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# Spectrographic Studies of Human Blood

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# SPECTROGRAPHIC STUDIES OF HUMAN BLOOD

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Many of the vitamins in the blood have been investigated by chemical and biological methods including vitamin A by photo-electric colorimetric analysis (1), vitamin $B_1$ by a fermentation method (2), nicotinic acid by a microbiological assay with Shigella paradysenteriae (3), pantothenic acid by measuring the growth of such bacteria as Proteus morganii (4), and Lactobacillus casei E (5), vitamin C by 2, 6-dichlorophenolindophenol (6), (7), (8), vitamin D by a colorimetric method (9), and vitamin K by measuring the clotting power of the blood (10). According to the above methods, these vitamins are present in blood and some of them cannot be found by any other means. Furthermore many of these reactions are not specific. Moreover, the constitution of blood is so complicated that it is almost impossible to apply any chemical analysis without altering its nature. Some of its constituents are present in such minute amounts that they are beyond chemical detection. Spectrographic analysis has the advantage of permitting the detection of small amounts of material and avoiding the previous chemical separation of compounds which do not absorb. Hence it was thought worth while to apply spectroscopy to the analytical study of blood. Both arterial and venous blood samples were taken from healthy humans approximately two hours after a meal and the blood was immediately separated into plasma and red cells by centrifugation in order to prevent hemolysis. The separated plasma and red cells were kept in a refrigerator at about zero degrees centigrade until the analyses were made. Exposure to light was avoided as far as possible during all of these manipulations. Aqueous solutions of the plasma proteins and also absolute alcoholic extracts of the plasma and red cells were examined spectrographically by means of a Hilger Medium Quartz spectrograph, using a hydrogen discharge tube as light source, and a Hilger logarithmic sector photometer for determination of extinction coefficients.

## SPECTOGRAPHIC STUDIES OF PLASMA PROTEINS

It was found that the water solutions of plasma, pure serum albumin and soluble globulin gave the same absorption band at the wave-length 2800 A, as shown in Figures I, II, and III respectively. In dilute sodium hydroxide solution, the absorption band of the above-mentioned proteins was shifted to a longer wave-length, 2900 A (see Figures I, II and III). The absorption band of these proteins was not affected by dilute hydrochloric acid or by phosphate buffer at paH

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7.35. The pure serum albumin and soluble globulin were prepared by the ammonium sulfate fractional precipitation method, involving a five days' dialysis. The final product contained about 13 mg. of ammonium sulfate per 100 ml. of solution. Since the position of the absorption band is characteristic of the molecular structure of a compound or of a certain chromophoric group, the serum albumin and globulin, which were isolated from plasma, apparently contain at least one common chromophore which gives the characteristic absorption band, although their molecular structures as a whole may be different as is shown by their different extinction coefficients. This common chromophore contained in both albumin and globulin is changed to the same isomer by the action of alkaline solution.

## SPECTROSCOPY OF ABSOLUTE ETHYL ALCOHOLIC EXTRACTS OF BLOOD PLASMA

The alcoholic extracts were prepared by adding one volume off1R plasma to ten volumes of absolute ethyl alcohol. The plasma was run into the alcohol in a slow stream and the flask was rotated during the process so that a finely flocculent precipitate of the protein was obtained. The solution, including the precipitate, was refluxed for five hours on a water bath. The clear alcoholic extracts were separated from the coagulated proteins by centrifuging and they were examined spectrographically. The first absorption band as will be seen from curve 1 in Figure IV, was located between the wavelengths 2900 and 2950 A and the second between 3200 and 3250 A. The former was stronger than the latter. The absorption bands of these alcoholic extracts were similar to those of pyridoxin (vitamin $B_6$) (see curve 2 in Figure IV). That pyridoxin was actually present was confirmed as follows: (I) The alcoholic extracts gave a positive ferric chloride reactions for phenol which had been used in estimation of pyridoxin in yeast (11). (II) After the extracts had been exposed to sunlight, the bands were no longer present, indicating that the substance producing them was destroyed by light, and pyridoxin is known to be destroyed by the effect of light (12). These alcoholic extracts were stable for a long time if they were kept in a dark place. Cold alcoholic extracts which had not been heated did not give these absorption bands, indicating that the pyridoxin in the plasma is probably bound in some way and that this complex is split by heating. It was found that the free form can be recombined with or adsorbed by the coagulated proteins to a certain extent and so it seems logical to suppose that the complex in the original plasma was a protein-pyridoxin combination of some sort. The amount of pyridoxin in venous plasma was lower than in arterial plasma, indicating that the compound was probably partly taken up by the body tissues. It was found that complete extraction was not obtained in five hours, but if the rate of refluxing was fairly well controlled, the amount of the pyridoxin extracted was quite constant for the same sample. If the time of extraction was increased, the amount of extracted pyridoxin was also increased, but another band near 2800 A appeared

which was probably due to a small amount of unprecipitated protein or possibly to some other vitamins. This new band dominated the whole spectrum if the extraction was prolonged for a considerable length of time.

## SPECTROGRAPHIC STUDIES OF ABSOLUTE ETHYL ALCOHOLIC EXTRACTS OF RED BLOOD CELLS

In this case, the extracts were prepared by allowing the red cells to remain in the alcohol at room temperature over night. The cells were then removed by centrifuging. These alcoholic extracts presented an absorption band at the wave-length 2600 A which was similar to that of nicotinic acid and the codehydrogenases as shown in curves 1 and 2 in Figure V. That nicotinic acid was present was confirmed by the cyanogen-bromide and p-amino acetophenone reaction which develops a yellowish-green color with free nicotinic acid. For details of the procedure, see Kodicek (13). No difference was observed between the arterial and venous blood cell extracts.

## SUMMARY

1. Proteins: Plasma, pure serum albumin and globulins give an ultra-violet absorption spectrum at the wave-length 2800 A in water solution, and at 2900 A in dilute hydroxide solution. The absorption spectrum of these proteins was not affected by dilute hydrochloric acid or phosphate buffer solution, paH 7.35. Therefore, the pure serum albumin and globulins which were isolated from plasma must contain at least one common chromophore or group which is changed to the same isomer by action of dilute sodium hydroxide solution. The molecular structures as a whole may be different, however, as is shown by the different extinction coefficients.

2. Pyridoxin: Pyridoxin was found in hot ethyl alcoholic extracts of blood plasma. The amount of pyridoxin is larger in arterial blood plasma than in venous blood plasma, probably due to utilization or storage by the body tissue. Pyridoxin in the blood plasma is bound to the proteins, but the free form was obtained by heating. Five hours' extraction with ethyl alcohol was found to be the most suitable. The extracted form can be recombined with or adsorbed on the coagulated protein precipitates to a certain extent.

3. Nicotinic Acid: Nicotinic acid, its amide and the codehydrogenases were found in the cold ethyl alcohol extracts of the red blood cells by spectrographic examination.

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LITERATURE

1. Yudkin, S., Biochem. J. 35, 551-56 (1941).
2. Goodheart, R., and Nitzberg, T. J., Chem. Investigation, 20, 625-30 (1941).
3. Isbell, H., Wooley, J. G., Butter, R. E., and Sebrell, W. H., J. Biol. Chem., 139, 499-510 (1941).
4. Pelczar, M. J., Jr., and Porter, J. R., Proc. Soc. Exptl. Biol. Med., 47, 3-7 (1941).
5. Pearson, P. B., J. Biol. Chem., 140, 423-26 (1941).
6. King, C. G., Ind. Eng. Chem., Anal. Ed., 13, 225-27 (1941).
7. Ott, M., Angew, Chem., 54, 170-75 (1941).
8. Kuether, C. A., and Roe, J. H., Proc. Soc. Exptl. Biol. Med., 47, 487-89 (1941).
9. Nield, C. H., Russel, W. C., and Zimmerli, A., J. Biol. Chem., 136, 73-79 (1940).
10. Evans, E. A., "The Biological Action of the Vitamins, A Symposium," p. 204, the University of Chicago Press, Chicago, Illinois (1942).
11. Greene, R. D., J. Biol. Chem., 130, 513 (1939).
12. Rosenberg, H. R., "Chemistry and Physiology of the Vitamins" p. 200, Interscience Publishers, Inc., New York, N. Y., (1942).
13. Kodicek, E., Biochem. J., 34, 713, 724, (1940).

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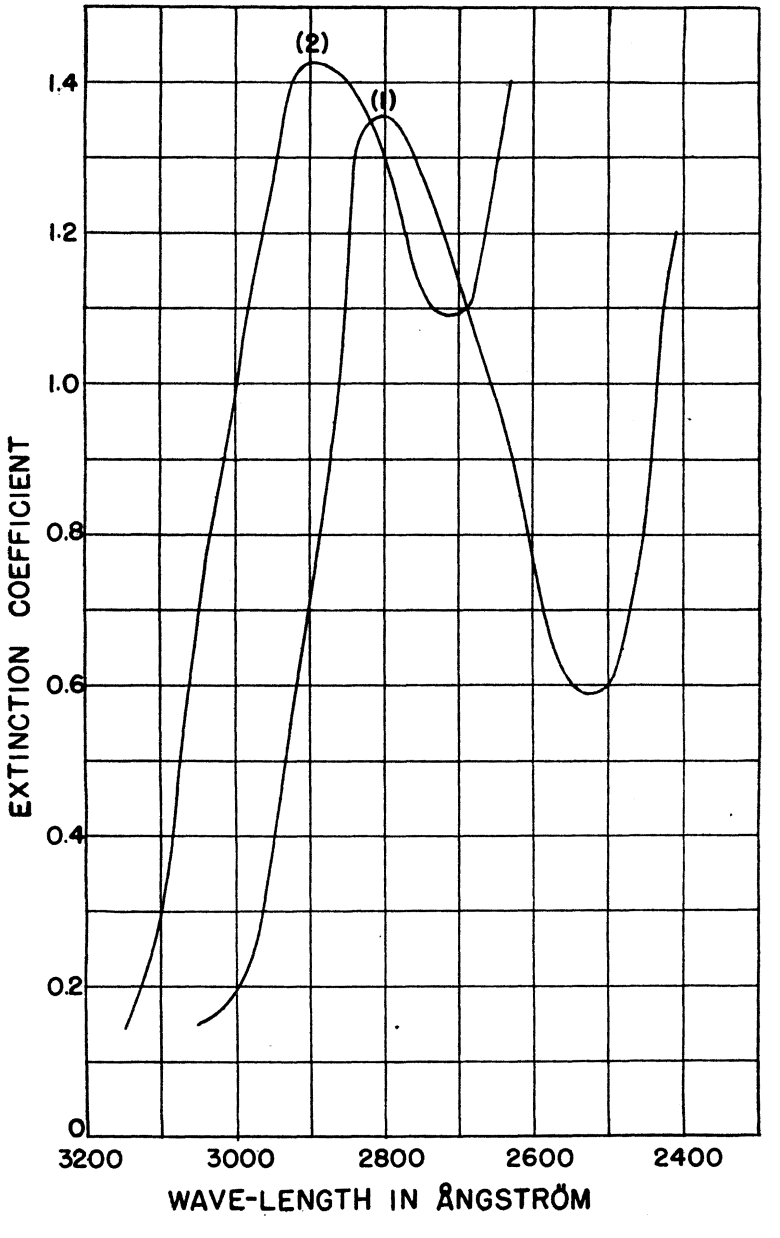


FIGURE 1

CURVE 1, WATER SOLUTION OF BLOOD PLASMA

CURVE 2, IN. NaOH SOLUTION OF BLOOD PLASMA

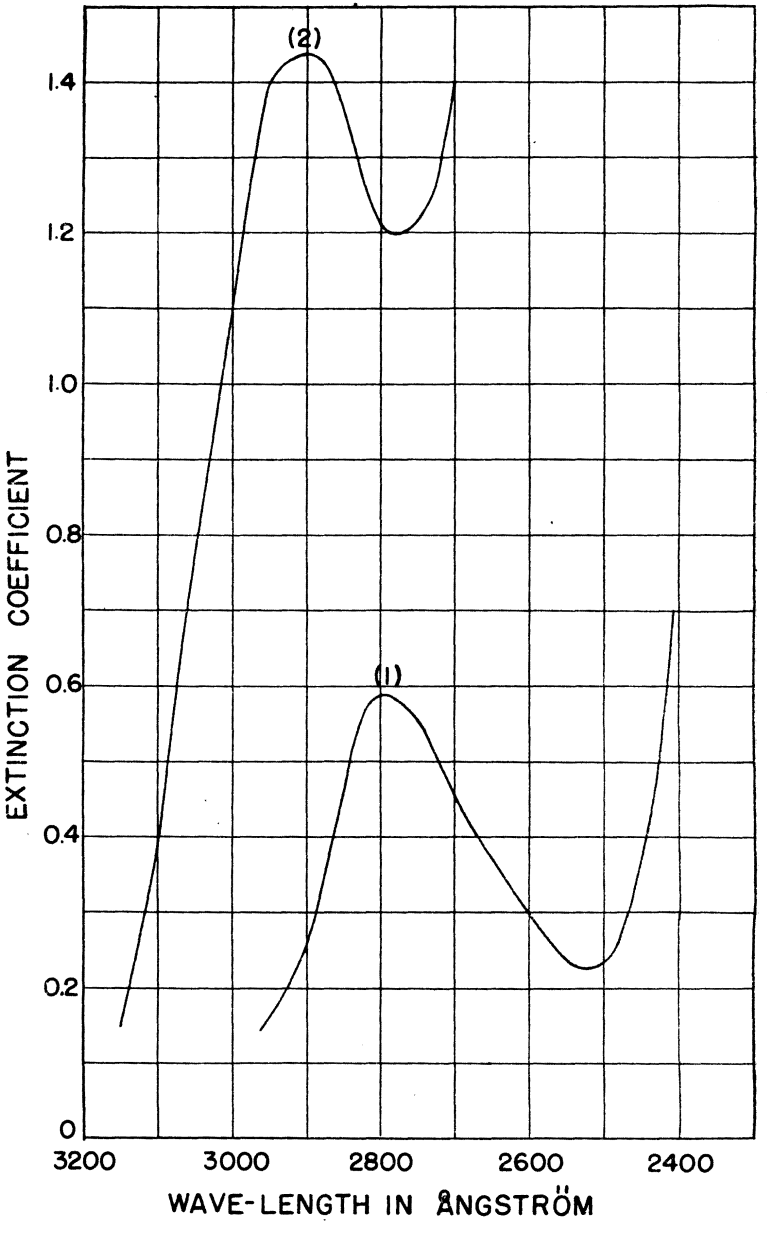


FIGURE 11

CURVE 1, 0.1% SERUM ALBUMIN IN WATER SOLUTION

CURVE 2, 0.15% SERUM ALBUMIN IN 5% NaOH SOLUTION

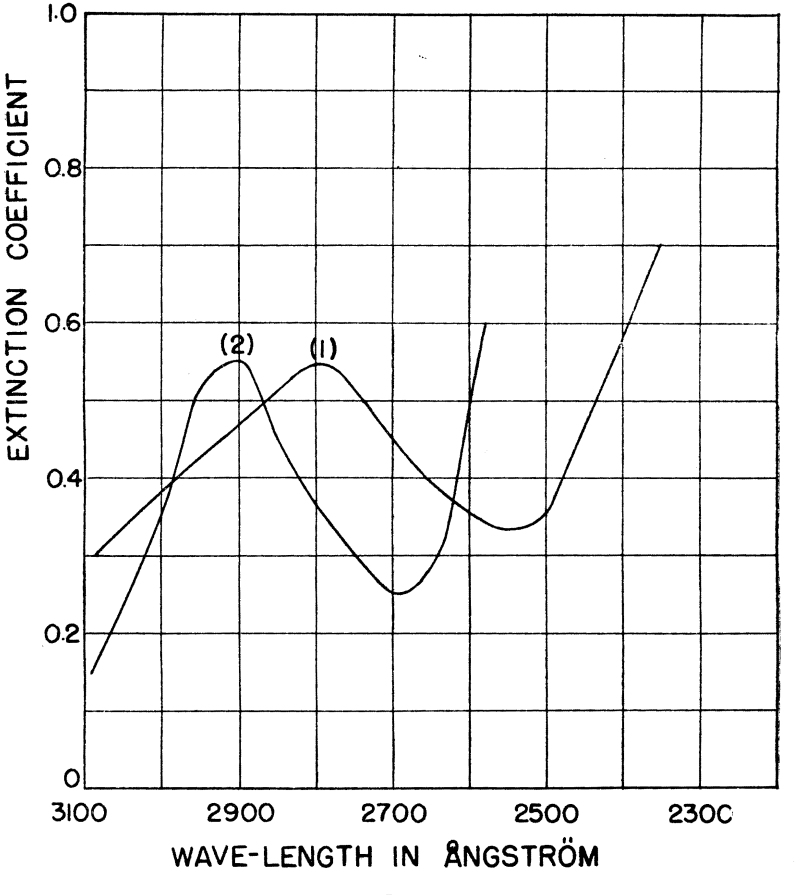


FIGURE III

CURVE 1, .02% SERUM GLOBULIN IN WATER SOLUTION

CURVE 2, .02% SERUM GLOBULIN IN 5% NaOH SOLUTION

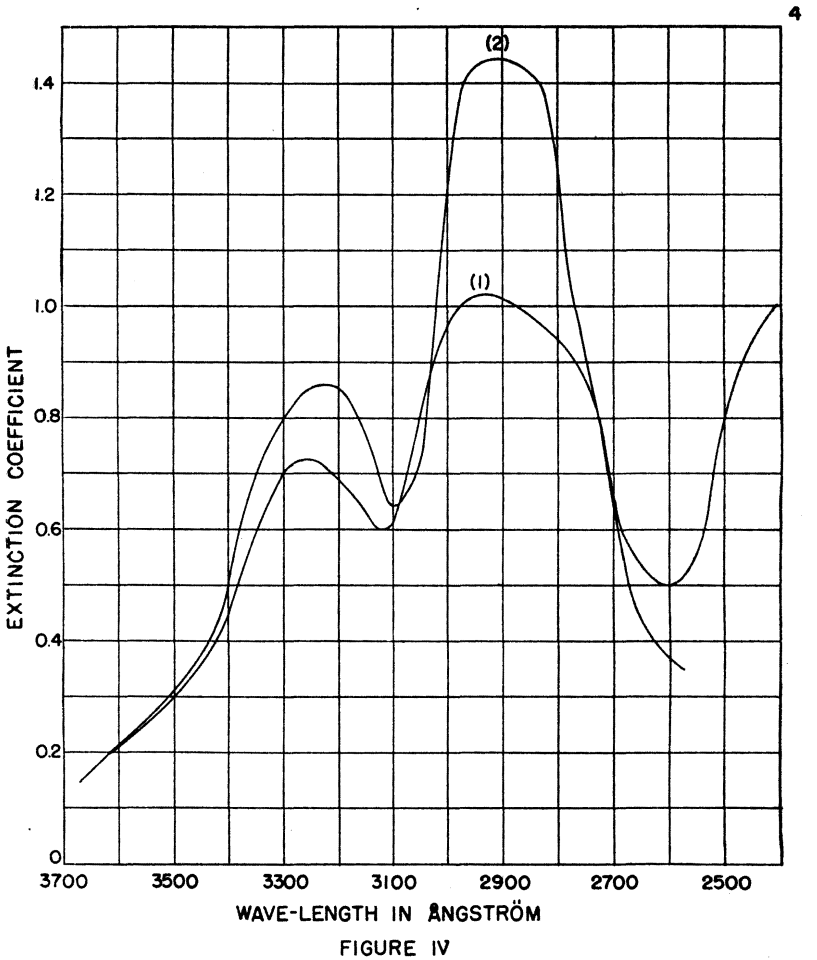


FIGURE IV

CURVE 1, ALCOHOLIC EXTRACT OF ARTERIAL BLOOD PLASMA
CURVE 2, PURE PYRIDOXIN HYDROCHLORIDE IN 91% ALCOHOL

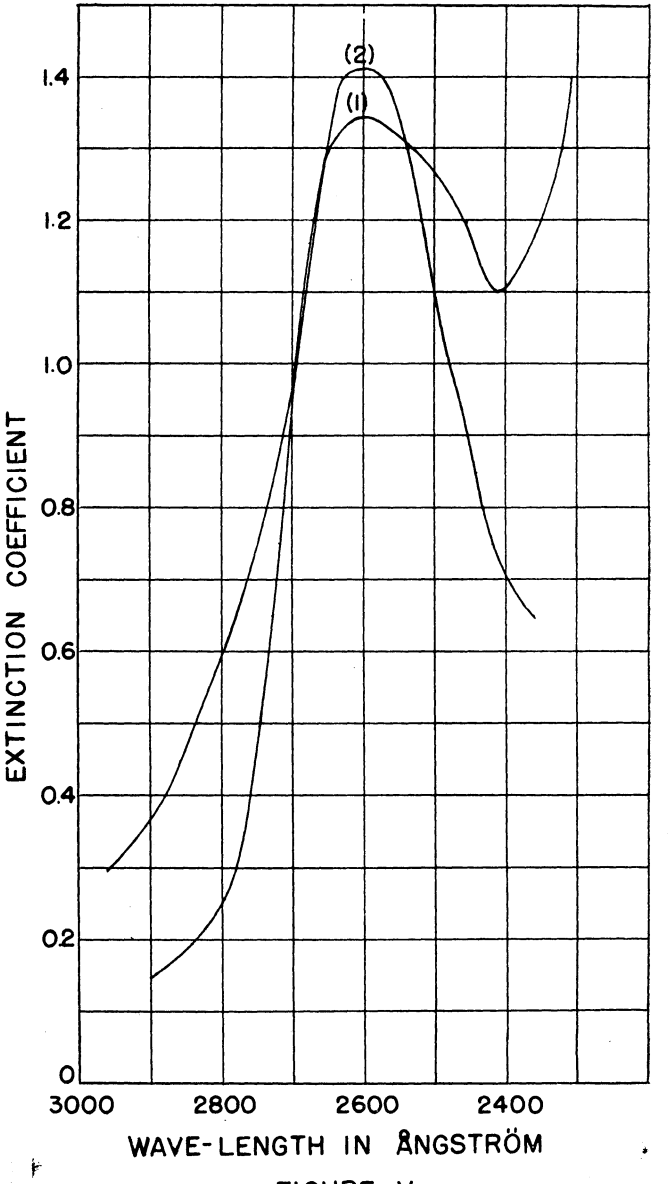


FIGURE V

CURVE 1, ALCOHOLIC EXTRACT OF ARTERIAL RED BLOOD CELL

CURVE 2, NICOTINIC ACID IN ALCOHOL SOLUTION