which were exhibited. Fat absorption is linked with the later appearance in the serum of the L-phenylalanine-sensitive isoenzyme of alkaline phosphatase.

NOTE ADDED IN PROOF: A recent interesting report by Langman *et al* (Nature, 1966, v212, 41) describes the relevance of the genetic background to the extent of fat-induced increases in serum "intestinal" alkaline phosphatase.

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Received April 14, 1966. P.S.E.B.M., 1967, v124.

Effect of Anthocyanin Pigments on Certain Enzymes.* (31829)

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Anthocyanins have been reported to be antibacterial against *Escherichia coli*(1,2) and *Salmonella typhosa*(3) but others have observed no effect on *Staphylococcus aureus*(4) or on pathogens(5). Charles and Niviere(6) found the anthocyanin pigments of red wine to be antiseptic and to inhibit fermentation. Lucia(7), in a review of the properties of wine, cited several reports on the antibacterial properties of wine or wine components. Pratt *et al*(8), Powers *et al*(9) and Hamdy *et al*(10) in separate studies, investigated the influence of anthocyanin pigments on *E. coli, S. aureus* and *Lactobacillus casei* and found that inhibition or stimulation of growth varied with the anthocyanin pigment and organism tested. Powers(11) reported on the effect of 24 anthocyanins, leucoanthocyanins and phenolic acids on the respiration and reproduction of 10 different bacteria.

Although it is well established that anthocyanin pigments do influence the growth of bacteria, the exact mechanism of influence is not understood. Leucoanthocyanin and anthocyanin compounds have been demonstrated to possess biologic activity toward enzymes and plant processes. Steward and Shantz(12) observed that cocoanut milk and horse chestnuts each contained a leucoanthocyanin which stimulated cell division of plants. Hulme and Jones(13) reported that anthocyanins and anthocyanidins inhibited succinoxidase and that leucoanthocyanins inhibited both succinoxidase and malic acid dehydrogenase.

From the work of Somaatmadja *et al*(14) it was established that the inhibitory action of anthocyanin pigments could be explained by their chelation of magnesium ions. It was postulated that the inhibitory action of anthocyanin pigments would affect certain metabolic enzymes in the bacteria. In order to determine the exact site of inhibition a systematic investigation of the enzymes of metabolism was begun. The following were selected because they represented 4 different classes of enzymes:

1. alpha glucan phosphorylase—phosphorylases; 2. glycerol dehydrogenase—dehydrogenases; 3. malate dehydrogenase—dehydrogenases; 4. hexokinase—kinases; 5. glutamic acid decarboxylase—decarboxylases.

* Supported in part by USPHS Grant EF-00349-06, Division of Environmental Engineering and Food Protection. Approved as Journal Paper 483, College Experiment Station, Coll. of Agri., Univ. of Georgia, Athens.

Other enzyme systems will be investigated in a continuing study so that the action of anthocyanin pigments on metabolic enzymes can be established.

The anthocyanin pigments used in this investigation are the 4 major pigments obtained from grapes: 1) petunidin-3-monoglucoside (P-3-M), 2) delphinidin 3-monoglucoside (D-3-M), 3) malvidin 3-monoglucoside (M-3-M), and 4) an unidentified pigment.

Materials and methods. Source of pigments. The extraction and isolation of pigments from the juice of Cabernet Sauvignon grapes was done according to the method of Pratt *et al* (8).

Enzyme assays. Enzymes were obtained from the Worthington Biochemical Corp. Alpha glucan phosphorylase activity was determined by the method of Illingworth and Cori(15) in which activity is measured by determination of the amount of inorganic phosphate formed. Glycerol dehydrogenase activity was measured by the method of Lin and Magasanik(16) based on the reduction of nicotinamide adenine dinucleotide (NAD) by substrate glycerol. The activity of malate dehydrogenase was measured by the method of Mehler *et al*(17) and is based on the oxidation of $NADH_2$ by substrate oxalacetate. Hexokinase activity was measured by the method of Darrow and Colowick(18). The assay for glutamic acid decarboxylase was conducted according to the manometric method outlined by Umbreit *et al*(19).

The 4 anthocyanin pigments, D-3-M, P-3-M, M-3-M and the unidentified pigment were used at concentrations of 0.01%, 0.10%, and 0.5%. For the spectrophotometric assays, 1 ml of the appropriate pigment solution was substituted for 1 ml of distilled water and blanks or control tubes were made by substituting 1 ml of enzyme diluent for the enzyme mixture. Control flasks for the manometric determination of glutamic acid decarboxylase were established as outlined by Umbreit *et al* (19).

Results are reported as mg of phosphorus produced for alpha glucan phosphorylase; change in optical density per unit time for glycerol dehydrogenase, malate dehydrogenase and hexokinase; and as microliters CO_2 produced for glutamic decarboxylase. Three replications of all treatments were made.

TABLE I. Alpha Glucan Phosphorylase Activity Expressed as mg % Inorganic Phosphate.*

	Concentration		
Pigment	.01	.10	.5
D-3-M	2.1	14.0	.5
P-3-M	7.5	6.4	6.6
M-3-M	14.5	14.0	13.7
Unidentified pigment	6.6	8.2	8.8

* Control value = 2.3.

Results and discussion. Alpha glucan phosphorylase. All pigments at all levels used had a stimulating effect on alpha glucan phosphorylase (Table I). According to Keller and Cori(20) phosphorylase exists in two forms: phosphorylase *a* (active enzyme) and phosphorylase *b* (inactive enzyme). Phosphorylase *a* can split into 2 molecules of phosphorylase *b* (Cori and Cori, 21,22). Krebs and Fischer(23) reported that the presence of AMP is needed for the activity of phosphorylase *a*, but when phosphorylase *b* is converted to phosphorylase *a*, phosphorylase *a* is fully active without addition of AMP. It is possible that anthocyanin pigments may catalyze the conversion of phosphorylase *b*, thereby increasing the activity of phosphorylase *a*.

Another possible explanation of the stimulation of phosphorylase by anthocyanin pigments is that inasmuch as phosphorylase is a sulfhydryl enzyme and subject to inactivation by heavy metals (Krebs and Fischer 23, Brown and Cori 24), the chelation properties of anthocyanin pigments reported by Somaatmadja *et al*(14) may prevent inactivation by heavy metals. This would be in agreement with Buell and Hansen(25) who reported that Versena, glycogen, and AMP had a protective effect on the activity of phosphorylase enzymes.

Glycerol dehydrogenase. Table II lists the effects of the anthocyanin pigments on glycerol dehydrogenase activity. At the 0.01% concentration, all pigments except M-3-M increased the activity of the enzyme. At all other concentrations, activity was decreased by the pigments. This is in general agreement with previous workers(8-10) who found that low concentrations of pigments slightly stim-

TABLE II. Glycerol Dehydrogenase Activity Expressed as Change in Optical Density at 340 mμ.*

Pigment	Concentration .01	.10	.5
D-3-M	.08	.01	.01
P-3-M	.05	.01	.01
M-3-M	.02	0	0
Unidentified pigment	.05	.03	.01

* Control value = .04.

ulated the growth of bacteria while higher concentrations inhibited them.

According to Lin and Magasanik(16), the overall reaction involving glycerol dehydrogenase is as follows:

$$\text{glycerol} + \text{NAD} \underset{\text{dehydrogenase}}{\overset{\text{glycerol}}{\longleftrightarrow}} \text{dihydroxyacetone} + \text{NADH}_2$$

In a biological system the availability of oxygen will influence the pathway of the reaction with aerobic metabolism favoring the glycerol kinase mechanism and the anaerobic system favoring glycerol dehydrogenase system. Since anthocyanin pigments may function as oxidants they may compete with the enzyme for the substrate glycerol *in vitro* or may influence the pathway or mechanism in a biological system.

Lin and Magasanik(16) also found that zinc ions were inhibitory and that ammonium ions increased the affinity of the enzyme for the substrate. The stimulation of this enzyme by anthocyanin pigments at low concentrations could have been due to chelation of the zinc ions. Increased concentrations of pigments may have chelated ammonium ions as well, thereby inhibiting the overall reaction.

Malate dehydrogenase. All concentrations of all pigments tested, except 0.01% D-3-M, decreased the activity of malate dehydrogenase (Table III). The activity of the enzyme in the presence of 0.01% D-3-M was approximately the same as the control.

TABLE III. Malate Dehydrogenase Activity Expressed as Change in Optical Density at 340 mμ.*

Pigment	Concentration .01	.10	.5
D-3-M	.1	0	0
P-3-M	.03	0	0
M-3-M	.05	0	0
Unidentified pigment	.06	0	0

* Control value = .10.

The reaction for assay of malate dehydrogenase activity is reported by Mehler *et al* (17) to be:

$$\text{oxalacetate} + \text{NADH}_2 \underset{\text{dehydrogenase}}{\overset{\text{malate}}{\longleftrightarrow}} \text{L-malate} + \text{NAD}$$

Wolfe and Neilands(26) reported that there are approximately 7 sulfhydryl groups per mole of malate dehydrogenase. Joyce and Grisolia(27) suggested that malate dehydrogenase is a coiled protein and that the coiling is related to activity. Because anthocyanin pigments are redox compounds they may have the ability to reduce the sulfide bonds thereby partially denaturing the enzyme causing it to uncoil and lose part or all of its activity. The possibility also exists that anthocyanin pigments are in competition with the substrate oxalacetate.

Hexokinase. All concentrations of all anthocyanin pigments tested decreased hexokinase activity (Table IV). Powers(11) in a limited study also observed that hexokinase was inhibited by M-3-M. Hexokinase catalyzes carbon-6-phosphorylation of hexoses (18):

TABLE IV. Hexokinase Activity Expressed as Change in Optical Density at 560 mμ.*

Pigment	Concentration .01	.10	.5
D-3-M	.15	.13	0
P-3-M	.15	.14	0
M-3-M	.15	.14	.01
Unidentified pigment	.15	.15	.15

* Control value = .27.

$$\text{ATP} + \text{hexose} \xrightarrow[\text{hexokinase}]{} \text{ADP} + \text{hexose-6-phosphate}$$

Evidence indicates that there is a direct interaction of ATP and hexose without the formation of intermediates; however, magnesium ions are required for enzyme activity (28). The inhibitory action of anthocyanin pigments could be explained by their chelation of magnesium ions as reported by Somaatmadja *et al*(14) and by Powers(11). Because anthocyanins have reactive groups in the form of hydroxyl and methyl groups they

TABLE V. Glutamic Acid Decarboxylase Activity Expressed as Amount of CO_2 Produced.*

Pigment	Concentration .01	.10	.5
D-3-M	54.3	51.5	41.7
P-3-M	45.7	44.0	39.0
M-3-M	39.1	40.2	56.3
Unidentified pigment	33.3	40.0	85.1

* Control value = 39.0.

may have the ability to form oxygen bridges with the substrate and thus inhibit hexokinase or other enzyme systems.

Glutamic acid decarboxylase. All concentrations of all pigments tested had either no effect or stimulated the activity of glutamic acid decarboxylase (Table V). This is in agreement with Hulme and Jones(13) who reported that several anthocyanins and anthocyanidins affected both oxygen uptake and carbon dioxide production of the Krebs cycle. It is possible that anthocyanin pigments act as intermediates or coenzymes for amino acid decarboxylases such as pyridoxal phosphate as suggested by White *et al*(29). The reaction mechanism with pyridoxal phosphate involves Schiff base formation on the enzymic surface between pyridoxal phosphate and the amino acid, followed by decarboxylation as a proton from the medium replaces the carboxyl carbon in its attachment to the alpha carbon.

The effect of different concentrations of pigments on the enzyme systems studied did not always follow a definite pattern. In general, however, the activity of the enzyme was inversely proportional to the concentration of the added pigment which agrees with work by other investigators using bacteria(8-11). The effects of different anthocyanin pigments on the same enzyme systems did not differ greatly. In most cases, if one pigment increased the activity of a particular enzyme, the other pigments at the same concentrations also increased activity. This indicates that the configuration of the side chain groups on the anthocyanin molecules did not influence or distinguish the chemical properties of a particular pigment in relation to its effect on the enzyme systems studied. Further studies are needed to elucidate the exact mechanism involved in the inhibition of these enzyme systems by anthocyanin pigments.

Summary. The activities of alpha glucan phosphorylase and glutamic acid decarboxylase increased when increasing amounts of 4 anthocyanin compounds were added to the substrate. The activities of glycerol dehydrogenase, malate dehydrogenase and hexokinase decreased except at the lowest concentration, 0.01%. At this level, the tendency was to increase slightly the activity of the enzymes studied. Possible explanations for the effect of anthocyanin pigments on the enzyme systems studied are discussed.

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Received May 27, 1966. P.S.E.B.M., 1967, v124.

Effects of Metaraminol Perfusion of Kidney, Liver, or Brain on Renal Response to Saline Infusion in the Dog. (31830)

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Exaggerated natriuresis occurs in hypertensive subjects in response to infusion of sodium(1). It appears that some feature of the hypertensive state itself is responsible for the natriuretic response since this phenomenon occurs in subjects with hypertension of varying pathogeneses(2-6) and can also be induced in man(7) or dog(8) made hypertensive pharmacologically with metaraminol.

It is uncertain, however, whether the factor responsible for exaggerated natriuresis in the hypertensive dog is some local pharmacologic effect of metaraminol such as increased vascular resistance in some key organ, or if increased systemic blood pressure itself acts to promote an augmented natriuretic response. Patients with labile plood pressure may exhibit exaggerated natriuresis even while normotensive(9), and sympathectomy may inhibit an exaggerated natriuretic response even in subjects whose hypertension persists(10). These observations suggest that the exaggerated natriuretic response may be related to arteriolar constriction within some organ rather than to hypertension itself. If the local effects of metaraminol such as vasoconstriction could be induced in the absence of systemic hypertension, the role of pharmacologic effects of this drug in specific organs apart from that of hypertension itself might then be examined. In the present study, therefore, metaraminol was delivered in subpressor doses into the kidney, liver or brain of the dog while an infusion of saline was given systemically. Exaggerated natriuresis was not observed.

Methods. Sixteen mongrel dogs were studied following withdrawal of food and fluids for 18 hours. Anesthesia was induced with pentobarbital, 30 mg per kilogram, which was supplemented as necessary during study. Aqueous vasopressin, 5 units, was given subcutaneously ½ hour prior to study and was continued intravenously with the saline infusion at a rate of 4 milliunits per minute. Blood pressure was determined with a mercury manometer connected to a femoral artery catheter and arterial blood samples were withdrawn at appropriate intervals for analysis.

In 5 of these dogs the renal response to sodium infusion was examined during administration of metaraminol directly into the renal artery. The ureters were catheterized through a lower abdominal incision to obtain urine samples from the separate kidneys. The left renal artery was exposed extraperi-