Photosensitive or Congenital Porphyria with Hemolytic Anemia

II. Isotopic Studies of Porphyrin and Hemoglobin Metabolism

By Moises Grinstein,* Ph.D., Robert A. Aldrich,† M.D., Violet Hawkinson, B.S., Paul Lowry, M.D. and Cecil James Watson, M.D., Ph.D.

AN UNUSUAL OPPORTUNITY has been afforded to carry out studies of porphyrin and hemoglobin metabolism with the aid of glycine containing N^{15} in a 4 year old girl with manifestations of the photosensitive or congenital type of porphyria. Detailed descriptions of the clinical and biochemical features of this patient are presented in paper I. At the time of the isotope studies anemia and splenomegaly were prominent and there was evidence that the anemia was hemolytic in character. This evidence consisted of heightened fecal urobilinogen excretion, elevated reticulocyte counts and a hyperplastic normoblastic bone marrow. In addition, it was repeatedly observed that the circulating hemoglobin level, after being elevated by transfusions to a level of from 9 to 12 Gm. per 100 cc. fell within a thirty day period to a level of 4 to 6 Gm. per 100 cc. This evidence, together with the difficulty of combatting the anemia, led us to determine upon splenectomy which was carried out uneventfully and with a very gratifying result.

A preliminary report[1] described the earlier data obtained during the first experiment in which glycine containing an excess of N^{15} was fed. The purpose of the present communication is to describe some additional results of this first experiment, and to compare them with the results obtained after a second feeding of N^{15} containing glycine during a later period in which the initial hemoglobin level was considerably higher.

Methods

Glycine containing N^{15} was given according to the method of Shemin and co-workers.[2, 3] In the first experiment 15 Gm. of glycine containing 32.2 atom per cent excess of N^{15} were given over a three day period (January 17 to 19, 1949, inclusive). A quantity of 14.5 Gm. of glycine containing 32.8 atom per cent N^{15} were given in the second experiment (April 26 to 28, 1949, inclusive). The glycine in each instance was prepared from potassium phthalimide‡ in the usual manner.[4] Blood samples were drawn at intervals of three to four days, sufficient in quantity to permit preparation of at least 10 mg. of protoporphyrin dimethyl ester according to the method previously described.[5] This also applied to 180 cc. of blood expressed

From the Departments of Medicine and Pediatrics, University of Minnesota Hospital, Minneapolis, Minn. Aided by grants from the Division of Research Grants and Fellowships, U. S. Public Health Service (RG No. 345), and the Medical Research Fund of the Graduate School, University of Minnesota.

* Special Research Fellow of the National Institutes of Health, now residing in Buenos Aires, Argentina.

† Senior Fellow in Pediatrics of the National Research Council. Some of this data is included in a thesis submitted to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for the Ph.D. degree.

‡ Eastman Kodak Company, Rochester, N. Y.

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from the spleen after splenectomy. Consecutive four day samples of urine and feces were collected for isolation of porphyrins and stercobilin. The latter was obtained in crystalline form by means of the method described in 1934[6] with minor modifications as given in paper I. Methods previously described[7, 9] were employed in purifying and crystallizing copro- and protoporphyrin methyl esters.

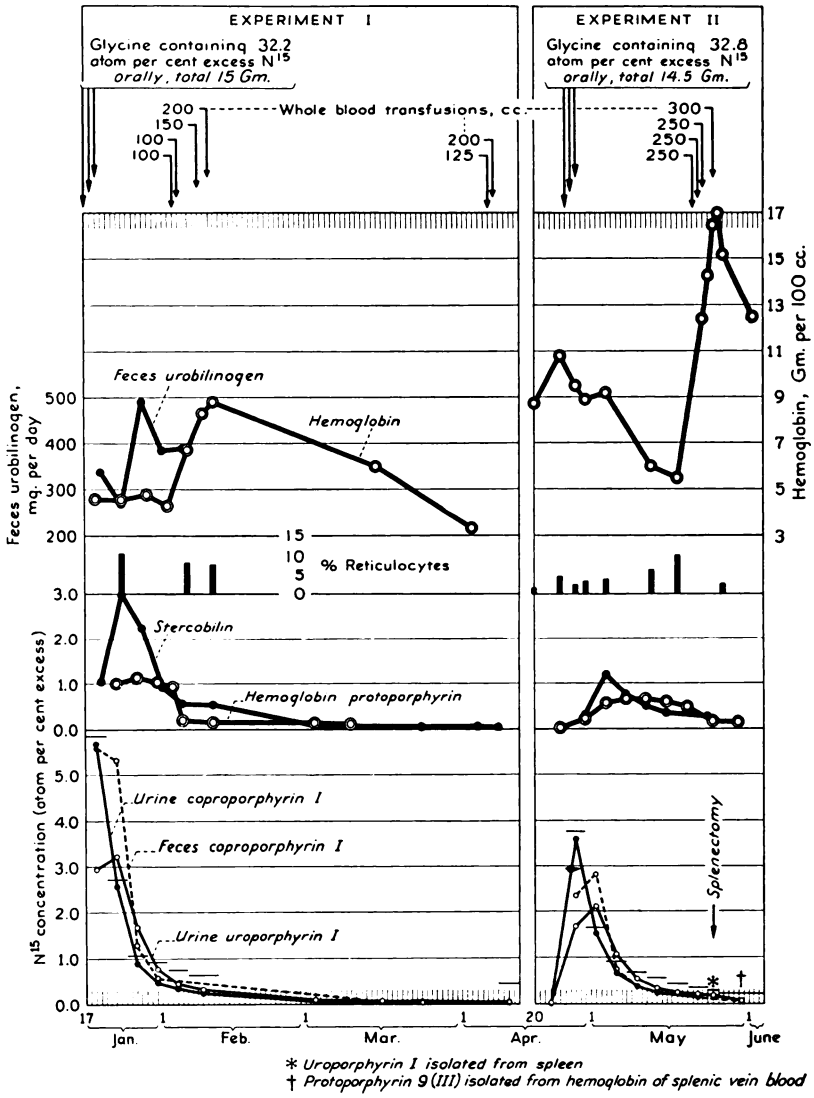

FIG. 1.—Results of N^{15} studies compared with other data relating to hemoglobin metabolism, in both experiments.

The fecal uroporphyrin was extracted from the fecal residue after the primary ether extraction, by means of methyl alcohol saturated in the cold with HCl gas. This effects esterification as well. From this point on the method was that usually employed.[8, 9]

The various porphyrins, i.e., proto-, copro-, and uro- were identified by means of absorption spectra and ester melting points, in addition to characteristic solubility differences which permitted their fractionation.

The urinary porphyrins were isolated and further purified by means of previously de-

scribed methods,[8, 9] the coproporphyrin first being removed by ether extraction, and the uroporphyrin by precipitation (see paper I).

TABLE 1.—*N^{15} Concentration of Porphyrins and Stercobilin After Feeding Glycine Containing an Excess of N^{15}*

Date	Days after glycine was commenced	N^{15} Concentration in atom per cent excess				
		Hemoglobin Protoporphyrin	Coproporphyrin I		Uroporphyrin I (urine)	Stercobilin (feces)
			Urine	Feces		
First Experiment: 15 Gm. of glycine containing 32.2 atom per cent excess N^{15} were fed in divided doses, 1-17 to 1-19-49, incl.						
1-20-49	4		5.686	5.614	2.959	1.057
1-23	7	1.006				
1-24	8		2.594	5.330	3.234	2.991
1-27	11	1.146				
1-28	12		0.897	1.289	1.687	2.234
1-31	15	1.055				
2-1	16		0.484	0.558	0.765	0.926
2-3	18	0.941				
2-5	20	0.205	0.373		0.458	0.551
2-11	26	0.169	0.260		0.319	0.554
3-3	46	0.162	0.076		0.085	0.102
3-10	53	0.110				
3-16	59		0.055	0.064	0.064	
3-24	68		0.062		0.059	0.057
4-4	79					0.056
4-8	83		0.051	0.047	0.053	0.050
4-25	100	0.017	0.021		0.026	
Second Experiment: 14.5 Gm. of glycine containing 32.8 atom per cent N^{15} were fed in divided doses, 4-26 to 4-28-49, incl.						
4-30	4	0.229	3.578	2.374	1.693	0.303
5-4	8	0.549	1.534	2.824	2.108	1.175
5-8	12	0.640	0.658	0.744	1.073	0.739
5-12	16	0.620	0.375	0.369	0.544	0.494
5-16	20	0.586	0.254	0.268	0.338	0.335
5-20	24	0.480		0.218	0.247	
5-24	28		0.169	0.177	0.201	0.232
5-25	29*	0.157†			0.182‡	
5-30	24	0.142		0.05	0.056	

* Splenectomy. † Splenic vein blood. ‡ Uroporphyrin I isolated from spleen.

Urine coproporphyrin III 1-20 to 1-24 contained 2.360 atom per cent excess N^{15}. The value for the feces protoporphyrin of the first 8 days was 0.140, and of the eighth to the twentieth days 0.766.

The method of isolating uroporphyrin from the spleen has also been described in paper I. Small amounts of proto- and coproporphyrin were present in the primary glacial acetic-ethyl acetate extract, but these were insufficient for N^{15} analysis.

The feces urobilinogen was determined by the ferrous hydroxide petroleum ether method.[10] The concentration of circulating hemoglobin was determined by the method of Evelyn.[11] The percentage of reticulocytes was determined in the usual manner by supravital staining of blood smears with brilliant cresyl blue in a moist chamber for ten minutes.

Results

The concentrations of N^{15} in the hemoglobin protoporphyrin, urinary and fecal coproporphyrin I, urinary uroporphyrin I, and fecal stercobilin, for the two study periods following administration of tagged glycine, appear in table 1, and are presented graphically in figure 1, where they may be compared with the data for circulating hemoglobin concentration, fecal urobilinogen excretion, and reticulocyte percentage. The urinary coproporphyrin III isolated in the period of January 20 to 24 contained 2.360 atom per cent excess N^{15}. The value for the feces protoporphyrin of the first eight day period (January 17 to 23 inclusive) was 0.140 atom per cent excess N^{15}, and that of the eighth to twentieth days, 0.766. From these data it is evident that the glycine N^{15} was incorporated, shortly after its administration, in the copro- and uroporphyrin I, coproporphyrin III, protoporphyrin 9 and stercobilin (both of which are also type III in configuration). This is in accord with our earlier report[1] and with more recent observations of Gray and co-workers,[12, 13] and London.[14]

In both of the present experiments the N^{15} concentration in the urinary porphyrins is seen to have been highest first in the coproporphyrin then, in the second four day period becoming higher in the uroporphyrin and slightly lower in the coproporphyrin. The concentration in the fecal corpoporphyrin, however, remained at a higher level in the second period, which may well be due to the relative lag in excretion. The data presented by Gray and co-workers[12] are quite similar. In London's study[14] the peak N^{15} concentration in the urinary uroporphyrin preceded that of the fecal coproporphyrin but again this may be due to delay in excretion.

Discussion

In the preliminary report[1] of the data from our first experiment, it was suggested that the sequence and relative levels of peak concentrations of N^{15} in the copro- and uroporphyrin of the urine, favored the view that the latter was formed by carboxylation of the former. There are, however, two important considerations[14, 15] which admittedly prevent conclusions. The first is the possibility that the uroporphyrin "pool" is larger than that of the coproporphyrin, and that as a consequence of this the peak N^{15} concentration of the uroporphyrin is smaller and later. The second consideration is purely theoretical, i.e., that a decarboxylation of uro- to coproporphyrin would be a more likely biochemical process than a carboxylation of copro- to uroporphyrin.[15] Nevertheless, the earlier and greater peak N^{15} concentrations of the copro- as compared with uroporphyrin in both of the present experiments undoubtedly have fundamental significance. Considered in relation to the closely similar slopes of decrease of their two curves, the data do not offer support for a transition of uroporphyrin I to coproporphyrin I.

The single value obtained for urinary coproporphyrin III N^{15}, during the fourth to seventh day of the first experiment, differs but slightly from that of the coproporphyrin I for the same period. This might be a coincidence but it seems much more probable that it indicates a parallel formation of the two porphyrins. Unfortunately, the amounts of coproporphyrin III obtained during other periods were too small for analysis.

It is evident that the glycine N^{15} was quickly utilized in stercobilin formation since in the first experiment the fecal stercobilin is seen to contain 1.0 and 3.0 atom per cent excess N^{15} during the first and second four day periods respectively. This may be due in part to formation from precursors, rather than from destroyed hemoglobin, in accordance with the recent findings of London and coworkers.[3] Nevertheless, there is little doubt that in this instance blood destruction was proceeding in random fashion and much more rapidly than normally, hence it is likely that at least a major proportion of the peak value for stercobilin N^{15} in both periods represented destroyed hemoglobin. There is also a distinct possibility that noncirculating hemoglobin, destroyed in the bone marrow, contributed to the early N^{15} increase in the stercobilin.

It is observed that the stercobilin N^{15} curve, unlike that in a normal individual,[3] exhibits but the one peak at the outset without further increase by the eighty-third day. At this time the hemoglobin protoporphyrin N^{15} had declined to a negligible level, so that a secondary rise in stercobilin N^{15} such as encountered normally could not have been anticipated. This is in accord with the belief that the red cell turnover was much more rapid than in the normal. Because of this random and rapid destruction, it is not possible to calculate the life span of the erythrocytes in this case, and for the same reason the percentage of N^{15} in newly formed heme and the proportion of the stercobilin not derived from destroyed circulating hemoglobin cannot be calculated. In London's case in which there was no evidence of an increased erythrocyte turnover the latter value was calculated to be 31 per cent. It is important to note that in both of the present experiments the slope of decrease of stercobilin N^{15} after its peak concentration, is not as steep as in the case of the porphyrins, the latter being regarded as wholly anabolic in character in contrast to stercobilin. Because of inherent lag it is probably better to compare the fecal coproporphyrin N^{15} with that of the stercobilin, but even here it is seen that the slope of decrease of the latter is not as steep. It may be noted further that the diluting effect of the transfusions is to make the slope steeper in the case of stercobilin; thus it is probable that the gradual slope between the sixteenth and twenty-sixth days of the first period represents a considerable increase in hemoglobin catabolism. Relatively little can be ascertained from the curve of N^{15} concentration of the hemoglobin protoporphyrin, due to the indeterminate dilution caused by the transfusions. In the second period, however, the downward slope of the curve from the twelfth day indicates a random destruction which is in good agreement with the fall in circulating hemoglobin.

The data for the fecal protoporphyrin N^{15} are too limited to permit conclusions as to the origin of this porphyrin. Three possibilities deserve consideration: (1) hemoglobin catabolism, from circulating erythrocytes; (2) synthesis in the bone marrow without having entered the hemoglobin molecule, or from hemoglobin formed and destroyed without having been in circulating erythrocytes; (3) free erythrocyte protoporphyrin of circulating erythrocytes. Comparison of the data with that for stercobilin, as already discussed, appears to favor the first of these possibilities somewhat more than the other two, though their participation cannot be excluded.

Mention was made at the outset of the striking reduction in porphyrin ex-

cretion which occurred following splenectomy. Although the operation was not carried out until nearly the end of the second experiment, the remarkable effect upon the porphyria is believed to have considerable significance with respect to interpretation of the present data. The complete disappearance of anemia, together with decline of the reticulocytes and fecal urobilinogen to normal, reveal that the hyperhemolytic factor was removed with the spleen. It is believed that with this the principal stimulus to excessive erythropoiesis was also removed, and, pari passu, the marked overproduction of copro- and uroporphyrin I. This, of course, relates their formation mainly to the bone marrow. Direct evidence of such formation is described in a separate communication. It is clear that the primary metabolic error still exists in this case, and it is highly probable that if other groups of cells take up the hyperhemolytic role of the spleen, an accelerated erythropoiesis may be anticipated which will then be associated with a corresponding increase in porphyrin formation and a return of symptoms.

Summary and Conclusions

1. Porphyrin and hemoglobin metabolism have been studied in a case of photosensitive or congenital porphyria with the aid of glycine containing N^{15}. The case was that of a 4 year old girl who also exhibited splenomegaly and hemolytic anemia. The reticulocytes and fecal urobilinogen were markedly increased and the bone marrow was hyperplastic-normoblastic. The urine contained large amounts of uroporphyrin I and lesser amounts of coproporphyrin I, while the reverse was true in the feces. Splenectomy resulted in disappearance of anemia, together with marked reduction of porphyrin excretion.

2. In two separate periods of study a rapid incorporation of glycine N^{15} was observed in the hemoglobin protoporphyrin, in both coproporphyrin isomers of the urine and in the type I isomer in the feces; also in the uroporphyrin I of the urine, and in the protoporphyrin and stercobilin of the feces.

3. The N^{15} data obtained for the urinary and fecal coproporphyrin and the urinary uroporphyrin, clearly reveal a synthesis similar in character to that of the hemoglobin protoporphyrin. The striking decline in formation of copro- and uroporphyrin after splenectomy is believed to be correlated directly with the reduced erythropoiesis incident to removal of the excessive hemolysis.

4. In both experiments the peak concentration of N^{15} was earlier and greater in the copro- than in the uroporphyrin. This, together with the closely similar slopes of decrease of the N^{15} curves for the two substances, does not appear to support a transition of uro- to coproporphyrin in the present case. The data are more consistent with the view that coproporphyrin I is a precursor of uroporphyrin I, or that both porphyrins are synthesized independently from common precursors.

5. The amount of coproporphyrin III in the excreta was too small to permit N^{15} analysis except in one period early in the first experiment; here the value was approximately the same as that of the copro- I for the same period, indicating a closely related synthesis.

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