FREE ERYTHROCYTE PROTOPORPHYRIN [1]

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INTRODUCTION

Porphyrins are widely distributed in living cells throughout nature and play essential roles in various metabolic processes such as photosynthesis, transportation of oxygen and cellular respiration. In man the most important of the porphyrins is protoporphyrin 9, type III, which in combination with iron and specific proteins forms such compounds as hemoglobin, myoglobin, cytochrome, peroxidase and catalase. In addition to its occurrence in these compounds, protoporphyrin has been found in apparently uncombined or free form in erythrocytes, hence the term "free erythrocyte protoporphyrin" (1–3).

Cartwright and associates (4) recently have summarized many of the salient features of previous work concerning free erythrocyte protoporphyrin. Suffice it to say here that the origin, function and fate of free protoporphyrin in erythrocytes remain uncertain. Indirect evidence suggests that it serves as an intermediary in synthesis of hemoglobin. Increased free erythrocyte protoporphyrin is thought to result from an interruption in normal synthesis of hemoglobin, as in the presence of iron deficiency states, increased erythropoiesis, or a disturbance of iron metabolism (5). Decreased levels have been ascribed to a deficiency in synthesis of protoporphyrin (6). There is evidence that reticulocytes contain more free protoporphyrin than do mature erythrocytes (7, 8).

This investigation has been concerned with the study of amounts of free protoporphyrin [2] in erythrocytes of normal subjects, in patients who had diseases of the liver and biliary tract, in patients who had a variety of other clinical conditions, and with certain details of the method of determination.

MATERIAL AND METHODS

All subjects were studied thoroughly in the various medical sections and hospital services of the Mayo Clinic. The studies included a medical history, physical examination, roentgenograms of the thorax, urinalysis, serologic test for syphilis, leukocyte, erythrocyte and reticulocyte counts, determination of hemoglobin, determination of volume of packed erythrocytes, and examination of the peripheral blood smear. Other tests appropriate to the patient's complaint or illness also were done.

The concentration of free erythrocyte protoporphyrin was determined by the method of Grinstein and Watson (10), with slight modification. Five ml. of oxalated whole blood were centrifuged in a test tube and the plasma was removed. Seven ml. of the mixture of ethyl acetate and glacial acetic acid were added to the cells and the mixture then was homogenized with a large stirring rod to make a very fine suspension. An additional 18 ml. of the ethyl acetate-acetic acid mixture were used to transfer quantitatively this suspension to a glass stoppered flask. The flask and its contents were shaken for one and a half minutes, then were allowed to stand for ten minutes. The contents of the flask were filtered by gentle suction, and the precipitate was washed five times with 5 ml. portions of the extraction mixture. Throughout the remainder of the determination the volumes of reagents were reduced to half those employed in the procedure as originally described by Grinstein and Watson (10), except that the volume of 5 per cent hydrochloric acid used in the final extraction of protoporphyrin was sufficient to yield 50 ml. of solution. The volume of erythrocytes in 5 ml. of whole blood was calculated from the results of a determination of the volume of packed erythrocytes obtained by the method of Sanford and Magath (11). The amount of protoporphyrin in the final 50 ml. of 5 per cent solution of hydrochloric acid was estimated by comparing its strength of fluorescence with that of a 5 per cent solution of hydrochloric acid containing 0.03 microgram of coproporphyrin per milliliter. A Pfaltz and Bauer fluorophotometer was employed for this purpose and electric current was supplied through a constant voltage regulator.

The dimethyl ester of protoporphyrin was prepared by the method of Grinstein (12). Two crystallizations from

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[2] It has recently come to our attention that Schwartz and Watson (9) have identified coproporphyrin in erythrocytes. The method used in this study for determination of protoporphyrin would include any coproporphyrin that might be present. The term "erythrocyte porphyrin" would perhaps be more appropriate. Data have not yet been reported as to the relative amount of coproporphyrin in the erythrocytes of individuals in health or disease.

chloroform-methanol yielded dark reddish-purple crystals which melted sharply at 227°C. Solutions of protoporphyrin were prepared by dissolving a weighed quantity of the dimethyl ester in 25 per cent hydrochloric acid and allowing it to stand for six hours to effect hydrolysis. Subsequent dilution with appropriate amounts of distilled water and 5 per cent hydrochloric acid yielded solutions of known concentrations of protoporphyrin in 5 per cent hydrochloric acid.

Eight solutions of protoporphyrin in 5 per cent hydrochloric acid were diluted serially with 5 per cent hydrochloric acid and the fluorescence of each solution was measured in the fluorophotometer. The initial concentrations of porphyrin were 0.0272 to 0.0392 microgram per milliliter and in the dilutions it was carried to concentrations of 0.0045 to 0.0094 microgram per milliliter. A linear relationship was found between concentration and strength of fluorescence over this range of concentration. However, since solutions of protoporphyrin are unstable, they are not satisfactory for use as standards, but coproporphyrin serves admirably for this purpose. Coproporphyrin, itself, deteriorates slowly when exposed for periods of time to ultraviolet light; therefore, fresh dilute standard solutions were prepared at frequent intervals from stock solutions containing 1 mg. of coproporphyrin per 100 ml. The stock solutions were kept in the dark, as were also the dilute standard solutions except when in use. Previous experience has shown that such stock solutions do not deteriorate appreciably within the period of time of this study. Standard solutions of coproporphyrin at the time of disposal did not show diminution in strength of fluorescence when compared with freshly prepared standard solutions of coproporphyrin.

Comparison was made between the strength of fluorescence of various solutions of coproporphyrin and protoporphyrin. These solutions were prepared to contain 0.03 microgram of protoporphyrin per milliliter. The fluorescence of solutions of protoporphyrin in 5 per cent hydrochloric acid exceeded that of the solutions of coproporphyrin by 1.19 times. However, the molecular weight of coproporphyrin exceeds that of protoporphyrin by 1.164 times. It is evident therefore that molecule for molecule the amount of fluorescence of the two porphyrins is very nearly the same. The small apparent difference may be well within the limits of error of the experimental procedures.

The final solutions of erythrocyte porphyrin which were placed in the fluorophotometer were not simple solutions of protoporphyrin in 5 per cent hydrochloric acid but were 5 per cent solutions of hydrochloric acid which had become saturated with ether in the process of extraction. It was found that solutions containing protoporphyrin in 5 per cent hydrochloric acid which had been saturated with ether exceeded the fluorescence of solutions of protoporphyrin in 5 per cent hydrochloric acid alone by 1.044 times. This discrepancy may have resulted from dilution of hydrochloric acid since it has been shown by others (10) that the strength of fluorescence of protoporphyrin in hydrochloric acid increases progressively as the concentration of hydrochloric acid decreases. Direct comparison showed that the fluorescence of solutions of protoporphyrin in 5 per cent hydrochloric acid saturated with ether exceeded the fluorescence of solutions of coproporphyrin in hydrochloric acid by 1.25 times. Consequently the factor 0.80 ($1 \div 1.25$) was introduced into the calculations since the standard used was coproporphyrin in 5 per cent hydrochloric acid.

To test the completeness of extraction from erythrocytes of free protoporphyrin, the once-extracted precipitate of erythrocytes, which is normally discarded after being washed with the extraction mixture, was returned to a test tube and extraction was repeated in the usual manner. The determination of free protoporphyrin was carried out in the routine fashion on the material obtained by this second extraction. Seven such experiments were performed. Amounts of protoporphyrin recovered by a second extraction varied from 0.5 to 1.5 micrograms per 100 ml. of erythrocytes, the average being 1 microgram. The amount recovered by a second extraction averaged 2.3 per cent of the amount obtained by the routine or first extraction.

To test the completeness of recovery of added protoporphyrin, the following study was performed on 16 samples of blood: the erythrocytes from 5 ml. of whole blood were extracted with the mixture of ethyl acetate and glacial acetic acid in the customary manner, and the material was filtered. The filtrate was freed of all naturally occurring free protoporphyrin by repeated extractions with 10 per cent hydrochloric acid. To this protoporphyrin-free filtrate was added a known amount of prepared protoporphyrin (0.1, 0.15 or 0.3 ml. of a 5 per cent hydrochloric acid solution containing 10 micrograms of protoporphyrin per milliliter). Solid sodium acetate was added in sufficient quantity to neutralize all hydrochloric acid present, and the mixture was agitated. The routine determination for free erythrocyte protoporphyrin was carried on from that point. Recovery of added protoporphyrin ranged from 94.2 to 100 per cent; the average recovery was 96.2 per cent.

Determinations of the quantities of free erythrocyte protoporphyrin were made in duplicate on 29 different samples of blood. Differences between results of duplicates in these 29 samples ranged from 0 to 7 per cent of the higher of the two values. The average difference was about 3 per cent, corresponding to a standard deviation, measured relatively, of about 2.5 per cent.

In nine other instances determinations were done in duplicate except that the erythrocytes of one part were washed once with 5 ml. of 0.9 per cent saline solution whereas those of the second part were not washed. In this series, differences ranged from 0 to 6.3 per cent. Neither of the two groups consistently exhibited higher values. The average difference was 3.8 per cent.

To determine the contribution to strength of fluorescence of small amounts of plasma which might contaminate unwashed erythrocytes a group of determinations were done for which 2 to 5 ml. of plasma were substituted for the erythrocytes in the usual determination. A final volume of 20 ml., instead of 50 ml., was used.

Values obtained are presented in Table I. For purposes of comparison the results are expressed as fluorescence equivalent to that yielded by a quantity of protoporphyrin, although it is most unlikely that any protoporphyrin was present under the circumstances. Studies were not done to establish the identity of the porphyrin responsible for the red fluorescence yielded by these samples of plasma. Previous studies of plasma porphyrins would suggest coproporphyrin as the most likely possibility (13–18).

These studies of the method enable one to estimate the accuracy of the determination. The completeness of extraction of the free protoporphyrin from erythrocytes, the good recovery of added protoporphyrin and the correspondence of values of duplicate determinations suggest that the total error due to factors discussed is less than 10 per cent. The necessity for thorough homogenization of the erythrocytes during the extraction process should be emphasized; also, the use of a suction filter afforded a marked saving of time. In the procedure employed in this study the erythrocytes were not washed except in cases of porphyria, lead poisoning or jaundice. It was felt that the results of the experiments in which the effect of washing of the cells was studied and the study of the plasma fluorescence, as well as consideration of the previously reported studies of plasma fluorescence (13–18), justified this omission.

Determinations of the quantity of free erythrocyte protoporphyrin were made on the blood of 77 normal adults, 26 patients who had obstructive jaundice, 28 patients who had parenchymatous disease of the liver, and 44 patients who had various other clinical conditions.

RESULTS

Values for free erythrocyte protoporphyrin for 77 normal adults ranged from 13 to 139 micrograms per 100 ml. of erythrocytes. The frequency distribution is shown in Figure 1. The mean was 43.0 ± 2.4 micrograms,[3] and the standard deviation was 21 micrograms. If twice the standard deviation is applied as the "normal variability," the upper limit of normal would be placed at 85 micrograms. Such limitation of "normal variability" is, of course, arbitrary, but has been found a satisfactory working rule. For three individuals in this group values were found to exceed 85 micrograms. These might be explained as the occasional extreme variations found in any normal series, or they may represent individuals whose free erythrocyte protoporphyrin values were elevated by some pathologic condition the presence of which was not recognized at the time of examination. It is possible that mild chemical toxicity, including in some individuals the use of alcohol, is capable of elevating the erythrocyte protoporphyrin value without causing any disturbance recognizable on the medical examination of these subjects.

In this normal group were 40 men; their values ranged from 13 to 92 micrograms and the mean was 44 micrograms per 100 ml. of erythrocytes. For the 37 women in the group, the range was 17 to 139 micrograms and the mean was 42 micrograms. Determinations were done on different days over a period of six weeks on blood from a normal individual; values were 43, 40, 43, 43, 40, 42, 40 and 39 micrograms per 100 ml. of erythrocytes.

TABLE I

Fluorescence yielded by samples of plasma subjected to the procedure for determination of free erythrocyte protoporphyrin: 17 cases studied

Diagnosis	Fluorescence calculated as protoporphyrin,* *micrograms per 100 ml. of plasma*
Normal	0.2
Normal	0.4
Normal	0.5
Normal	0.4
Normal	0.4
Cirrhosis	2.0
Cirrhosis	1.9
Obstructive jaundice; carcinoma of common duct	5.6
Obstructive jaundice; metastatic carcinoma	4.6
Obstructive jaundice; carcinoma of pancreas	5.1
Obstructive jaundice; stricture of common duct	4.0
Obstructive jaundice; stone in common duct	2.0
Obstructive jaundice; stone in common duct	1.7
Anorexia nervosa	0.2
Chronic porphyria	8.4
Chronic porphyria	4.4
Hemolytic anemia with jaundice	4.6

* This does not imply that protoporphyrin actually was present; the fluorescence obtained from these samples of plasma probably was yielded by coproporphyrin.

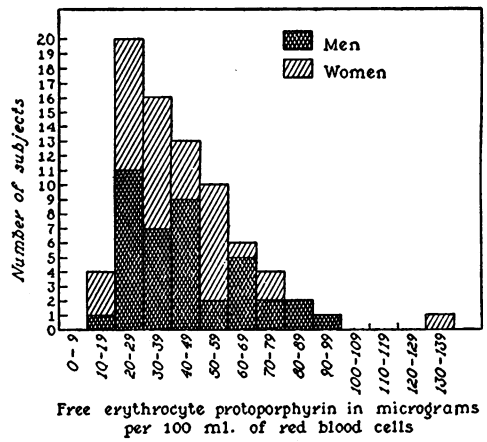

FIG. 1. DISTRIBUTION OF VALUES OF FREE ERYTHROCYTE PROTOPORPHYRIN IN 77 NORMAL PERSONS

[3] The figure 2.4 represents the standard error of the mean.

TABLE II

Values of free erythrocyte protoporphyrin in cases of obstructive jaundice and in cases of parenchymatous liver disease

Diagnosis	Cases	Free erythrocyte protoporphyrin, *micrograms per 100 ml. of erythrocytes*
Obstructive jaundice		
Stricture of common duct	12	23, 24, 33, 35, 36, 47, 49, 49, 55, 61, 65, 71
Stricture and stones of common duct	2	35, 35
Stone in common duct	5	27, 34, 45, 51, 73
Malignant lesions obstructing common duct	6	27, 29, 44, 52, 61, 128
Cholecystitis	1	31
Parenchymatous liver disease		
Cirrhosis	14	33, 33, 36, 39, 43, 45, 49, 60, 72, 93, 98, 138, 141, 249
Cholangiolitic cirrhosis	2	38, 58
Infectious hepatitis	5	30, 35, 37, 55, 205
Serum hepatitis	3	22, 40, 46
Hemochromatosis	1	42
Melano-epithelioma metastatic to liver	1	29
Chronic constrictive pericarditis with jaundice	1	50
Constitutional hepatic dysfunction	1	20

In 26 cases of obstructive jaundice values for free erythrocyte protoporphyrin ranged from 23 to 128 micrograms per 100 ml. of erythrocytes (Table II and Figure 2), the mean being 47 micrograms. Of these 26 patients, one exhibited a rather high value of 128 micrograms. This man had abdominal carcinomatosis with severe anemia as well as obstructive jaundice; therefore he may have been subject to factors other than obstructive jaundice which might alter his free erythrocyte protoporphyrin level. For the other 25 patients values ranged from 23 to 73 micrograms, the mean being 44 micrograms and the standard deviation being 15 micrograms. In two cases the determination was repeated after surgical relief of jaundice; no significant change was observed. In six cases it was thought that considerable damage to the parenchyma of the liver had occurred as the result of prolonged obstruction. In these cases the values of free erythrocyte protoporphyrin were 44, 71, 49, 35, 45, and 65 micrograms.

In 28 cases of parenchymatous disease of the liver without evidence of extrahepatic obstruction values for free erythrocyte protoporphyrin ranged from 20 to 249 micrograms per 100 ml. of erythrocytes, with an average of 66 micrograms (Table II and Figure 2).

It will be noted that the values in four cases were considerably higher than in the other 24 cases of parenchymatous hepatic disease. If these four cases are eliminated, the mean for the remainder is 46 micrograms and the standard deviation 19 micrograms.

Values for free erythrocyte protoporphyrin in 44 cases of other clinical conditions are presented in Table III.

In two cases of previously untreated pernicious anemia intramuscular injections of vitamin B_{12} were given. In one of these the level of free erythrocyte protoporphyrin before treatment was 44 micrograms per 100 ml. of erythrocytes. Under treatment the level rose to 375 micrograms during the phase of reticulocytosis, then fell to 38 micrograms as treatment was continued past that phase. In the second case the pretreatment level was 27 micrograms. Two determinations were done subsequently during the phase of reticulocytosis. The first of these, at the peak of the reticulocytosis (31.7 per cent reticulocytes), yielded a value of 81 micrograms; the second, two weeks later, after the reticulocyte count had fallen to 4.0 per cent, yielded 120 micrograms.

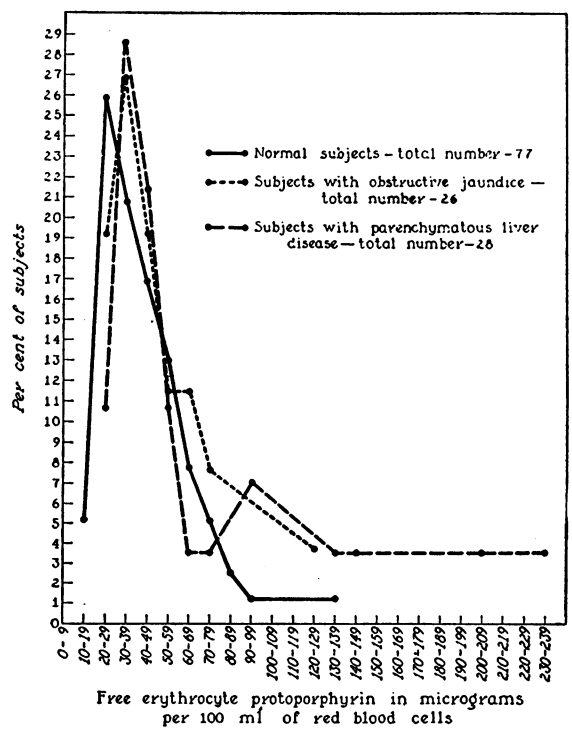

FIG. 2. COMPARISON OF DISTRIBUTION OF CONCENTRATIONS OF FREE ERYTHROCYTE PROTOPORPHYRIN IN NORMAL SUBJECTS, PATIENTS WHO HAD OBSTRUCTIVE JAUNDICE AND PATIENTS WHO HAD PARENCHYMATOUS HEPATIC DISEASE

TABLE III

Values of free erythrocyte protoporphyrin in patients who had miscellaneous diseases

Diagnosis	Number of cases	Free erythrocyte protoporphyrin, *micrograms per 100 ml. of erythrocytes*
Acute leukemia	5	21, 25, 48, 55, 68
Acute lupus erythematosus	4	28, 59, 77, 85
Myxedema	5	31, 43, 49, 72, 119
Nontropical sprue	4	52, 119, 122, 232
Pernicious anemia	4	23, 26, 27, 44
Postgastrectomy microcytic, hypochromic anemia	1	137
Postgastrectomy macrocytic, normochromic anemia	2	19, 35
Chronic porphyria	2	36, 54
Acquired hemolytic anemia	2	72, 198
Paroxysmal cold hemoglobinuria	1	40
Congenital hemolytic anemia	1	39
Hemolytic anemia secondary to acute leukemia	1	52
Lymphoblastoma	1	58
Myelosclerosis	1	57
Multiple myeloma	1	62
Essential thrombocytopenic purpura	1	66
Anorexia nervosa	1	34
Myelophthisic anemia	1	111
Aplastic anemia	1	40
Carcinoma of lip	1	110
Hypochromic anemia	4	82, 91, 113, 144

COMMENT

In our series of 77 normal adults, the mean and the variation of values of free erythrocyte protoporphyrin correspond reasonably well with those found by Cartwright and co-workers (4) in a series of 66 normal subjects whose levels ranged from 13 to 140 micrograms, with a mean of 35 micrograms. These results are in general agreement with those obtained by Watson, Grinstein and Hawkinson (3), although variability in their series appeared to be less. Earlier workers have reported values for normal free erythrocyte protoporphyrin which in general tend to be lower and to have a narrower range than those reported herein. The methods employed by those groups doubtless did not have as high a degree of accuracy as the present method, and, with the exception of van den Bergh and Grotepass (19), their results were based on rather small series. In Table IV the normal values for free erythrocyte protoporphyrin given by various investigators are compared (3–5, 19–23). In these normal subjects no correlation could be found between free erythrocyte protoporphyrin and such factors as the amount of hemoglobin, percentage of reticulocytes, volume of packed erythrocytes, appearance of the blood smear, and age or sex of the subjects.

Previous studies of the amount of free erythrocyte protoporphyrin in cases of disease of the liver and biliary tract have not been extensive (3, 4, 21–24). It might be anticipated that, if the liver has a part in synthesis of protoporphyrin, the quantity of free erythrocyte protoporphyrin might be diminished in association with hepatic disease. Earlier work, however, has not suggested such an effect. In the present study the values of free erythrocyte protoporphyrin in cases of obstructive jaundice did not differ significantly from those of normal individuals (Figure 2). Hence it might be concluded that the presence or absence of jaundice, in itself, does not exert an appreciable effect on the level of free erythrocyte protoporphyrin.

In cases of parenchymatous disease of the liver due to portal cirrhosis, cholangiolitic cirrhosis, hemochromatosis, homologous serum jaundice, infectious hepatitis, metastatic melano-epithelioma, chronic constrictive pericarditis, and constitutional hepatic dysfunction, the values of free erythrocyte protoporphyrin varied considerably. In most of the cases values corresponded to those for normal subjects (Figure 2). In a few cases the values appeared to be significantly high. In none were there values which could be considered abnormally low. These results suggest that hepatic disease does not cause obvious alteration of level of free erythrocyte protoporphyrin in most instances. Even severe degrees of hepatic damage did not result in a decrease in the concentration of

TABLE IV

Values of free erythrocyte protoporphyrin in normal subjects

Series	Number of subjects	Free erythrocyte protoporphyrin, *micrograms*			
		Per 100 ml. of erythrocytes		*Per 100 ml. of blood*	
		Range	Mean	Range	Mean
van den Bergh and Grotepass (19)	?			2–12	?
Grotepass and Defalque (20)	?			10–15	?
Angeleri and Vigliani (21)	3	11–20	16	4–8	6
Schumm (22)	6			11–17	13
Lageder (23)	13			10–120	60*
Seggel (5)	7	16–47	30	7–18	12
Watson and associates (3)	12	19.7–45.7	30		
Cartwright and associates (4)	66	13–140	35		
Present series	77	13–139	43		

* This value is estimated from data presented in a chart in Lageder's paper (23).

free erythrocyte protoporphyrin. It may not be justified to conclude from this fact that the liver does not have a role in synthesis of protoporphyrin, since it is conceivable that the liver might be able to carry out its part, if any, in this process despite serious impairment of many of its other functions. Furthermore, serial determinations before, during and, if possible, after parenchymatous disease of the liver might reveal changes which are not made evident by a single determination.

Evaluation of the occurrence of concentrations of free erythrocyte protoporphyrin which seem to exceed those found in normal subjects is difficult. In some cases this elevation may be related to the increase seen in iron-deficiency and blood-loss anemias, since some of the patients with hepatic disease had suffered loss of blood. The patient with cirrhosis who had the highest level of free erythrocyte protoporphyrin among the patients with hepatic disease had had bleeding from esophageal varices. That the explanation is not so simple in all instances is indicated by the fact that the second highest value was obtained from a patient with acute infectious hepatitis in which there was no bleeding nor any evidence of anemia.

The nature of the disease process affecting the liver did not appear important in determining the level of free erythrocyte protoporphyrin, nor could any correlation be found between that level and the results of the various tests of liver function, the severity of the anemia, the degree of reticulocytosis, or the presence of macrocytic erythrocytes in the blood smear.

The finding in pernicious anemia of increased free erythrocyte protoporphyrin during the phase of reticulocytosis resulting from treatment with vitamin B_{12} bears obvious relationship to similar findings in cases of pernicious anemia treated with liver extract or folic acid (3–5).

In three of the four cases of nontropical sprue the values of free erythrocyte protoporphyrin were greatly in excess of the normal mean, although only one value exceeded the highest value found in a normal subject. Certainly the average in the four cases, 131 micrograms, is far in excess of the normal mean, 43 micrograms. In this small series of cases it was not possible to correlate the value of erythrocyte protoporphyrin with any of the clinical or laboratory data bearing upon the severity of the disease. All four patients exhibited macrocytic anemia.

The concentration of free erythrocyte protoporphyrin was determined a number of times in the same subject over a period of six weeks. There was very little difference between any of the values obtained. This observation is in agreement with the results of repeated determinations reported by Schumm (25) and by Cartwright (4). Frequent determinations on a subject during the course of a single day have not been reported. It would not be anticipated that much variation would be found.

Values of free erythrocyte protoporphyrin in cases of acute disseminated lupus erythematosus, acute leukemia, lymphoblastoma, myelosclerosis, multiple myeloma, aplastic anemia, myxedema, essential thrombocytopenic purpura, anorexia nervosa, postgastrectomy macrocytic anemia, and chronic porphyria fell within the range of values exhibited by normal subjects. It should be pointed out that the number of cases in each of these groups was not great, that serial determinations were not done, and that the level prior to onset of the illness was not known. Therefore it cannot be stated that these diseases had no effect on free erythrocyte protoporphyrin.

SUMMARY

Results of re-extraction and recovery experiments and of duplicate determinations indicate that the error in the determination of free erythrocyte protoporphyrin by the method employed probably does not exceed 10 per cent.

In 77 normal adults values for free erythrocyte protoporphyrin ranged from 13 to 139 micrograms per 100 ml. of erythrocytes. The mean was 43 micrograms, and the standard deviation was 21 micrograms. The possible significance of values exceeding two times the standard deviation was discussed.

In 26 cases of obstructive jaundice values for free erythrocyte protoporphyrin ranged from 23 to 128 micrograms per 100 ml. of erythrocytes. The mean was 47 micrograms. The range in 25 of these cases was 23 to 73 micrograms, with a mean of 44 micrograms and a standard deviation of 15 micrograms. Jaundice, in itself, apparently does not cause abnormality of the level of free erythrocyte protoporphyrin.

Values for free erythrocyte protoporphyrin in 28 cases of parenchymatous liver disease were, for the most part, similar to values found in the normal group. In a few of these cases the values were much higher than any seen in normal subjects.

Patients who had pernicious anemia under treatment with vitamin B_{12} showed significant elevation in values for free erythrocyte protoporphyrin during the phase of reticulocytosis and there appeared to be a tendency for values of free erythrocyte protoporphyrin to be increased in cases of nontropical sprue.

Values of free erythrocyte protoporphyrin in cases of acute disseminated lupus erythematosus, acute leukemia, lymphoblastoma, myelosclerosis, multiple myeloma, aplastic anemia, myxedema, essential thrombocytopenic purpura, anorexia nervosa, postgastrectomy macrocytic anemia, and chronic porphyria generally fell within the range of values exhibited by normal subjects.

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