tion oxidants do indeed have absorption bands which overlap the blue absorption band of chlorophyll. The evidence that methylene blue is an electron acceptor is conflicting in the literature.

It may well be asked where the energy for electron transfer at an energy level corresponding to the 420-mμ absorption band (68 kcal/mole) comes from, since the Hill reaction proceeds, as does photosynthesis, in red light (44 kcal/mole). It will be noted that at least 24 kcal/mole remain unaccounted for.

Another light absorption step may provide the additional energy needed. No such two-step process which uses only the short-lived singlet states can occur. Excitation of chlorophyll to a "chemically active species" may, however, be explained as occurring in a two-step process analogous to the one occurring in the flash photolysis of anthracene and related substances (*5*). This is based on the existence of a metastable state (triplet) of chlorophyll with a life-time of about 10^{-3} sec (*6*). The energy of the metastable state is slightly below that of the first (singlet) excited state of chlorophyll; experimental evidence which indicates its participation in photosynthesis has been obtained by Calvin (*7*) and by Commoner (*8*).

Thus we picture radiant energy to be converted to chemical energy in the course of the Hill reaction in the following two-step process. The first step is excitation to either the first or second excited singlet state, but only the molecules remaining in the long-lived metastable state are of further interest. These may be promoted by absorption of another quantum of red light to provide the additional energy required for ultimate reduction of the oxidant (*9*).

One consequence of this two-step energy absorption process is that the quantum requirement for the formation of molecular oxygen cannot be less than eight. Two quanta are required to promote each of the four electrons involved for wavelengths corresponding to either the red or the blue absorption bands of chlorophyll (*10*).

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3 September 1957

Serotonin, Norepinephrine, and Related Compounds in Bananas

The present study was initiated following a personal communication from J. A. Anderson (*1*) that ingestion of bananas produces an increased urinary excretion of the serotonin (5-hydroxytryptamine) metabolite, 5-hydroxyindoleacetic acid. This observation was reported recently by Anderson, Ziegler, and Doeden (*2*). The chemical studies reported here were undertaken to explain this phenomenon; they have led to the rather surprising finding that bananas contain large amounts of two physiologically important agents, serotonin and norepinephrine.

Table 1. Serotonin and catecholamines in banana. The values presented are the averages of a number of analyses on ripe, yellow bananas. In the case of serotonin, 14 assays were done on pulp (range, 8 to 50 μg/g) and 5 on peel (range, 47 to 93 μg/g). In the case of norepinephrine and dopamine, the values represent the results obtained on three bananas.

Pulp*		Entire peel†	
μg/g	mg/ banana	μg/g	mg/ banana
Serotonin			
28	3.7	65	3.9
Norepinephrine			
1.9	0.25	122	7.3
Dopamine			
7.9	1.0	700	42

* Average wt. of pulp, 130 g.
† Average wt. of peel, 60 g.

The studies started with an attempt to find serotonin in bananas. Extracts of banana pulp were prepared by homogenization in 0.1*N* HCl followed by alkalinization and extraction as described by Weissbach *et al.* (*3*). Homogenates of banana peel were precipitated with $Zn(OH)_2$ and the filtrates were used for assay (*3*). Spectrophotofluorometric analysis of these extracts revealed large amounts of material having the characteristic activation and fluorescence spectra of serotonin (*4*). The amounts found are shown in Table 1. The serotonin in these extracts was identified further by subjecting it to chromatography on paper. As is shown in Table 2, the R_f values in two solvent systems and the colors obtained with several spray reagents were identical with those obtained with an authentic sample of serotonin.

The presence of related 5-hydroxyindole compounds was also investigated. No 5-hydroxyindoleacetic acid was found by use of the quantitative assay procedure (*5*). Chromatography of acetone-HCl extracts (see below) revealed the presence of at least two other 5-hydroxyindole substances. No further identification of these substances was attempted in the present study.

The presence of serotonin, coupled with the fact that bananas produce melanin-like material on ripening, suggested the possibility of oxidation of 5-hydroxyindoles to "melanin-precursors," 5,6-dihydroxyindole compounds (*6*). The presence of "melanin-precursors" was further suggested by the ob-

servation that extracts adjusted to neutral or basic *p*H values blackened rapidly. To investigate the presence of such compounds, banana pulp and peel were homogenized in five volumes of acetone-2*N* HCl (12:1) and centrifuged. The supernatant fluid was concentrated by heating it on a water bath at 70°C. The volume was then adjusted so that 1 ml represented 0.5 g of original tissue. Portions of these extracts were applied to Whatman No. 3 paper, and chromatograms were developed overnight with phenol (see Table 2).

The chromatograms were sprayed with ferricyanide reagent which gives a blue color with 5,6-dihydroxyindole compounds. Although little, if any, such material was revealed, a large number of substances appeared which had the characteristic pink color of oxidized catecholamines. None of these were indoles, as was shown by their failure to give the characteristic color with Ehrlich's reagent. A comparison of the chromatographic behavior of these substances with known catecholamines (see Table 2) revealed the presence of norepinephrine and 3,4-dihydroxyphenylethylamine (dopamine). In addition to having R_f values identical with those of the authentic compounds, these substances gave the same colors and fluorescence following spraying with ferricyanide reagent. Each substance was eluted from the paper with dilute HCl and converted to its respective fluorophor by a modification (7) of the Lund procedure (*8*). The activation and fluorescence spectra were identical with those formed from reference standards (activation, 390 mμ; fluorescence, 510 mμ). The amounts of norepinephrine and dopamine in pulp and peel were determined by chromatographing measured aliquots of the acetone-2*N* HCl extracts, eluting the appropriate areas, and assaying fluorometrically (7).

Table 2. Chromatographic identifications.

Compound	Phenol system*			N-Propanol-1*N* ammonia (5:1)		
					Color	
	R_f	Color†	Fluorescence‡	R_f	Ehrlich reagent	Nitroso-Naphthol reagent
1	0.55	Yellow	Pink	0.52	Blue	Purple
Serotonin	0.56	Yellow	Pink	0.53	Blue	Purple
2	0.38	Pink	Blue			
Dopamine	0.38	Pink	Blue			
3	0.12	Pink	Yellow			
Norepinephrine	0.12	Pink	Yellow			

* 80 g of phenol, 20 ml of 0.02*N* HCl, several milligrams of KCN, saturated with SO_2.
† After spraying with ferricyanide reagent (0.4 percent ferricyanide in 0.1*M* phosphate buffer, *p*H 7).
‡ As seen under ultraviolet light after spraying with ferricyanide reagent.

The results shown in Table 1 indicate that banana contains large amounts of norepinephrine and the related substance dopamine. The biologic activity of these catecholamines was studied as further proof of their identity and to ascertain whether the norepinephrine was the physiologically active, L-form. Aliquot portions of banana eluates containing known amounts of these substances as determined fluorometrically were injected into dogs, and the effects on arterial blood pressure were compared with those obtained with synthetic L-norepinephrine and dopamine. As is shown in Fig. 1, the pressor responses obtained with 10 μg of extracted norepinephrine and the synthetic L isomer were comparable. In the case of dopamine, 1 mg of both the extracted material and the standard produced an identical rise in blood pressure, amounting to 120/60 mm-Hg.

The presence of related catecholamines was also investigated. Large amounts of material having chromatographic properties similar to 3,4-dihydroxyphenylalanine (DOPA) were found in all the extracts examined. Although it is likely that the banana is rich in DOPA, further identification of this substance is required. There appears to be at least one additional catecholamine substance in banana. This material had an R_f in the phenol system of about 0.05, unlike any of the available reference standards. No epinephrine has as yet been demonstrated in banana extracts. It was found that the extracts do not contain histamine.

Although 5-hydroxyindoleamines have been found in plants previously, they have usually been associated with plants regarded as toxic to animals (*9, 10, 11*). To our knowledge this is the first report of the finding of norepinephrine in plant material. The significance of these two amines in the metabolism of the banana is not obvious although they may play a role in the browning reaction.

The presence of these potent physiologic agents in a food as widely used as the banana is of clinical interest. Serotonin is known to inhibit gastric secretion and to stimulate smooth muscle in the intestine (*12*) and elsewhere; norepinephrine is an important mediator of autonomic function and is used extensively as a vasoconstrictor agent. Whether the oral administration of these amines through banana feeding can have effects on the gastrointestinal tract or on the cardiovascular system remains to be determined. One might also speculate about whether some of the reported therapeutic uses of bananas (*13*) (in celiac disease, peptic ulcer, constipation, and so forth) may be due to the presence of these amines. As a result of these findings, we were encouraged to administer large amounts of serotonin to patients. It was found that at least 20 mg of serotonin can be taken orally without any apparent physiologic effect. In contrast, as little as 1 mg of serotonin produces marked effects when it is administered intravenously. Although no comparable information is available concerning oral administration of norepinephrine, Richter has administered as

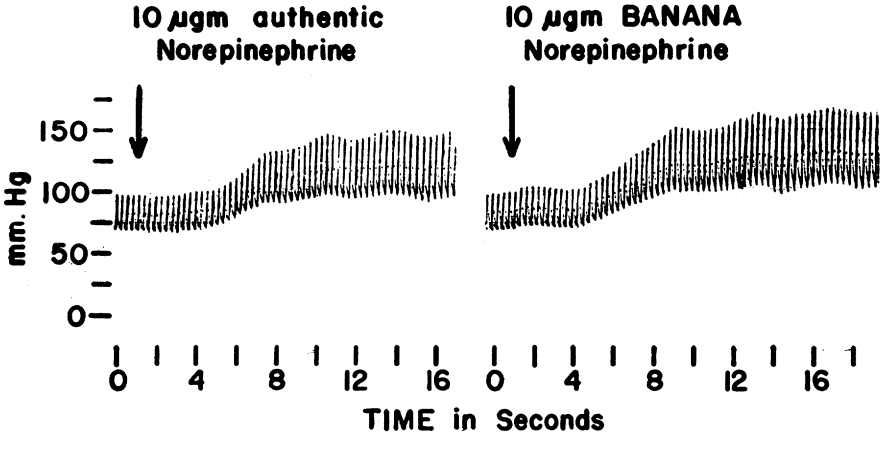

Fig. 1. Effects of authentic and banana norepinephrine on arterial blood pressure in a 14-kg dog under pentobarbital anesthesia. The agents were administered intravenously in 5 ml of isotonic NaCl solution.

much as 30 mg of epinephrine to human beings via the oral route (*14*). However, physiological changes were observed at this level.

Of immediate clinical significance is the fact that ingestion of bananas may lead to erroneous chemical diagnoses of carcinoid tumors (*2*) and pheochromocytoma by producing an increased urinary excretion of serotonin and norepinephrine and their metabolites. Also, bananas should be eliminated from the diets of patients whose urinary indoles and catecholamines are being measured for other purposes—for example, in mental disease. It remains to be determined whether other edible plants contain these agents.

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2-Thiazolidinethione-4-Carboxylic Acid from the Reaction of Captan with Cysteine

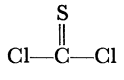
In studies of the mechanism of fungitoxic action of captan [N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide] (*1*), it was found that certain sulfhydryl compounds react with this fungicide to form ultraviolet absorbing products. A compound with an absorption maximum at 272 mμ is formed when cysteine is reacted with captan or trichloromethylsulfenyl chloride ($ClSCCl_3$). The evidence presented in this paper demonstrates that the ultraviolet absorbing compound formed in the reaction between captan and cysteine is 2-thiazolidinethione-4-carboxylic acid.

This compound was made by reacting captan and L-cysteine in aqueous solutions. Tetrahydrophthalimide formed in this reaction was removed by extraction at *p*H 6.0 with ethyl acetate, and 2-thiazolidinethione-4-carboxylic acid was then removed at *p*H 1.5 with the same extractant. The latter was further purified by passing it through ethyl acetate and sodium carbonate solutions in the manner described for purifying the hydrazones of keto acids (*2*). The purified compound was converted to the sodium salt and recrystallized from ethanol by addition of excess ethyl ether. The sodium salt was dissolved in water and converted to the acid form, which was then extracted with ethyl acetate. White to pale yellow crystals formed upon evaporation of the ethyl acetate.

The following evidence was obtained for the identity of the compound. The equivalent weight was determined by base titration. Ninhydrin and nitroprusside tests were negative but were positive when cysteine was reformed upon base hydrolysis or partial reduction of the compound. Values obtained by analyses for carbon, hydrogen, nitrogen, and sulfur were comparable to the theoretical values (*3*). The ultraviolet absorption of the compound is similar to that for 2-thiazolidinethione (Fig. 1). Infrared analysis suggested the presence of a secondary amine, a thioureide, and a carboxyl structure in the molecule.

The melting point of the compound was 180 to 181°C. Chatterjee *et al.* (*4*) and Behringer and Zillikens (*5*) have reported different syntheses of the compound or its isomers in which the reported melting point or decomposition temperature was 161°C and 190° to 194°C, respectively.

Thiophosgene



is apparently an intermediate in the reaction of captan or $ClSCCl_3$ with cysteine, since a compound with the same ultraviolet absorption spectrum and the same chromatographic R_f value as 2-thiazolidinethione-4-carboxylic acid is formed when thiophosgene is reacted with cysteine. Barron (*6*) reported that cysteine reacts with phosgene to form 2-thiazolidone-4-carboxylic acid. Apparently thiophosgene behaves the same as phosgene in its reactivity with cysteine.

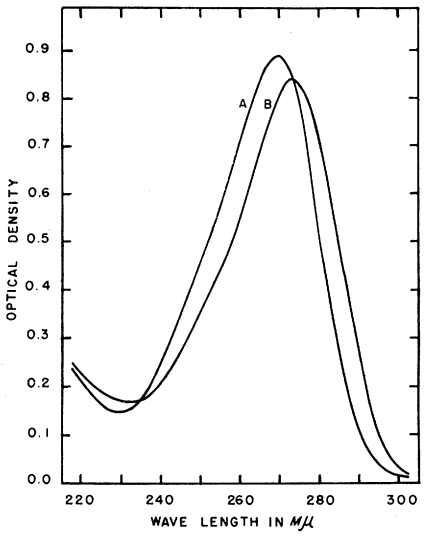


Fig. 1. Comparison of the ultraviolet absorption spectrum of 2-thiazolidinethione with that of 2-thiazolidinethione-4-carboxylic acid. Curve *A*, 2-thiazolidinethione, $6.1 \times 10^{-5} M$; curve B, 2-thiazolidinethione-4-carboxylic acid, $6.1 \times 10^{-5} M$.

A description of other products of the reaction of captan with cysteine and the significance of the reaction in the fungitoxic mechanism of captan is presented elsewhere (*7*).

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