(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.

GEORGE A. ZENTMYER
CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN, CONN.

[11] L. F. Green, *et al., Jour. Biol. Chem.*, 128: 447, 1939.
[12] E. Stotz, *et al., Jour. Biol. Chem.*, 119: 511, 1937.

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

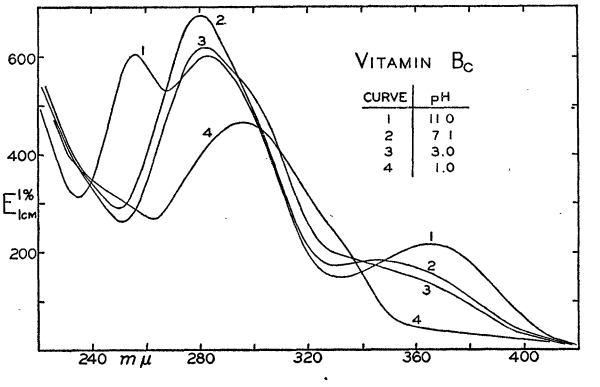

FIG. 1

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,

[1] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, SCIENCE, 97: 404, 1943.
[2] E. L. R. Stokstad, *Jour. Biol. Chem.*, 149: 573, 1943.
[3] H. K. Mitchell, *Jour. Am. Chem. Soc.*, 66: 274, 1944.

since the pH value of his recorded curve was not indicated and the absorption intensities of his concentrates were low, we were unable to draw any inferences as to the possible identity of the "folic acid" factor and vitamin B_c.

The ultraviolet absorption characteristics of vitamin B_c, coupled with the nitrogen content of the compound[1] suggest the presence in the molecule of a pyrimidopyrazine ring structure (*e.g.*, flavins, alloxazines, pterins). In this connection it is of interest that the pterins have had a long though somewhat uncertain association with nutritional anemia. Their possible role in hematopoiesis was emphasized some time ago by Sir Frederick Gowland Hopkins.[4] In 1936 Tschesche and Wolf[5] reported that the pterins and particularly xanthopterin had hematopoietic activity when administered to rats rendered anemic on a goat's milk diet. More recently Simmons and Norris[6] reported that xanthopterin causes a hematopoietic response in fingerling salmon. On the other hand, O'Dell and Hogan[7] were unable to replace vitamin B_c in the diet of the chick with xanthopterin. The only ultraviolet absorption data on pure xanthopterin available in the literature is a curve for the barium salt in glacial acetic acid.[8] Since this curve showed a general resemblance to that of vitamin B_c (Fig. 1) at pH 3 and 7 we considered it desirable to make a study of the ultraviolet absorption of xanthopterin. Synthetic xanthopterin was prepared by Purrmann's method,[9] and natural xanthopterin was prepared by the method of Schöpf and Becker[10] from the wings of butterflies (Colias philodice).[11] It was necessary to determine the coefficients of pure natural xanthopterin because it was our experience that synthetic products may give acceptable analytical figures and yet be grossly impure. Xanthopterin has no melting point and little crystallization tendency. Identical curves were obtained with analytical ash-free specimens of the synthetic and natural products. In Fig. 2 are recorded the data obtained at approximately the same pH levels as reported for vitamin B_c (Fig. 1).

A comparison of the curves for the two compounds at the different pH levels brings out some very striking dissimilarities, although in general the ultraviolet

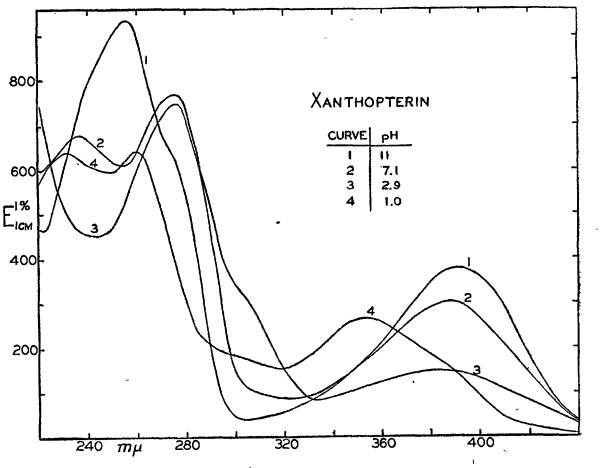

FIG. 2

absorption curves of xanthopterin and vitamin B_c are similar. However, the ultraviolet absorption characteristics of the flavins, alloxazines and pterins are all similar, and the relationship of vitamin B_c to these classes of compounds must await chemical study.

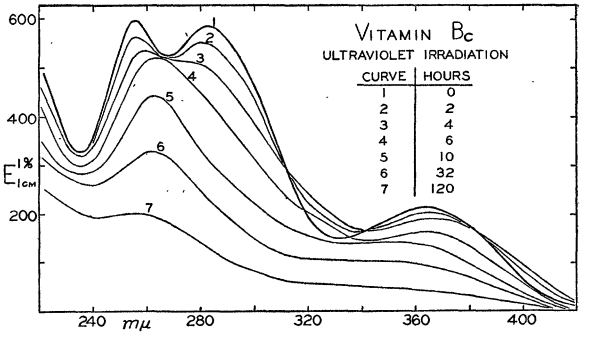

FIG. 3

Recently Totter and Day[12] reported that xanthopterin and "folic acid" concentrates counteract the leucopenia encountered in succinylsulfathiazole treated rats. They later reported[13] that xanthopterin delays the onset of nutritional cytopenia in monkeys and causes a certain hematopoietic response in cytopenic animals. The results of Totter and Day[12] prompted Mitchell[14] to point out that his "folic acid" concentrates may contain several per cent. of xanthopterin as an impurity, but the possibility of the presence of xanthopterin as a contaminant is not discussed in his more recent paper[3] in which he emphasizes the marked similarity of the absorption characteristics of xanthopterin to those of "folic acid" concentrates. The absorption data recorded by him indicate that his prepa-

[4] F. G. Hopkins, *Proc. Roy. Soc. B.*, 130: 359, 1942.
[5] R. Tschesche and H. J. Wolf, *Z. Physiol. Chem.*, 244: I, 1936; *ibid.*, 248: 34, 1937.
[6] R. W. Simmons and E. R. Norris, *Jour. Biol. Chem.*, 140: 679. 1941.
[7] B. L. O'Dell and A. G. Hogan, *Jour. Biol. Chem.*, 149: 323, 1943.
[8] C. Schöpf and E. Becker, *Ann.*, 507: 273, 1933; W. Koschara, *Z. physiol. Chem.*, 240: 138, 1936.
[9] R. Purrmann, *Ann.*, 546: 98, 1940.
[10] C. Schöpf and E. Becker, *Ann.*, 524: 49, 1936.
[11] We wish to thank Dr. G. W. Rawson of this laboratory for his assistance in collecting the insects during the summer of 1943 and for the species identification.
[12] J. R. Totter and P. L. Day, *Jour. Biol. Chem.*, 147: 257, 1943.
[13] J. R. Totter, C. F. Shukers, J. Kolsom, V. Mims and P. L. Day, *Jour. Biol. Chem.*, 152: 147, 1944.
[14] H. K. Mitchell, SCIENCE, 97: 442, 1943.

ration of xanthopterin was 50–60 per cent pure. The use of impure xanthopterin may account for recent conflicting reports concerning its role in nutrition.

The effect of ultraviolet irradiation upon the ultraviolet absorption of the vitamin is shown in Fig. 3. The irradiation was carried out with an H-4 lamp without filter for the periods of time indicated. Both the irradiation and corresponding absorption curves were made in aqueous solution (conc. = 0.002 per cent.) at pH 11. It is apparent that the compound is only moderately sensitive to ultraviolet illumination. Our purest specimens of the vitamin have a barely detectable trace of blue-green fluorescence in ultraviolet light. Since this fluorescence increases markedly on exposure to ultraviolet light, we can not be certain that the original very faint trace of fluorescence is not due to a decomposition product. We shall discuss the chemistry and biological activity of the irradiation products in a later paper.

E. S. BLOOM
J. M. VANDENBELT
S. B. BINKLEY
B. L. O'DELL
J. J. PFIFFNER

RESEARCH LABORATORIES,
PARKE, DAVIS AND COMPANY,
DETROIT, MICH.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW DIFFERENTIAL STAIN FOR MOUSE PITUITARY[1]

THE need for a reliable and consistent differential stain for the pituitary gland of the mouse in certain experimental work provoked the search for such a method. The literature[2] on the mouse pituitary staining technique was studied and several methods were tried. The technique that was found to be most successful was a combination of several methods rather than any one method previously used. The combination method which has been developed is given in detail below.

(1) Fix tissues in modified Zenker-formol from six to twenty-four hours. Modified Zenker-formol contains only one half the normal amount of corrosive sublimate and one cc less than the normal amount of formalin.

(2) Wash in running water for twenty-four hours and then change to 50 per cent. alcohol for three hours.

(3) Change to 70 per cent. alcohol and leave overnight.

(4) Change to 80 per cent. alcohol for at least two hours or until needed.

(5) Change to 95 per cent. and 100 per cent. alcohols for an hour each.

(6) Change to a 1: 1 mixture of 100 per cent. alcohol and xylol for twenty minutes, to xylol for forty minutes and to a 1: 1 mixture of xylol and paraffin for twenty minutes.

(7) Change to 56°–58° paraffin for one hour and then embed.

The sections of the gland were cut at four micra and were then stained as follows:

(1) Carry sections from xylol through the alcohols to water in the usual manner, using iodine in the 70 per cent. alcohol to remove the corrosive sublimate crystals.

(2) Stain with haematoyxlin 15 to 20 seconds. (Mayer's haematoxylin was used).

(3) Rinse in water and then blue the sections in an aqueous solution of lithium carbonate.

(4) Stain the sections in a solution of picro-orange until they are even yellow. Picro-orange[3] consists of 0.25 gms of Orange G and 100 cc of 80 per cent. alcohol saturated with picric acid.

(5) Rinse in running water until only the red blood cells remain yellow.

(6) Stain in a 1: 1 mixture of 1 per cent. ponceau de xylidine and 0.5 per cent. acid fuchsin as long as the red blood cells remain yellow, about 2 to 5 minutes. (Mcfarlane used ponceau red instead of ponceau de xylidine.)

(7) Rinse in 2 per cent. acetic acid in water.

(8) Differentiate to desired redness in a solution consisting of 40 cc of 95 per cent. alcohol, 20 cc of water and 40 cc of a stock solution. Stock solution includes 25 gms phosphotungstic acid, 2.5 gms picric acid and 100 cc per cent. alcohol.[4]

(9) Rinse in water.

(10) Stain for about 3 minutes in a solution of 100 cc of 2 per cent. Orange G in 1 per cent. phosphomolybdic acid.

(11) Rinse in water and transfer to a 1 per cent. aqueous solution of anilin blue for 15 to 20 minutes.[5]

(12) Rinse in water and dip quickly in 95 per cent. and 100 per cent. alcohols, clear in xylol and mount in balsam.

The technique described above gives results comparable to those of various triple stains on the pituitary glands of other animals. The acidophiles are orange to pink and their nuclei are reddish purple, the basophiles are an intense blue and the nuclei are pur-

[1] This work has been aided by grants from the Commonwealth Fund, Anna Fuller Fund, International Cancer Research Foundation, Jane Coffin Childs Memorial Fund and the National Advisory Cancer Council.

[2] Alexei Koneff, *Stain Tech.*, 13: 2, April, 1938; I. H. Perry and M. S. Lockhead, *Stain Tech.*, 15: 3, July, 1940; Miriam Reed, SCIENCE, 98: 2553; Earl B. Scott, *Stain Tech.*, 15: 2, April, 1940; Aura E. Severinghaus, *Anat. Rec.*, 53: 1, June, 1932.

[3] David McFarlane, *Stain Tech.*, 19: 1, January, 1944.

[4] *Idem.*

[5] C. R. Stockard and E. M. Vicari, *Amer. Anat. Memoirs*, No. 19, Sect. 5, 1941. Detailed suggestions from E. M. Vicari, unpublished material.