2. The following 20 amino-acids were found in every hydrolysate examined; phenylalanine, leucine, isoleucine, valine, alanine, glycine, threonine, serine, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, hydroxyproline, $\alpha\epsilon$ -diaminopimelic acid, α -amino-*n*-butyric acid, β -alanine, γ -aminobutyric acid and tyrosine. The sulphoxide derived from methionine and glucosamine were found in most of the hydrolysates.

3. Cystine, methionine and tryptophan could not be detected.

4. D-Amino-acids were detected in acid hydro-

lysates of soil, but the small amounts found could have arisen by racemization during hydrolysis.

5. No free amino-acids could be detected in any of the soils studied.

6. The results indicate that the protein materials in different soils are similar in their amino-acid composition.

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Studies in Congenital Porphyrria

3. THE INCORPORATION OF ^{15}N INTO THE HAEM AND GLYCINE OF HAEMOGLOBIN

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In the two preceding papers of this series (Gray & Neuberger, 1950; Gray, Neuberger & Sneath, 1950) results were reported concerning the incorporation of ^{15}N into the coproporphyrin, uroporphyrin, stercobilin and hippuric acid of a patient suffering from congenital porphyria who had been fed isotopically labelled glycine. The present paper deals with the

isotope content of the haem of the circulating haemoglobin and of the glycine from the globin as well as with haematological and other data concerning the patient. An attempt will be made on the basis of the new facts elicited during these investigations to define more clearly the nature of the metabolic lesion in congenital porphyria.

EXPERIMENTAL

General details of the patient and of the two feeding experiments have been given by Gray & Neuberger (1950). Most of the chemical methods used have also been described (Gray & Neuberger, 1950; Gray *et al.* 1950).

'Urobilinogen' estimation. 'Urobilinogen' in faeces was estimated by the method of Schwartz, Sborov & Watson (1944) which measures all substances soluble in light petroleum and reacting with *p*-dimethylaminobenzaldehyde under specified conditions, and in addition any substances which behave similarly after reduction with ferrous ions in an alkaline medium.

Isolation of glycine from a hydrolysate of haemoglobin

The isolation of glycine from protein hydrolysates by precipitation with potassium trioxalatochromate has been described by Bergmann & Niemann (1938) and been used for various proteins. In applying this method to human haemoglobin, it was found that in the precipitation of the complex, aspartic acid was carried down with the glycine. Separation could be effected by dissolving the mixture of the benzamido acids in *n*-butanol followed by fractional extraction with dilute alkali. The first fractions contained the more acidic benzoylaspartic acid. Hippuric acid, in rather poor yield, was obtained in a later fraction. In the second experiment aspartic acid was removed by the Foreman (1914) method before the precipitation of the glycine.

All melting points are uncorrected.

Packed red cells were washed three times with 0.9% NaCl and laked with distilled water. The haemoglobin was precipitated with acetone, washed with acetone and ether and dried. Dry haemoglobin (19 g.) was hydrolysed by refluxing with 150 ml. conc. HCl and 150 ml. glacial acetic acid for 18 hr. The excess acid was removed by evaporating the solution twice to dryness *in vacuo*. The residue was taken up in 1 l. water and 10% (w/v) phosphotungstic acid added until there was no further precipitation. The precipitate was filtered after standing overnight at room temperature, and washed with 1% (w/v) phosphotungstic acid. The filtrate and washings were combined and extracted thrice with 0.3 vol. of a 1:1 mixture of amyl alcohol and ether. Excess Cl⁻ was removed by treating the solution with silver acetate until no longer acid to congo red. The AgCl was removed by filtration and washed with hot water. Excess Ag⁺ was removed as Ag₂S, which was washed with hot water. The filtrate and washings were combined and boiled with a little charcoal. On evaporating the solution to about 50 ml. some crystals appeared, which were filtered off and discarded. The filtrate, after concentration to 20 ml., was treated with 1.75 ml. conc. HCl and 9 g. potassium trioxalatochromate (Inorganic Syntheses, Vol. 1). A solution of 5 ml. 10% (w/v) HCl in 35 ml. ethanol was then added and the mixture shaken for 10 hr. The precipitate was filtered and washed with a solution of 25 ml. 0.5N-HCl in 75 ml. ethanol, and finally with ethanol, and dried (wt. 7.76 g.). The benzoylation was carried out by the procedure of Bergmann & Niemann (1938) using 2.4 ml. redistilled benzoyl chloride. At the end of the reaction the solution was acidified with HCl, extracted thrice with benzene and then four times with equal volumes of ethyl acetate. The ethyl acetate solution was evaporated to dryness and the residue dissolved in water, treated with a little charcoal, filtered and evaporated in a desiccator until crystals appeared. Yield 808.1 mg., m.p. 165–168°. On re-

crystallization the melting point rose to 173°. The equivalent weight was 128.2 indicating admixture of a dicarboxylic acid. 769.2 mg. of the mixture were dissolved in 100 ml. *n*-butanol saturated with water. The butanol solution was extracted with 10 portions of 30 ml. 0.02N-NaOH. The emulsions which formed were dispersed by centrifuging. The ten alkaline extracts were acidified to congo red, and each extracted thrice with 30 ml. ethyl acetate. The extracts were dried and evaporated to dryness. Each residue was crystallized from water. The first four fractions contained benzoylaspartic acid, m.p. 181° alone or when mixed with an authentic specimen. (Found: C, 55.8; H, 5.1. Calc. for C₁₁H₁₁O₅N: C, 55.8; H, 4.7%.) The sixth fraction contained 80.4 mg. hippuric acid, m.p. 189–190° alone or when mixed with an authentic specimen. (Found: N, 7.3; Calc. for C₉H₉O₃N: N, 7.8%.)

In the second experiment 20 g. dried protein were used. After precipitation with phosphotungstic acid and removal of excess reagent, the solution was treated with Ca(OH)₂ and ethanol. The precipitate was washed with 85% ethanol. Filtrate and washings were combined and excess Ca⁺⁺ precipitated with oxalic acid. The solution was then evaporated almost to dryness, and the residue dissolved in 20 ml. 0.5N-HCl. Potassium trioxalatochromate (9 g.) was added and the reaction carried out as before. Hippuric acid was isolated directly from the reaction mixture by extracting with ethyl acetate. It was recrystallized three times from water; m.p. 190°.

RESULTS

Isotope contents of circulating haem

First glycine feeding. In the first experiment (Fig. 1) the ¹⁵N content of the circulating haem rose sharply from the 4th to the 12th day of the experiment, when the isotope content was 0.303 atom %

Fig. 1. ¹⁵N contents of haemin samples obtained from a case of congenital porphyria who had been given isotopic glycine over days 1–4 (first experiment). Glycine (12 g.) containing 31.65 atoms % excess ¹⁵N was fed.

excess ¹⁵N. Since no fall of isotope content was expected at this stage of the experiment, no further blood samples were taken until the 25th day, when it was found, rather surprisingly, that the ¹⁵N content of the porphyrin had decreased to 0.187 atom % excess. Both analyses were obtained with twice recrystallized protoporphyrin methyl ester. The isotope content then increased very slightly to 0.205

atom % excess in the following 2 weeks. Between the 40th and 76th day there was a definite decrease, well outside the limits of error of the method, followed by a much greater fall in ^{15}N content between the 87th and 150th day of the experiment.

The last part of the curve (80–150 days) has the shape as first observed in normal man by Shemin & Rittenberg (1946) and it would appear, therefore, that a large part of the population, possibly a half, of cells formed during the period immediately following the first feeding of isotopic glycine had a normal mean life span of about 110–120 days. However, an appreciable number of red cells must have had life spans of less than 20 days. It would also appear that a much smaller proportion of cells died between the 40th and 75th day, i.e. probably had an average life span of about 50 days. It thus seems that the cell population was heterogeneous falling into three distinct groups: one with a normal life span, a second group with an intermediate life span and a third, large, group which died within 20 days.

Fig. 2. ^{15}N contents of haemin samples obtained from a case of congenital porphyria who had been given isotopic glycine over days 1–2 (second experiment). Glycine (12 g.) containing 31.68 atoms % excess ^{15}N was fed. The full line indicates experimental values; •, experimental points. The broken line gives values corrected for the decrease in ^{15}N of the haem of those cells labelled in the first glycine feeding. For details of this correction see text, this page.

Second glycine feeding. The results obtained in the second experiment were completely different and are shown in Fig. 2. As pointed out in an earlier paper (Gray & Neuberger, 1950) the exact interpretation of these results is somewhat complicated by the fact that the haem of the patient at the beginning of the second experiment still contained 0.044 atom % excess ^{15}N . We have corrected for the decrease in ^{15}N in the old cells still labelled from the first experiment (see Gray & Neuberger, 1950) by assuming that the decrease in ^{15}N content after the 200th day is linear and amounts to 0.00045 atom % excess/day under the conditions of our experiment. The general conclusions obtained are not greatly affected by the

somewhat arbitrary character of this correction. The maximum isotope content was reached on the 12th day, the value then remaining constant up to the 30th day. There was a definite fall in isotope content between the 30th and 80th day, but the last part of the curve (80–150 days) was again normal in shape, showing the greatest rate of fall between the 110th and 140th day. The results suggest that at least 80 % of the cells formed after the second glycine feeding had normal life spans (approximately 120 days). The fall of ^{15}N content of the haem between the 30th and 80th day suggests that a small fraction had life spans varying between 20 and 80 days, a phenomenon which is not found in normal man. However, there was no evidence that cells of extremely short life span (less than 20 days) were present in the circulating blood in the second experiment.

Haematological findings

The haematological investigations were carried out by Dr W. M. Davidson of the Department of Clinical Pathology, King's College Hospital, London.

First glycine feeding. Blood counts and blood films were first made 20 days after the patient had received the first dose of isotopic glycine. The following values were obtained: red cells, $4.0 \times 10^6/\text{mm}^3$; haemoglobin, 11.9 g./100 ml.; leucocytes, $3.1 \times 10^3/\text{mm}^3$. A differential leucocyte count gave the following figures: polymorphs, 57; lymphocytes, 37 and monocytes, 6%. In the blood films the red cells showed anisocytosis, poikilocytosis, polychromasia and punctate basophilia. Three nucleated red cells were seen in counting 100 white cells. Thirteen days later red cell count and haemoglobin content were unchanged and leucocytes were $4.2 \times 10^3/\text{mm}^3$. 4.8 % of red cells were reticulocytes. The blood film showed marked polychromasia, with some anisocytosis and poikilocytosis. No nucleated red cells were seen. About 2.5 months later the haemoglobin content had risen to 12.7 g./100 ml. and the relative reticulocyte count had fallen to the normal value of 0.7%. In the film the red cells still showed some anisocytosis and poikilocytosis.

The almost normal red cell count and haemoglobin content are in striking contrast to the cytological findings. Thus the occurrence of nucleated red cells in the circulation, the marked reticulocytosis, polychromasia, punctate basophilia, the pronounced anisocytosis and poikilocytosis are generally associated with severe anaemias in which the bone marrow is hyperactive. Our patient had already been the subject of careful investigations by Mackey & Garrod (1922, 1926) and of Garrod (1936), who described their haematological findings obtained about 25 years ago in some detail. The patient then had a very slight anaemia, the haemoglobin content varying between 80 and 85 % and a red cell count of

$4.5 \times 10^6/\text{mm}^3$. However, there was polychromasia, punctate basophilia and anisocytosis. Nucleated red cells, both normoblasts and megaloblasts, were also seen at various times. The authors concluded 'that excessive haemolysis was in progress and was balanced by an abnormal production of red cells'. This interpretation of the haematological characteristics of the patient which have remained virtually unchanged for 25 years is now strongly supported by the isotope data. There is no doubt that a haemolytic crisis occurred during and immediately after the period during which the patient was fed isotopic glycine on the first occasion. This haemolysis of circulating red cells was counterbalanced by an increased production resulting in an almost normal haemoglobin content. The low leucocyte count was also observed in a normal subject under similar conditions and may be associated with the low protein diet given in the early stages of the experiment.

Second glycine feeding. The patient was again examined about 6 weeks after the beginning of the second experiment. Red cell count was $5.0 \times 10^6/\text{mm}^3$, haemoglobin was 12.4 g./100 ml. and leucocyte count was $4.0 \times 10^3/\text{mm}^3$. There was slight anisocytosis and a mild degree of punctate basophilia. The isotope data suggest that most of the red cells formed in the beginning of the second experiment had normal life spans and thus agree with the haematological observations. The data of Mackey & Garrod (1926) also indicate that the haematological findings varied from time to time in this patient.

'Urobilinogen' excretion

'Urobilinogen' as estimated by the method of Schwartz *et al.* (1944) measures not only stercobilin and its hydrogenation product but also other bilenes and bilanes as well as other end products of haem

Table 1. *Daily faecal excretion of urobilinogen of the patient at various times*

(The values represent daily averages of 4-day collections.)

Date	Faecal urobilinogen (mg./day)
15-19 Feb. 1949	316
19-23 Feb. 1949	226
23-27 Feb. 1949	350
1-5 Dec. 1949	418
9-13 Dec. 1949	252
19-24 Jan. 1950	238

catabolism. Biladienes and bilatrienes such as bilirubin and biliverdin, however, are not included and there may well be other breakdown products of haem excreted in the faeces which are not estimated by this method. Table 1 gives results of faecal 'urobilinogen' for selected periods during both experiments. Values for the daily faecal excretion of 'urobilinogen' in normal subjects vary between 40

and 280 mg. (Watson, 1938) and thus the figures obtained in this investigation are distinctly raised above normal.

^{15}N Contents of stercobilin

The isotope contents of a few stercobilin samples collected in the second experiment are given in Table 2. The values are similar to those found in the first experiment (Gray *et al.* 1950) high values (1.2-1.3 atom % excess) being found immediately after feeding of isotopic glycine. At the time when the red cells were broken down, the isotope content of the stercobilin was only about 0.1 atom % excess. This is very different from what is observed in normal man and similar to our findings in the first experiment.

Table 2. *^{15}N contents of crystalline stercobilin hydrochloride collected after the second glycine feeding*

Day of experiment	^{15}N content of stercobilin (atom % excess ^{15}N)
3-8	1.24
9-16	0.79
126-132	0.096
133-139	0.101

The ^{15}N content of the glycine in the globin

Two glycine samples were obtained after the first glycine feeding. The first sample on the 12th day of the experiment had 0.156 atom % excess ^{15}N as compared with 0.303 atom % excess for the protoporphyrin. The glycine obtained on the 39th day had 0.211 atom % excess ^{15}N , whilst the protoporphyrin isolated from the same blood sample had 0.205 atom % excess.

DISCUSSION

Comparison of the isotope contents of the excreted coproporphyrin I and uroporphyrin I with that of protoporphyrin IX

If the nitrogen of all porphyrins is derived to an equal extent from that of glycine, the protoporphyrin IX, the uroporphyrin I and the coproporphyrin I, which are synthesized at the same time, should have equal isotope contents. It has already been pointed out (Gray & Neuberger, 1950) that uroporphyrin and to a lesser extent coproporphyrin are stored in the tissues of subjects suffering from congenital porphyria and that the porphyrins excreted immediately after feeding isotopic glycine may therefore contain an unknown proportion of material synthesized before the ^{15}N was administered. However, as a first approximation we shall neglect this possibility and assume that the ^{15}N content of the excreted coproporphyrin is identical with that of material synthesized during the preceding 24-48 hr. The haem synthesized after feeding isotopic glycine mixes with a large amount of non-labelled haem, but the isotope

content of the newly synthesized material can be calculated from that of the total circulating haem by equations first employed by Shemin & Rittenberg (1946). Assuming a mean life span of 120 days, which was observed in our second experiment, it can be calculated that the mean ^{15}N content of the haem synthesized during the first 5 days following the administration of glycine was about 4 atom % excess. The corresponding value for faecal coproporphyrin during the same period was 2.4 atom % excess (Gray & Neuberger, 1950). The calculation for the protoporphyrin is not very accurate, since it implies that the isotope was introduced all at once into the body. In fact feeding took place over 2 days.

Fig. 3. Comparison of the ^{15}N contents of circulating haem with the theoretical values calculated from the faecal coproporphyrin results. The upper (full) line represents the experimental values corrected as in Fig. 2 and is a measure of the increase of ^{15}N in the haem due to the second glycine feeding. The broken line represents the curve expected on the basis of the coproporphyrin results assuming that the isotope contents of the newly formed circulating haem and of the excreted coproporphyrin I are identical and that the life span of the red cells is 120 days.

A more satisfactory way of comparing the isotope contents of haem and coproporphyrin is to start with the assumption that the newly formed protoporphyrin and the excreted coproporphyrin have the same isotope content and that the daily haem production is constant and equal to $A/\bar{T}$, where A is the total circulating haem and $\bar{T}$ is the mean life span. If X_t is the isotope content of the faecal coproporphyrin on day t , $X_t/\bar{T}$ is the calculated increment of the isotope content of the total circulating haem on day t . By adding up the values of $X_t/\bar{T}$ for the first 40 days, a calculated isotope curve for the haem is obtained which can be compared with the experimental curve (Fig. 3). It can be seen that the two curves are similar, but that the curve calculated on the basis of the coproporphyrin results reaches a maximum which is about 15% lower than the experimental figure. This difference might be explained by assuming that the nitrogen atoms of type I porphyrin, in contrast to those of protoporphyrin IX, are not derived exclusively from glycine. However, the fact that the calculated curve is still rising

at a time when the experimentally determined isotope content of the haemin is constant, suggests that the newly formed coproporphyrin is diluted somewhat by preformed material. The results obtained are thus compatible with the assumption that the nitrogen atoms of porphyrins of both series I and III are derived to an equal extent from that of glycine.

Comparison of the isotope content of the haem with that of the glycine contained in the globin

The fact that the isotope content of the glycine obtained from the globin hydrolysate of the second large blood sample (taken on the 29th day) was almost identical with that of the protoporphyrin from the same sample of red cells agrees with the assumption that all four nitrogen atoms of porphyrins are derived from glycine. With the blood sample obtained on the 12th day of the experiment the ^{15}N content of the porphyrin was almost twice as high as that of the glycine. As Thorell (1947) has shown, the formation of protein in the erythroblast precedes the synthesis of haem or porphyrin. Thus, when isotopic glycine first appears in the bone marrow, it will be utilized for protein synthesis by very young erythroblasts and for porphyrin synthesis by relatively mature cells. The latter will appear in the circulating blood very soon after administration of glycine and will contain a large amount of the isotope in the porphyrin and little in the globin part of the haemoglobin molecule. The isotope content of the free glycine of the body decreases steadily and therefore the cells released into the circulation at a later stage will contain decreasing amounts of ^{15}N in the porphyrin, but the globin glycine will be rich in isotope, since it was largely formed immediately after administration of the isotopic compound. Thus, provided the daily production of haem is constant, the isotope contents of the two fractions should become equal some time after the isotope content of the haem has reached its maximum value. The findings reported here are of considerable importance in the interpretation of other isotope data (Muir & Neuberger, 1950) concerned with the elucidation of porphyrin synthesis.

Congenital porphyria and haemolysis

The haematological findings indicate that the bone marrow of our patient is in an hyperactive state suggesting increased destruction of cells, a conclusion already reached by Mackey & Garrod (1926). Similar observations of the occurrence of nucleated red cells in the circulating blood and of punctate basophilia, polychromasia and poikilocytosis together with an almost normal blood count and haemoglobin content have been made in other cases of congenital porphyria (Ashby, 1926; Gray, 1926; Schmidt-La Baume, 1927). In other cases marked anaemia and enlargement of the liver and spleen

have been noted (Sato & Takahashi, 1926; Duesberg, 1931; Dunskey, Freeman & Gibson, 1947). It seems that the anaemia often appears later in life. Thus the patient described by Gray (1926) had a normal blood count and haemoglobin content at that date, but had a severe anaemia 10 years later (Garrod, 1936). The patient Petry also developed an anaemia a few years before he died (Weiss, 1925; Guenther, 1925) and the post-mortem findings in this case were similar to those commonly observed in untreated pernicious anaemia (Borst & Koenigsdörffer, 1929). However, in many other cases of congenital porphyria no abnormal haematological findings have been reported. In some of these cases cytological abnormalities of circulating red cells may have been present, but were not sought for owing to a normal red cell count or haemoglobin content.

These deductions regarding haemolysis based on haematological findings are fully supported by the isotope data in our patient and in that of Grinstein, Aldrich, Hawkinson & Watson (1949). Our results and the reports in the literature suggest that such haemolysis may not take place continuously, but occurs intermittently or is at least periodically exacerbated. The combination of haemolysis and porphyria is unlikely to be fortuitous.

The mechanism of congenital porphyria

A rational explanation of the mechanism of congenital porphyria must account not only for the greatly increased excretion of porphyrin, mainly of type I, in urine and faeces, but also for the very large amount of isotope found in the stercobilin immediately after feeding labelled glycine and for the excessive haemolysis which has been shown to occur in at least some cases.

It is suggested that congenital porphyria, in contrast to acute porphyria, consists of a disturbance of porphyrin formation, not in the body as a whole, but in the erythropoietic system. All the clinical manifestations may be ascribed either to an excretion and deposition of porphyrins in various tissues especially the skin, or to a disturbance of blood formation. The autopsy findings in the case of Petry led Borst & Koenigsdörffer (1929) to the conclusion that the large amounts of porphyrins or iron-containing pigments in the erythroblasts were related to their formation in these cells, whilst the presence of such pigments in many other tissues was due to secondary deposition. Such a disturbance in porphyrin metabolism, which is probably caused by a genetically determined abnormality of the enzyme systems concerned (Rimington, 1936, 1938), produces greatly increased quantities of type I porphyrin within the developing red cell. Such porphyrins cannot be utilized for haemoglobin synthesis and their presence may interfere with the normal process of erythropoiesis. Developing red cells containing excessive

amounts of type I porphyrins may be broken down in the marrow, and the porphyrins of type I, which cannot give rise to the bile pigments, are excreted as such, whilst those of type III are converted to bilirubin and ultimately to its reduction products, one of which is stercobilin. Isotope experiments have indicated that even in the normal a small part of the stercobilin is derived from non-circulating haem (London, West, Shemin & Rittenberg, 1948; Gray *et al.* 1950). This was explained as being caused by a destruction of haemoglobin accompanying its synthesis in the bone marrow. In congenital porphyria owing to the presence of large amounts of type I porphyrin the rate of catabolism of haem or haemoglobin may be increased relative to that of haemoglobin synthesis with the result that the proportion of stercobilin which is derived from non-circulating haem is vastly greater than in the normal. Thus, unutilized porphyrins or stercobilin or both may diffuse out of the immature red cells without haemolysis taking place.

On the other hand Borst & Koenigsdörffer (1929) found numerous porphyrin-containing immature red cells in phagocytes in preparations of the bone marrow of the case Petry; this is in accord with the view that haemolysis within the bone marrow is an important feature of congenital porphyria.

It appears that under certain circumstances, the haemolysis does not take place until the primitive red cell has matured and reached the circulating blood. There is no evidence that the circulating red cells of patients with congenital porphyria contain porphyrins of type I or their corresponding haem compounds, and haemolysis in the circulation cannot be explained in this way. However, as has been suggested above, the presence of large amounts of porphyrin of type I interferes in an unspecified manner with the production of normal red cells. A proportion of red cells which leave the bone marrow may be defective and have a life span appreciably shorter than normal. But there is an alternative explanation. Hausmann (1910) found that 'haematoporphyrin' sensitized red cells to haemolysis by light, and the presence of porphyrins in the circulating plasma may have a similar effect *in vivo*. On the other hand, the haemolytic crisis observed in our patient occurred in January, when the intensity of sunlight was insufficient to produce acute skin changes.

The high ^{15}N content in the stercobilin observed immediately after glycine feeding and the relatively low ^{15}N content at a time when the labelled cells were broken down suggest that most of the stercobilin excreted by this patient was not derived from circulating haem. Since the breakdown of the circulating haemoglobin must produce an amount of bile pigment at least equal to that found in normal subjects, it would be expected that the total amount of bile

pigment excreted would be greatly increased. The quantity of 'urobilinogen' found at various periods was not as great as would be anticipated on the basis of the isotope results. This is probably due to changes of bile pigments occurring in the gut and giving rise to products not estimated by the method used. There is no indication of any excessive excretion of urobilin or urobilinogen in the urine, and the possibility that there has been an extensive diversion of bile pigments to the urinary tract can therefore be excluded. Watson (1938) has already shown that there is no quantitative relationship between haemoglobin breakdown and bile pigment excretion.

SUMMARY

1. Glycine labelled with ^{15}N was fed on two separate occasions to a patient suffering from congenital porphyria. Changes of isotope content of the circulating haem indicated marked haemolysis during the first experiment and slight haemolysis during the second experiment. These findings are supported by haematological data.

2. Isotope contents were estimated in glycine isolated from the two globin samples. The results suggest that globin synthesis precedes that of haem, and that all four nitrogen atoms of protoporphyrin are derived from that of glycine.

3. The 'urobilinogen' excretion was raised above normal values, but not to levels anticipated on the basis of the isotope results.

4. It is suggested that in congenital porphyria the excessive excretion of porphyrin of type I is associated with a disturbance of blood formation leading to increased breakdown of haem in the bone marrow and in the circulating blood.

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