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Studies in Congenital Porphyria

1. INCORPORATION OF ^{15}N INTO COPROPORPHYRIN, UROPORPHYRIN AND HIPPURIC ACID

BY C. H. GRAY AND A. NEUBERGER

Department of Chemical Pathology, King's College Hospital, Denmark Hill, London, S.E. 5, and the National Institute for Medical Research, Hampstead, London, N.W. 3

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Like other rare inborn errors of metabolism, congenital porphyria is of considerable genetic interest and might be expected to give valuable information about normal metabolism in man. Congenital porphyria has been the subject of a large number of investigations, but the reason why large amounts of highly carboxylated porphyrins of series I are formed and excreted has so far not been elucidated. Shemin & Rittenberg (1946) have shown that glycine is the specific precursor of at least some and probably all the nitrogen atoms of the protoporphyrin in circulating haemoglobin. Later work of Wittenberg & Shemin (1949) and Muir & Neuberger (1949) has shown that at least one of the nitrogen atoms of rings I and II and one of rings III and IV, respectively, are derived from glycine. It is therefore to be expected that, if the porphyrins of series I and III have part of their metabolic pathway in common, glycine should also be the precursor of the nitrogen atoms of coproporphyrin and uroporphyrin I. Owing to the fact that the labelled newly formed haem is greatly diluted with pre-existing unlabelled haem, the ^{15}N content of the newly formed protoporphyrin present in haemoglobin can only be calculated by a mathematical analysis (Shemin & Rittenberg, 1946). In a porphyric, on the other hand, we may assume that excreted porphyrins are not diluted to any large extent by material formed prior to the date of administration

of labelled glycine and we might thus expect to get an approximate but direct measure of the ^{15}N content of newly formed porphyrins.

The present paper deals with the incorporation of ^{15}N into the porphyrins, into the haem of circulating haemoglobin and into the urinary hippuric acid after feeding with labelled glycine a patient suffering from congenital porphyria. We have also investigated the incorporation of ^{15}N into the haem of the circulating haemoglobin of a normal person given a similar amount of labelled glycine.

Some of the results reported here were the subject of a preliminary communication (Gray & Neuberger, 1949).

EXPERIMENTAL

General

Experiments with a porphyric

The subject was a 32-year-old male, G.L. (60 kg. body weight), who had suffered from porphyria since birth. His clinical history up till the age of 9 has been described by Mackay & Garrod (1926). Since then, he has continually excreted mahogany-coloured urine and the skin condition has progressed each summer. As a result of secondary infection he has now lost an eye, both pinnae of the ears and all the terminal phalanges of his hands. The skin of the exposed parts is a mass of scar tissue intermingled with patches of deeply pigmented skin. The patient was given labelled glycine in two separate experiments.

Experiment 1. The subject was put on a diet containing only 20 g. of animal protein/day, beginning 26 January 1949. On the morning of 30 January 1949 he was given the first of 12 doses of 1 g. of labelled glycine, which were given every 6 hr. during the following 3 days. The glycine contained 31.65 atom % excess ^{15}N . On 10 February 1949 all restriction on his diet was discontinued. The urine and faeces were collected separately in 24 hr. batches for the first 12 days after glycine feeding. From the 2nd to the 9th day and on the 12th day after glycine feeding, 3 g. of Na benzoate were given by mouth at 6 a.m. The urine excreted during the succeeding 4 hr. periods was collected separately from the remaining 20 hr. urine of the 24 hr. collection. These 4 hr. collections were used for the isolation of hippuric acid. Subsequently, faeces were collected and worked up for pigments in 4- or 6-day batches during the succeeding 160 days except for a few batches collected between the 40th and 60th day. An occasional 24 hr. collection of urine was also investigated during this period. Blood samples (usually 10 ml.) for preparation of haemin were taken at convenient intervals.

Experiment 2. On 4 September 1949 (that is 217 days after the beginning of the first glycine feeding) the patient was again put on a diet, this time containing 40 g. animal protein/day, and on 7 September 1949 he was given another 12 doses of 1 g. of glycine containing 31.68 atom % excess ^{15}N . This time the glycine was given every 2.5 hr. Urine was not collected on this occasion, but faeces passed from the 2nd to the 9th day after glycine feeding were collected and worked up for stercobilin. Blood samples for the isolation of haemin were taken first at 3-day, then at 4-day intervals, and 40 days after the glycine feeding at fortnightly intervals.

Experiment with normal male

A normal male subject (67.6 kg. body weight) was put on a diet containing only 20 g. protein/day beginning 14 April 1949. On the morning of 21 April he was given the first of 12 doses of 1 g. of labelled glycine which was given every 6 hr. during the next 3 days. The glycine contained 31.65 atom % excess ^{15}N . On 27 April the restriction on his diet was discontinued. Na benzoate (3 g.) was given by mouth at 8 a.m. on 25 April and an identical dose again at 11.15 a.m. on 27 April. The urine was collected for hippuric acid isolation in 4 hr. batches following each dose of Na benzoate. Blood samples were taken at suitable intervals and the protoporphyrin isolated as described below.

Chemical methods

Isolation of urinary porphyrins. The urine was acidified with 1/20 of its volume of glacial acetic acid and extracted with equal volumes of ether three times. The small amounts of emulsion were separated by centrifuging, the resulting interfacial precipitate and aqueous layer then being added to the ether-extracted urine. The coproporphyrin was transferred to 2.7 N-HCl, and after adjusting with NaOH and K acetate to pH 3.0-3.2, taken back into ether. The ether layer was washed with water containing a trace of K acetate, and evaporated to dryness. After esterification with methanolic HCl, the coproporphyrin tetramethyl ester was transferred to CHCl_3 in the usual way, and finally crystallized four times from hot CHCl_3 -methanol. Typical crystals of coproporphyrin I tetramethyl ester m.p. 253-257° (corr.) were obtained.

The ether-extracted urine, to which all interfacial precipitates had been added, was allowed to stand for about 5 days during which time a deposit of impure uroporphyrin settled at the bottom of the vessel. The supernatant was siphoned off and separated from the crude uroporphyrin by centrifuging. This precipitate was then washed by stirring with 2% (w/v) acetic acid followed by centrifuging. The precipitate was repeatedly extracted with small quantities of 2.7 N-HCl until the extracts were no longer red in colour. K acetate solution was added to the deep red solution until the pH was approximately 3.2. On standing, the uroporphyrin settled out practically quantitatively and was centrifuged off and dried in a vacuum desiccator over P_2O_5 . The porphyrin was then esterified with methanolic HCl, and the resulting ester, after transfer to CHCl_3 , recrystallized six times from hot CHCl_3 -methanol. Typical crystals of uroporphyrin I octamethyl ester m.p. (corr.) 290-293° were obtained.

Isolation of faecal coproporphyrin. The faeces were ground in a mortar with about a quarter of their bulk of glacial acetic acid and then with an equal volume of ether. On standing for a short while, a deeply pigmented layer separated out and could be decanted through a large filter paper. The residue was repeatedly extracted with further quantities of ether, fresh acetic acid being added at about every fourth ether extraction, until the extracts were pale in colour. The ether was then distilled on the water bath from the combined filtered ether extracts and the dark brown residue containing much acetic acid was added to ten times its volume of 0.27 N-HCl. After standing overnight the dark brown fatty material was filtered off through a large Büchner funnel, and to the filtrate saturated K acetate solution added until congo red paper no longer turned blue. The solution was then repeatedly extracted with ether until the ether extracts showed only weak porphyrin absorption bands with the hand spectroscope. A bulky interfacial precipitate formed and was roughly separated from the aqueous phase, which, after saturation with solid $(\text{NH}_4)_2\text{SO}_4$, was exhaustively extracted with repeated small volumes of CHCl_3 until the CHCl_3 extracts were only pale yellow in colour. The CHCl_3 extracts were worked up for stercobilin (Gray, Neuberger & Sneath, 1950). An interfacial precipitate again formed and, together with that formed at the ether-water interface, dissolved in 2.7 N-HCl, giving a dark violet-red solution. The porphyrin in the combined ether extracts was transferred to 2.7 N-HCl and united with the acid solutions of the two interfacial precipitates. The resulting solution was neutralized to pH 3.2 with concentrated NaOH solution and K acetate, and on standing the coproporphyrin separated practically quantitatively; after washing with 2% (v/v) acetic acid it was esterified in the usual way. The final coproporphyrin I tetramethyl ester was recrystallized six times from hot CHCl_3 -methanol and gave typical crystals m.p. (corr.) 257-259°.

Isolation of hippuric acid. The 4 hr. urine samples were concentrated *in vacuo* to about a quarter of their original volume, acidified to pH 1.0 with approx. 10 N-HCl and extracted with a small amount of CHCl_3 which was discarded. The solution was then extracted three times with 2 vol. of ethyl acetate. The ethyl acetate extracts were dried and concentrated to dryness *in vacuo*. The residue, after treatment with charcoal, was recrystallized repeatedly from water until the m.p. (uncorr.) was 190°.

Isolation of haemin. The blood samples were centrifuged and the red cells washed three times with 0.9% (w/v) NaCl. The cells were lysed with distilled water to which a little

saponin was added. In the earlier experiments, crystalline haemin was obtained in the usual way by treatment of the haemoglobin solution with hot glacial acetic acid containing NaCl. Most of the haemin samples were recrystallized from pyridine- CHCl_3 -acetic acid. In later experiments protoporphyrin methyl ester was prepared by the method of Grinstein (1947) and recrystallized from CHCl_3 -methanol.

Isotope analyses. The samples, containing at least 0.5 mg. N, were digested by heating for 24 hr. with 1.5 ml. conc. H_2SO_4 containing SeO_2 , HgSO_4 and K_2SO_4 . They were then distilled in the apparatus described by Sprinson & Rittenberg (1949) and the NH_3 converted to N_2 . The excess of ^{15}N was determined in the mass spectrometer.

RESULTS

^{15}N Content of coproporphyrins

The average excretion of porphyrins in the urine was approximately 50 mg./day and the amount of coproporphyrin eliminated in the faeces was also about 50 mg./day. There was no apparent change following the administration of glycine.

Faecal coproporphyrin. The isotope content of the faecal coproporphyrin (Table 1) rose from 0.243 atom % excess ^{15}N on the 2nd day of glycine feeding

Table 1. *Isotope contents of faecal coproporphyrin following the administration of isotopic glycine*

(Glycine (12 g.) containing 31.65 atom % excess ^{15}N was fed on days 1-4.)

Days of experiment	^{15}N content (atom % excess)
1	0.07
2	0.243
3	1.56
4	2.41
5	2.92
6	2.51
7	2.21
8	1.42
10	1.10
11	0.92
12	0.78
14-16	0.451
17-20	0.243
21-24	0.177
25-28	0.128
35-39	0.089
75-78	0.031
89-93	0.026
129-135	0.020

to the maximum value of 2.92 atom % which was reached on the 5th day of the experiment, i.e. the 1st day after the feeding of the labelled glycine had been completed. It may be assumed that the ingested glycine mixes very quickly with the glycine 'pool' of the body, and the latter will have the highest ^{15}N content at the end of the 4-day feeding period. The two facts, that the faecal coproporphyrin collected during the 2nd day already contained a large amount of ^{15}N , and that the maximum isotope con-

tent was found on the 5th day, would indicate that the time required for the incorporation of the labelled glycine into coproporphyrin and the elimination of this porphyrin into the gut is only between 1 and 3 days.

The isotope content in the faecal coproporphyrin fell steadily after the 5th day, but even after 130 days there was still a small amount of ^{15}N present in this

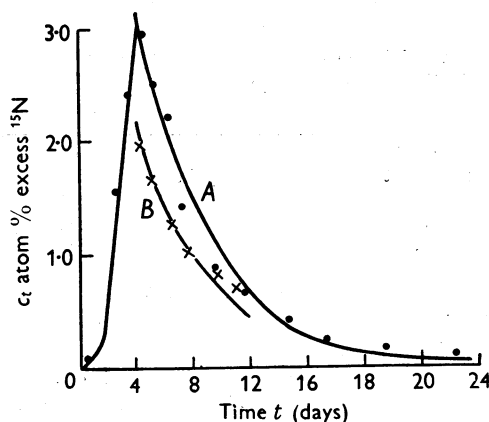


Fig. 1. Comparison of calculated and experimental values for the ^{15}N contents of urinary uroporphyrin and faecal coproporphyrin during the days 4-12 and days 4-24, respectively, after administration of isotopic glycine to a porphyric subject. \times , represents experimental values for uroporphyrin; \bullet , experimental values for coproporphyrin. Curve A represents the theoretical equation for coproporphyrin: $c_t = 3.25e^{-0.20(t-t_0)}$; curve B represents the theoretical equation for uroporphyrin:

$$c_t = 2.20e^{-0.18(t-t_0)}$$

t_0 is the 4th day of the experiment.

fraction (Table 1). The values for the period of 5-30 days can be represented reasonably well (Fig. 1) by an equation of the type

$$c_t = 3.25e^{-0.20(t-t_0)} \quad (1)$$

where c_t is the isotope content at time t (in days after the beginning of the experiment); t_0 is the time at which the isotope concentration in the newly formed coproporphyrin is maximal, i.e. some time during the 5th day of the experiment. The values obtained for coproporphyrin after 30 days do not fit equation (1).

Urinary coproporphyrin. Table 2 gives the isotope contents of the urinary coproporphyrin of the first 12 days of the experiment in three 4-day periods and for comparison the corresponding values for faecal coproporphyrin (calculated from the daily figures given in Table 1). The two sets of values for the corresponding periods are of the same order of magnitude, but the figure of ^{15}N for the urinary coproporphyrin during the first 4-day period is higher than for the second 4-day period. This is not

the case with the faecal coproporphyrin. It is probable that this difference is caused by a faster elimination of the porphyrin through the urinary tract than through the alimentary tract.

Table 2. Comparison of the isotope contents of urinary and faecal coproporphyrin collected during the first 12 days of the experiment

(Glycine (12 g.) containing 31.65 atom % excess ^{15}N were given over the first 4 days. The faecal coproporphyrin values (4-day periods) are mean values calculated from the daily values given in Table 1.)

Days of experiment	^{15}N content (atom % excess) in	
	Urinary coproporphyrin	Faecal coproporphyrin
1-4	2.23	1.07
5-8	1.10	2.50
9-12	0.499	0.90

^{15}N Content of uroporphyrin

The isotope content of the urinary uroporphyrin (Table 3), rises more quickly than that of faecal coproporphyrin, the ^{15}N contents on the 2nd day being 1.00 and 0.243 atom % excess respectively.

Table 3. Isotope contents of urinary uroporphyrin following the administration of isotopic glycine

(Glycine (12 g.) containing 31.65 atom % excess ^{15}N was fed on days 1-4.)

Days of experiment	^{15}N content (atom % excess)
1	0.370
2	1.00
3	1.50
4	1.90
5	1.95
7	1.26
8	1.08
9-10	0.93
11-12	0.775
75-76	0.086
83	0.054
121-124	0.024
135-137	0.029

This again is most probably explained by the fact that elimination through the kidney is faster than through the gut. However the maximum value for uroporphyrin is definitely lower than that found with coproporphyrin. The values for the days 5-12 can tolerably well be expressed by an exponential equation (Fig. 1).

$$c_t = 2.20e^{-0.18 t - t_0} \quad (2)$$

As with faecal coproporphyrin, the values for the isotope contents of uroporphyrin collected between 75 and 130 days do not fit the exponential equation (2). In fact, the ^{15}N figures for uroporphyrin found for the period 75-85 days are much higher than those found for faecal coproporphyrin collected during the same period.

Incorporation of ^{15}N into the haem of circulating haemoglobin in congenital porphyria

The isotope contents of haemin samples obtained in the first experiment indicated that the patient produced at that time a certain proportion of red cells of an abnormally short life span. Most of the cells, however, had a life span of 100-150 days. These haemin results, together with haematological findings indicating abnormal haemolysis, will be published in a subsequent paper (Gray, Muir & Neuberger, 1950). When a second dose of isotopic glycine was given 7 months later, there was now no haematological evidence for a haemolytic anaemia, and the curve, which was obtained by plotting the ^{15}N contents of haemin samples against time (Fig. 2),

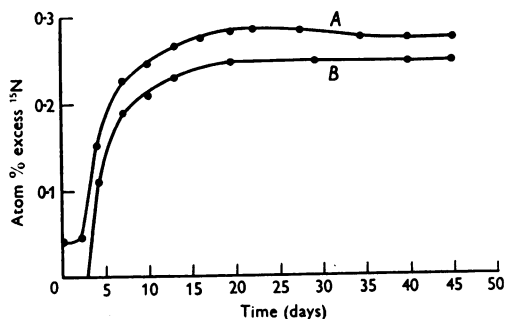


Fig. 2. Experimental and corrected values for the ^{15}N content of the circulating haemin of the porphyric subject at various times after feeding isotopic glycine. Glycine (12 g.) containing 31.65 atom % excess ^{15}N was fed during the first 27.5 hr. of the experiment. Curve A, experimental values; curve B is corrected for the slow decay of the isotope content of the haemin already present before the second dose of labelled glycine was given. For details of the correction see text.

resembles more closely in shape those reported by Shemin & Rittenberg (1946), by London, Shemin, West & Rittenberg (1949) and by ourselves (see p. 85) for normal man. The protoporphyrin isolated from the blood immediately before the second administration of labelled glycine still contained 0.044 atom % excess ^{15}N (mean of three estimations; individual values were: 0.040, 0.048, 0.044). The uncorrected haemin curve represents therefore both the formation of new cells with high ^{15}N content and the slow destruction of very old cells still labelled from the first experiment. We have assumed, on the basis of the data of Shemin & Rittenberg (1946) and those of London *et al.* (1949), that the fall of ^{15}N in the circulating haem after the 200th day is approximately linear and would amount to 0.0045 atom % excess ^{15}N /day under the conditions of our experiment. We have therefore assumed that the fraction of the ^{15}N of the total circulating haem which is due to old cells decreased linearly from 0.044 to 0.026

atom % excess in the 40 days immediately following the second administration of labelled glycine. The data thus corrected are shown in Fig. 2. The error which might arise from the rather arbitrary character of the correction is not serious for the greater part of the curve.

As shown by Shemin & Rittenberg (1946), the initial part of a normal haem curve fits fairly well an equation of the type

$$c_t = c_0 (1 - e^{-\lambda t}), \quad (3)$$

where λ is a constant.

The corrected data of Fig. 2 are fairly well represented by this equation if the values $c_0 = 0.250$ and $\lambda = 0.25$ are used.

Incorporation of ^{15}N into the haem of circulating haemoglobin in normal man

Fig. 3 shows the haem curve obtained on feeding an identical amount of ^{15}N in the form of glycine to a normal male subject of body weight similar to that of our porphyric patient. The curve resembles those given for normal man by Shemin & Rittenberg (1946) and London *et al.* (1949). The American workers and ourselves used glycine of very similar isotope content, but the dose given to our two subjects was only about a quarter of that used by the Columbia workers. It is reasonable, therefore, that the maximum ^{15}N content observed by Shemin & Rittenberg (1946) and by London *et al.* (1949) was between 4 and 5 times as great as the corresponding figure in our normal subject.

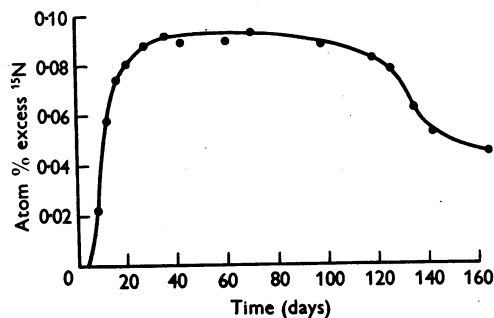


Fig. 3. ^{15}N contents of haemin samples obtained from a normal subject who had been given isotopic glycine over days 1-4. Glycine (12 g.) containing 31.65 atom % excess ^{15}N was fed.

The absolute values of our haemin ^{15}N contents are rather small, and the relative accuracy of the individual analyses is therefore not as high as that attained by the American workers. However, by using the treatment of Shemin & Rittenberg (1946) it can be deduced that the average life span of the red cells in our normal subject is about 130 days. The ascending part of the curve seems to fit an equation

$$c_t = 0.090 (1 - e^{-0.15t}), \quad (4)$$

from which, as shown by Shemin & Rittenberg (1946), the ^{15}N content, $f(t)$, of the haem formed at time t can be calculated by equation (5)

$$f(t) = \frac{\bar{T}}{100} c_0 \lambda e^{-\lambda t}, \quad (5)$$

where \bar{T} is the average life span.

Using the very approximate figures given above, it can be calculated that the ^{15}N content of the haem formed in the 4-5 days immediately following the administration of the glycine to our normal subject was initially of the order of 1.0-1.2 atom % excess ^{15}N falling to about 0.6-0.7 atom % excess ^{15}N at the end of that period.

Incorporation of ^{15}N into hippuric acid in the normal and porphyric subject

The urine obtained in the two experiments carried out with the normal subject was collected over short periods, and the hippuric acid samples isolated and analysed separately. As shown in Table 4, the bulk of the hippuric acid is excreted over the first 4 hr.

Table 4. *Isotope contents of hippuric acid samples obtained from a normal subject following the administration of isotopic glycine*

(Glycine (12 g.) containing 31.65 atom % excess ^{15}N was fed over days 1-4. In the first benzoate experiment, the subject took by mouth 3 g. Na benzoate at 8 a.m. on the 5th day after the beginning of glycine feeding. In the second benzoate experiment, the subject took by mouth the same dose of benzoate at 11.15 a.m. on the 7th day after the beginning of glycine feeding.)

Periods of urine collection after benzoate administration (hr.)	Yield of hippuric acid (g.)	^{15}N Content of hippuric acid (atom % excess)
Experiment 1		
0-4	2.213	0.880
4-8	0.180	0.650
8-12	0.080	0.544
12-16	0.030	0.561
16-24	0.213	0.470
Experiment 2		
0-4	2.07	0.306
4-8	0.076	0.257
8-12	0.015	0.250
12-20	0.014	0.353

The isotope content in the first benzoate feeding falls steadily with time, whilst in the second benzoate experiment this effect is not marked. A direct comparison of the isotope contents of newly formed porphyrin and of hippuric acid is not possible in the normal, but the values calculated from the initial part of the haem curve (Fig. 3) indicate that the protoporphyrin formed on the 5th and the 7th day had about 1.0-1.2 and 0.65-0.85 atom % excess

respectively. These values are about 1.5–2.5 times greater than the ^{15}N contents of the hippuric acid excreted on these days.

In the porphyric the excreted porphyrins may be expected, as discussed above, to give a direct but approximate measure of the isotope contents of newly formed porphyrins. A comparison between ^{15}N contents of coproporphyrins and of hippuric acid is shown in Fig. 4. The values for coproporphyrin

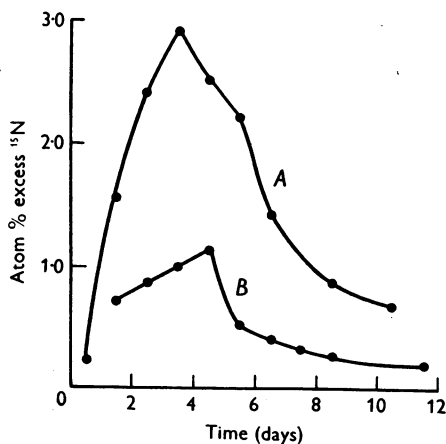


Fig. 4. Comparison of ^{15}N contents of hippuric acid (B) and of faecal coproporphyrin (A) after feeding glycine (12 g.) containing 31.65 atom % excess to a porphyric over days 1–4.

have been plotted on the assumption that the interval between the formation of coproporphyrin and its elimination in the faeces is 1 day. The conclusion would not be greatly affected, if the delay is somewhat longer, i.e. 2–3 days. It is clear from Fig. 4 that the isotope contents of the hippuric acid samples are much smaller than those of coproporphyrin produced at similar times of the experiment.

DISCUSSION

Comparison of the isotope contents of the different porphyrins of the porphyric

When isotopically labelled glycine is administered it will mix with the free glycine present in the tissues, be synthesized into proteins, partake in various reversible reactions such as synthesis of glutathione and serine and also take part in irreversible reactions such as the formation of uric acid and porphyrins. In biological isotope experiments, one or more of such metabolic products of glycine are isolated at various times after the administration of the labelled compound, and the isotope contents estimated. For the interpretation of the results of such experiments it is convenient to use the term glycine 'pool'. This glycine 'pool' will consist not only of free glycine

present as such in the organism, but will also include a proportion of glycine contained in proteins and a certain fraction of the other compounds which can be converted into glycine. In fact, the glycine 'pool' cannot at present be identified with any definite fraction of compounds present in tissues.

If all four nitrogen atoms of protoporphyrin IX, of coproporphyrin I and uroporphyrin I are derived exclusively from glycine, and if all these porphyrins are formed in the bone marrow, then the isotope contents of these porphyrins formed at the same time should be identical and reflect accurately the isotope content of the glycine present in the marrow at that time. The ^{15}N content of the excreted coproporphyrin and uroporphyrin is not necessarily an exact measure of the isotope content of the newly formed porphyrin. If the two porphyrins are stored at all in the body then the labelled newly formed material will be diluted to some extent by pre-formed porphyrin. Evidence, in fact, exists for such a storage (Fischer, Hilmer, Lindner & Pützer, 1925) and the experimental data on the excreted porphyrins therefore represent minimal figures for the ^{15}N contents of the newly formed porphyrins. Such a storage would also explain the high ^{15}N contents found particularly for uroporphyrin between the 70th and 135th day of the experiment, when the label must have almost completely disappeared from the glycine 'pool' of the body. The difference in isotope contents of the two porphyrins indicates that storage of uroporphyrin is more extensive than that of coproporphyrin.

A comparison of ^{15}N contents of the excreted porphyrins with those of the newly formed porphyrin of the circulating haem will be made in a subsequent paper.

Comparison of the glycine 'pool' of the normal with that of the porphyric

In the normal subject the highest isotope content of the newly formed protoporphyrin was calculated to be about 1.0–1.2 atom % excess. The porphyric who received an identical amount of ^{15}N showed a maximal isotope content in his various porphyrins of about 3.0–3.5 atom % excess. This estimate is given either by the coproporphyrin results directly or by the haem values. The difference between the isotope content of the haem in the porphyric and in the normal is also apparent from a comparison of Figs. 2 and 3. It would appear that the ingested labelled glycine is diluted to a greater extent in the normal than in the porphyric, so that the glycine 'pool' of the latter appears to be only about half that of the normal subject investigated. It may also be significant that the rate of decrease of the isotope content of the newly formed haem and of the other porphyrins (which is given by the constant λ of the exponential equations (2)–(4)) is greater than those

found in normals by Shemin & Rittenberg (1946) and London *et al.* (1949). This would indicate that the turnover rate of the glycine 'pool' is greater in the porphyric than has been found so far in normal subjects.

Comparison between the isotope contents of hippuric acid and those of porphyrins

In man, hippuric acid is formed mainly in the liver. This organ contains appreciable amounts of free glycine and other substances like glutathione which can be rapidly converted into glycine. It was expected that the glycine in the liver, which is probably the main precursor of hippuric acid, would have the same isotope content as the glycine in bone marrow which is utilized for the synthesis of porphyrins. However, the present results show clearly that this assumption is not correct. It is likely that benzoic acid mobilizes glycine of low isotope content or induces synthesis of glycine from nitrogen-free precursors. This would also explain the decrease of ^{15}N in successive fractions of hippuric acid isolated in the first experiment with the normal subject. Benzoic acid will probably first combine with free glycine in the liver and reserves will only be mobilized

when the liver glycine is largely used up. The ^{15}N content of hippuric acid cannot, at least with the dose used in the present experiments, be employed for estimating the isotope content of the glycine 'pool' of man.

SUMMARY

1. Glycine containing 31.65 atom % excess ^{15}N was fed to a patient suffering from congenital porphyria. The faecal and urinary coproporphyrin samples isolated during the first few weeks of the experiments had high ^{15}N contents which decreased with time. The urinary uroporphyrin samples had somewhat lower ^{15}N contents.

2. It is suggested that the differences in isotope content observed may be explained by differences in the degree of storage of uroporphyrin and coproporphyrin in the body.

3. Sodium benzoate was administered to a normal man and to the porphyric. The ^{15}N contents of the hippuric acid isolated indicate that administration of benzoic acid stimulates a mobilization of glycine from proteins, or the synthesis of glycine from nitrogen-free precursors, or both.

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Studies in Congenital Porphyria

2. INCORPORATION OF ^{15}N IN THE STERCOBILIN IN THE NORMAL AND IN THE PORPHYRIC

By C. H. GRAY, A. NEUBERGER AND P. H. A. SNEATH

Department of Chemical Pathology, King's College Hospital, Denmark Hill, London, S.E. 5, and the National Institute for Medical Research, Hampstead, London, N.W. 3

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In the course of the experiments described in the preceding paper (Gray & Neuberger, 1950), samples of stercobilin were isolated from the faeces of the porphyric. The surprising observation was made that the isotope content of the stercobilin was extremely high during the first few days of glycine feeding. This observation, together with similar observations in the normal, are the subject of the

present paper. Some of the results have already been reported (Gray, Neuberger & Sneath, 1949).

EXPERIMENTAL

Details of the two experimental subjects have been given in the first paper of this series (Gray & Neuberger, 1950).

Isolation of stercobilin hydrochloride from faeces of the porphyric. The CHCl_3 extract from the preparation of the