known, this phosphatide contains phosphoric acid esterified with inositol suggesting it to be an anionic agent. The effects of heparin on electrophoretic mobility of lipoproteins *in vivo* remain unexplained. However, the effects on both alpha and beta lipoprotein are qualitatively the same as those seen after incubation with anionic agents *in vitro*(4).

Lipoprotein densities studied ultracentrifugally are unrelated to ionic charges of surface active agents; however, density changes suggest binding of agents by lipoproteins.

The prealbumin fraction observed after incubation with anionic agents has also been reported after injection of heparin(9) and after incubation of serum with sodium oleate(3). Reasons for the marked difference between the effects of these agents on lipid and protein patterns are not apparent. If all lipids are bound to only a small portion of total serum, protein changes in the migration of this small portion might not be detected on paper electrophoresis.

Summary. The effects of incubation of serum with various surface active agents on serum lipids and protein patterns have been studied on electrophoresis and in the ultracentrifuge. Direction of migration of lipid in electrophoresis depends upon the charge of surface active agents. Anionic surface active agents increase migration rate of both alpha and beta lipoproteins toward the anode. Cationic agents either decrease migration rate of lipids toward the anode or cause them to move toward the cathode. Non-ionic agents cause complete loss of mobility of all lipids toward either electrode. These effects could be explained by assuming that surface active agents are bound to lipoproteins and contribute their charge in the migration of these lipoproteins. Effects of surface active agents on protein patterns are less pronounced. Surface active agents either increase or decrease density of serum lipoproteins as studied in the ultracentrifuge suggesting that these agents are bound to lipoproteins. However, prediction of effects of these agents on lipoprotein densities cannot be made from knowledge of the charge present on the agent.

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Received March 17, 1958. P.S.E.B.M., 1958, v98.

Measurement of Compounds of Vit. B_6 Group in Blood.* (24029)

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Using a spectrophotofluorometric technic (1), Duggan and Udenfriend have shown that the Vit. B_6 group could be detected in concentrations as low as 0.001 μg/ml(2). We confirmed these observations, and extended the use of this technic to demonstrate effects of hydrogen peroxide and ultraviolet irradiation on these fluorescent curves. Initial studies established optimal conditions for fluorescence of these compounds to be in 0.1 molar phosphate buffer of a pH 6.75. The ideal concentration for studying activity was found in the range of a 1 micromole/l. Table I outlines the fluorescence of a 1 micromolar stand-

* This work supported in part by grant from Hoffman LaRoche Labs.

TABLE I. Fluorescent Characteristics of Compounds of Vit. B_6 Group under Selected Physical Chemical Conditions. The individual substances were of 1 micromolar concentration in phosphate buffer of pH 6.75. Photomultiplier readings at .001 sensitivity.

Physical chemical reaction	Compounds of the vit. B_6 group		
	Pyridoxine	Pyridoxamine	Pyridoxal
Activating, mμ	340	335	330
Fluorescent, mμ	400	400	385
Fluorescence, %	44	56	35
H_2O_2, 10 lambda			
3%	No effect	No effect	Partial destruction
30%	"	"	Complete
Ultraviolet			
1 min.	Partial destruction	Partial destruction	Partial destruction
5 "	Complete	Complete	Complete

ard solutions of each of the free forms of Vit. B_6 to Xenon lamp activation; after exposure to 10 lambda quantities of hydrogen peroxide (3% and 30% concentration); and after exposure to ultraviolet irradiation (1 and 5 minute duration) using a quartz mercury vapor lamp at 3 cm. Hydrogen peroxide does not affect activity of pyridoxine or pyridoxamine. However, there is partial destruction of pyridoxal by a 3% solution and complete destruction by a 30% concentration. The effects of ultraviolet light were comparable in all 3 forms with partial destruction at 1 minute and progressive deterioration with time to complete disappearance of activity at 5 minutes. These experimental data suggested that if the minute quantities of Vit. B_6 present in blood could be isolated, these procedures would facilitate their identification and quantitation. Concern over the possibility of altering the fluorescent patterns led to exploration of a number of physical chemical approaches to the problem with ion exchange columns and extraction procedures. Of these, the most successful has been with acetone extraction of heparinized fresh human whole blood.

Method. A 1 ml sample of fresh whole blood was extracted with 25 ml of reagent grade acetone in an amber glass container. The mixture was shaken 30 minutes and then evaporated to dryness. The residue was taken up in 5 ml of 0.1 molar phosphate buffer of pH 6.75. The sample was cleared either by high speed centrifugation (20,000 rpm for 30 minutes) or by a Morton sintered glass filtration. A 1 ml aliquot was activated by the Xenon lamp at 335 mμ with simultaneous scanning showing a peak value for total fluorescence at 400 mμ. A 10 lambda quantity of a 30% solution of hydrogen peroxide was added to the sample to destroy pyridoxal activity, and the reading procedure repeated. The sample was then exposed to ultraviolet irradiation for 5 minutes to destroy pyridoxamine, and fluorescent output again determined. A comparison of readings obtained at these 3 peaks with known standards that had been similarly treated provided direct quantitative measurement of pyridoxal and pyridoxamine in the blood. The possibility of pyridoxine and pyridoxic acid being present in the sample was considered. However, studies with pyridoxic acid have shown it to be excited at an activating wave length of 330 mμ with a peak fluorescence at 430 mμ. Conceivably, traces of pyridoxine could be present, but well documented experiments with microbiological assay and ion exchange columns have repeatedly failed to demonstrate the presence of this form in blood(3,4).

Results. This procedure has permitted quantitative measurement of individual members of the Vit. B_6 group in standard solutions, and in whole blood *per se*. Furthermore, it has made possible recovery of known amounts of these compounds that have been added to whole blood. Blood samples were obtained on 3 occasions from a healthy male adult to establish the normal range of Vit. B_6 activity for the patient. Under fasting conditions, he was given 30 mg of pyridoxine hydrochloride orally every 4 hours for 6 doses total 180 mg. Blood samples were obtained at 4 and 24 hours after start of medication. Fig. 1 shows the fluorescence present in patient's blood prior to medication. Total emission is represented by the solid line. The effect of 30% H_2O_2 with destruction of pyridoxal and the residual pyridoxamine is shown by dashed line. Complete destruction of activity by ultraviolet irradiation is seen in the dotted line. Fig. 2 shows change in fluores-

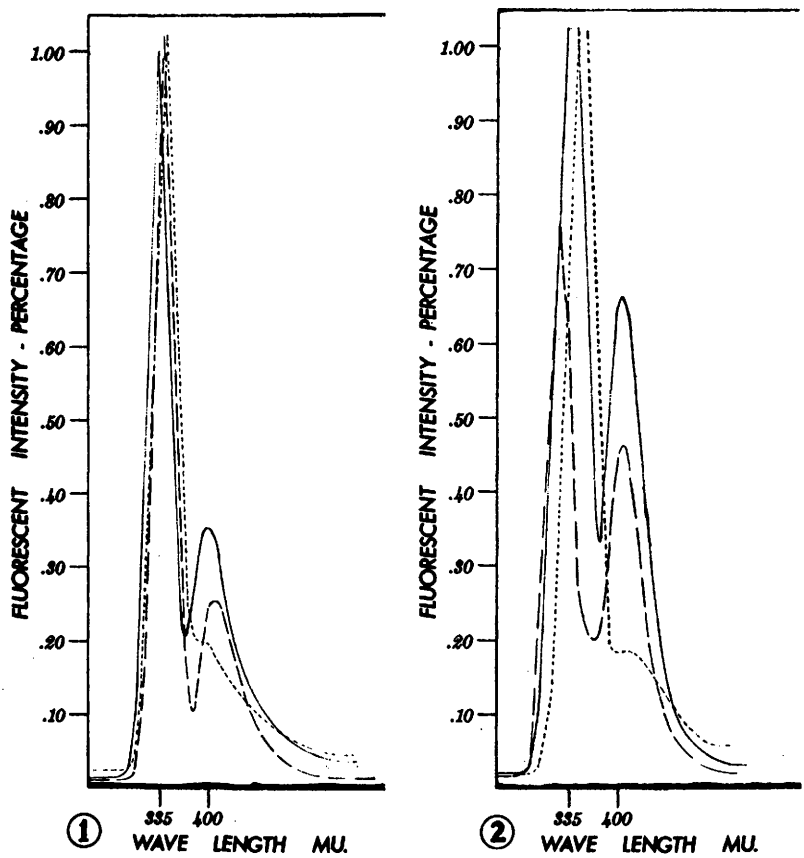

FIG. 1. Whole blood sample prior to ingestion of pyridoxine. Solid line (———) is total fluorescence transmission. Dashed line (– – –) represents fluorescence of pyridoxamine remaining after destruction of pyridoxal with 30% hydrogen peroxide. Dotted line (- - - -) is the blank produced by irradiation with ultraviolet light to destroy the pyridoxamine.

FIG. 2. Whole blood sample 4 hr after ingestion of 30 mg of pyridoxine. Solid line (———) shows increased total B_6 content. Dashed line (– – –) represents increased pyridoxamine following destruction of larger quantity of pyridoxal. Dotted line (- - - -) is residual blank after irradiation with ultraviolet light to destroy the pyridoxamine.

cence 4 hours after ingestion of 30 mg of pyridoxine. The total (solid line) is definitely increased. There are also higher values for pyridoxal and pyridoxamine as evidenced by height of curve following treatment with 30% H_2O_2 (dashed line). The effect of ultraviolet exposure (dotted line) provides a blank wave comparable to those seen before medication. These studies were done with samples that had been cleared with high speed centrifugation—leaving enough activating scatter to cause a minor effect on readings of fluorescent output. Ultrafiltration removed this material completely, and produced a zero blank.

It is of interest to note that the blood sample at 24 hours revealed curves and values slightly lower, but comparable to those for 4 hours. This would suggest that the blood transport of Vit. B_6 had reached a saturation point beyond which it could not carry additional quantities of the compounds. Concurrently, the patient's urinary excretion of pyridoxic acid increased markedly.

Summary. A study of fluorescent characteristics of pyridoxine, pyridoxal, and pyridoxamine under selected conditions has been presented. These have been applied to acetone extracts of standard solutions and whole blood with quantitative identification of all 3 components of the Vit. B_6 group. Data show that human blood contains pyridoxal and pyridoxamine. These two components can be

made to increase to a limited maximal concentration of saturation upon the oral administration of pyridoxine.

Authors express their appreciation to Drs. S. Bessman, S. Udenfriend, and W. Curtis for valuable advice.

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Received March 17, 1958. P.S.E.B.M., 1958, v98.

Decrease in Hexosamine Content of Epiphyseal Plates in Experimental Lathyrism.* (24030)

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Lathyrism is caused by ingestion of excessive amounts of sweet pea seeds (*Lathyrus odoratus*). The most characteristic lesions are deformities of bones, enlargement of epiphyseal plates, dissecting aneurysm of the aorta, and loss of weight. Research in this field has increased greatly since Ponseti *et al.* (1-2) showed the possible relations between lathyrism and many human bone diseases of unknown etiology. The earlier literature on lathyrism has been reviewed recently by Selye(3). The disease can be reproduced experimentally by injection of beta-aminopropionitrile, the active principle of sweet pea, or by aminoacetonitrile which is still more active in reproducing the bone lesions. The nature of metabolic block produced by aminonitriles remains to be elucidated. Since the most evident lesions appear in ground substance of the epiphyseal plates, some interference with mucopolysaccharide or collagen metabolism can be supposed. For this reason we studied the content of hexosamine, a component of mucopolysaccharides, and hydroxyproline, characteristic of collagen, in normal and lathyric epiphyseal plates.

Methods. Twenty rabbits, 7-10 days old, were used. Ten were injected with aminoacetonitrile sulfate,§ the others were controls. Each experimental animal received subcutaneously 10 mg of aminoacetonitrile sulfate neutralized with $NaHCO_3$, daily for 6 days. With this dosage the animals showed very severe skeletal lesions; the epiphyseal plates were very wide and distorted as described by Ponseti and Shepard(1). Longer treatment resulted in death of animals. After 6 days all rabbits were killed with ether; epiphyseal plates (about 200 mg each) were excised from proximal and distal ends of long bones (radius, ulna, tibia, femur) after removal of muscles and periosteum. The tissue was dried at 104°C to constant weight, then homogenized with 4N HCl in a Potter-Elvehjem homogenizer and refluxed at 100°C for 15 hours; a large excess of acid was used (500-600 times the quantity of tissue) to reduce humin formation. After hydrolysis, the acid solution was put in a vacuum desiccator with NaOH and $CaCl_2$ and the air evacuated. The dry residue was taken up in 25 ml H_2O. Hexosamine was determined by Schloss' method(4). Eastoe's method(5), using Dowex 50, was tried for a few hydrolysates, to separate glucosamine and galactosamine. Hydroxyproline was determined by the method of Neuman and Logan(6) according to suggestions of

* Aided by grant from Nat. Inst. of Health.

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‡ Idem Chim. Generale.

§ The aminoacetonitrile sulfate has been kindly furnished by Dr. S. Wawzonek, Chem. Dept., State University of Ia.