmi*) used in these tests. Hence, if 8-hydroxyquinoline prevents growth by removing zinc from solution, growth should be resumed when the system is saturated by supplying additional zinc. This was found to be the case. Addition of 25 mg of zinc per liter permitted nearly normal growth of the fungus in the presence of 40 mg of the fungicide per liter. (Table 2.)

TABLE 2

SATURATION OF 8-HYDROXYQUINOLINE SYSTEM WITH ZINC

Mgs. of zinc per liter	Milli-mols 8-hydroxyquinoline per liter	Milli-mols metals added per liter purified nutrient	Calculated molar ratio metal/fungicide*	Dry wt. of fungus mat (mgs)
1	0.2758	.00789	.028	0
5	0.2758	.0848	.308	74
25	0.2758	.3925	1.43	160
1	0	.00789	∞	206.5

*Subsequent chemical analysis showed the presence of metal contaminants in the "purified" nutrient solution, so that these ratios are below actual values.

(3) Increasing amounts of the fungicide should be required to inhibit growth of *Fusarium* in the presence of increasing amounts of zinc. In the presence of 0.5 mg of zinc per liter of nutrient solution, 30 mg of 8-hydroxyquinoline per liter inhibited growth of the fungus. With 5 mg of zinc per liter, 60 mg of the fungicide prevented growth; 30 mg did not.

The above evidence strongly supports the view that 8-hydroxyquinoline owes its fungistatic action to the phenomenon of forming inner complex salts with metal ions and thus rendering them unavailable to microorganisms. A chemical with this type of mechanism of action would obviously be fungistatic rather than fungicidal. The importance of traces of zinc in the metabolism of the vascular fungi indicates that a zinc-protein enzyme may be involved; zinc may be the prosthetic group. Further support for the theory that this fungicide affects metal-enzyme systems is seen in the fact that it inactivates copper-enzyme systems in *Chlorella* and in extracts of higher plants.[11, 12]

The possibility is evident that other known fungicides may be effective because of a similar precipitation of metals essential to fungi. Conversely, analytical reagents other than 8-hydroxyquinoline should be found to be good fungicides. As an example of the latter, ammonium nitrosophenyl hydroxylamine ("Cupferron"), a well-known organic reagent, also forming chelate inner complex salts with metals, has been tested in this laboratory recently and found to have considerable fungistatic value.

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THE ULTRAVIOLET ABSORPTION OF VITAMIN B_c AND XANTHOPTERIN

IN the course of the isolation of crystalline vitamin B_c[1] from liver, we observed that concentrates containing 5 to 10 per cent. of the compound exhibited specific absorption in the ultraviolet region. This property, which proved to be characteristic of the vitamin, was used along with biological criteria in the isolation work. It was apparent from the study of even grossly impure preparations that the intensity and position of the absorption bands were dependent on the hydrogen-ion concentration.

The absorption curves of pure vitamin B_c at four selected pH levels are shown in Fig. 1. Analytical

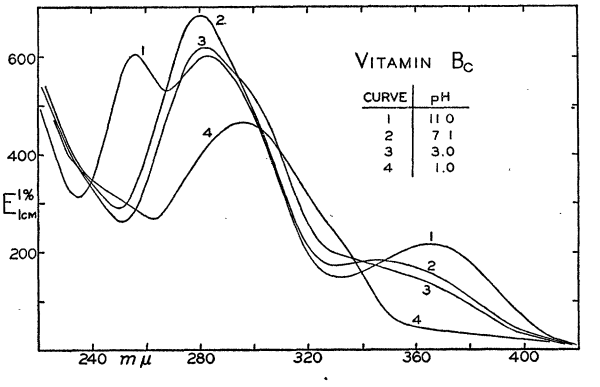

FIG. 1

ash-free specimens isolated from liver and from yeast exhibit identical absorption characteristics.

Stokstad[2] reported the preparation of a *Lactobacillus casei* ε growth factor in purified form from liver and expressed the view that his substance was identical with the previously isolated crystalline vitamin B_c. The shape of the ultraviolet absorption curves which he recorded for his preparation at several pH levels support his view that he was dealing with vitamin B_c, but a comparison of the extinction coefficients with those of the pure vitamin indicate that his preparation was approximately 70 per cent. pure. Mitchell[3] has recently recorded ultraviolet absorption data on "folic acid" concentrates from spinach. We have tried to compare his data with those in Fig. 1. However,

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