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.

REFERENCES

Fischer, H., Hilmer, H., Lindner, F. & Pützer, B. (1925). *Hoppe-Seyl. Z.* **150**, 46.
Gray, C. H., Muir, H. M. & Neuberger, A. (1950). *Biochem. J.* (in the Press).
Gray, C. H. & Neuberger, A. (1949). *Biochem. J.* **44**, xlvii.
Gray, C. H., Neuberger, A. & Sneath, P. H. A. (1950). *Biochem. J.* **47**, 87.
Grinstein, M. (1947). *J. biol. Chem.* **167**, 515.
London, I. M., Shemin, D., West, R. & Rittenberg, D. (1949). *J. biol. Chem.* **179**, 463.
Mackay, L. & Garrod, A. E. (1926). *Quart. J. Med.* **19**, 357.
Muir, H. M. & Neuberger, A. (1949). *Biochem. J.* **45**, 163.
Shemin, D. & Rittenberg, D. (1946). *J. biol. Chem.* **166**, 621.
Sprinson, D. B. & Rittenberg, D. (1949). *J. biol. Chem.* **180**, 707.
Wittenberg, J. & Shemin, D. (1949). *J. biol. Chem.* **178**, 47.

Studies in Congenital Porphyria

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

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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

faecal coproporphyrin (Gray & Neuberger, 1950) was extracted with water (30 × 1/50 vol.). The combined aqueous extracts were rendered 3% (w/v) in respect of HCl by the addition of the required volume of 36% (w/v) HCl. The stercobilin hydrochloride was extracted with $CHCl_3$, which was then filtered through a $CHCl_3$-moistened paper and evaporated to dryness on the water bath. On the addition of a small volume of acetone, the stercobilin hydrochloride precipitated out and was centrifuged from the mother liquor containing brown impurities. After six recrystallizations from $CHCl_3$, crystalline stercobilin hydrochloride was obtained, the yield being higher in the porphyric than in the normal. This method is a modification of that of Watson (1934).

Isolation of stercobilin hydrochloride from faeces of a normal subject. The faeces were worked up in 4- and 6-day batches in a similar manner to that employed with the faeces of the porphyric. However, as the amount of porphyrins present was so much smaller, only four ether extractions were necessary before $CHCl_3$ extraction of the stercobilin. $(NH_4)_2SO_4$ was not added to assist $CHCl_3$ extraction of the stercobilin, since tarry impurities were more readily extracted when $(NH_4)_2SO_4$ was present. Before recrystallization, the stercobilin hydrochloride was precipitated from $CHCl_3$ solution with light petroleum.

RESULTS

The incorporation of ^{15}N into faecal stercobilin of normal man

The stercobilin curve of the normal subject (Fig. 1) shows two distinct peaks, one occurring immediately after the glycine feeding and the other with its maximum at or near the 138th day. Between these two peaks, the curve is quite flat, but the stercobilin excreted during the whole of this period contained significant amounts of ^{15}N.

In order to interpret the results obtained with stercobilin it is necessary to follow the simultaneous changes in the ^{15}N content of the haem of the circulating haemoglobin. The haemin curve of our normal subject (Gray & Neuberger, 1950, Fig. 3) shows that on the 5th day of the experiment, i.e. on the 1st day after glycine feeding had been completed, there was no detectable excess of ^{15}N in the haem. On the 8th day of the experiment the haem contained 0·022 atom % excess ^{15}N. This means that there is an interval of about 3 days between the peak of the isotope content of the glycine pool (which occurs presumably at the end of the 4th day) and the appearance of a significant amount of ^{15}N in the circulating haem. This delay is probably associated with the production of protoporphyrin in the primitive red cell and the maturation of this cell in the bone marrow. In contrast, the stercobilin excreted during the first 4-day period, during which isotopic glycine was being fed, had 0·085 atom % excess ^{15}N. The sample collected during the second 4-day period during which the ^{15}N in the haem began to rise, had 0·161 atom % excess ^{15}N. During the next 12 days the stercobilin fell while the haem curve rose steeply. The second flat part of the stercobilin curve corresponded to the flat part of the haemin curve (30–90 days) and apparently during this period no breakdown of haemoglobin occurred. However, stercobilin samples collected during this period contained an average of 0·020 atom % excess ^{15}N.

During the third period (90–160 days) circulating haemoglobin was destroyed as shown by the decrease of ^{15}N. The highest rate of breakdown was between the 120th and 150th day of the experiment. Our values for ^{15}N in the haem are too small to allow accurate plotting of the function dc/dt, the rate of change of the isotope content of the haem against time t. But it appears that in our normal subject such a function would be represented by a curve with a maximum at or near $t = 135$ days, and it is likely from the results of Shemin & Rittenberg (1946) that such a curve would be symmetrical.

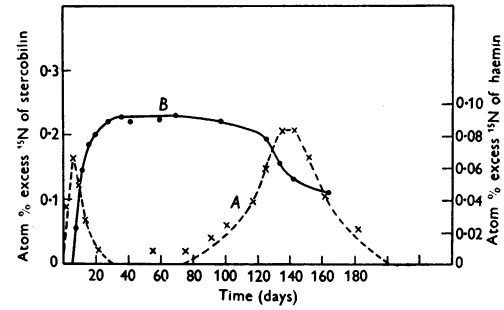

Fig. 1. ^{15}N contents of stercobilin hydrochloride samples (curve *A*) obtained at various times after the administration to a normal subject of glycine (12 g.) containing 31·65 atom % excess ^{15}N. Experimental points (×) represent mean values of 4- or 6-day collections. Glycine was given over days 1–4. The broken line for the values of 80–180 days is a calculated normal distribution curve with a mean value of 0·210 at the 140th day and a standard deviation of 18 days. The ^{15}N contents of the haemin samples (full line; experimental values ●) of the same subject (curve *B*) are given for comparison. The values are taken from the earlier paper (Gray & Neuberger, 1950, Fig. 3).

After the 90th day the ^{15}N content of the stercobilin rose steadily to reach a maximum between the 135th and 140th day. This was almost concomitant with the greatest rate of fall in the ^{15}N content of the circulating haem. The correlation between the two sets of values is so good that there can be no doubt that most or all of the labelled stercobilin excreted between the 100th and 160th day was derived from the breakdown of circulating haemoglobin. No data are available beyond the 180th day, but it appears that the values of Fig. 1 lying between 110 and 160 days can be represented

by a normal distribution curve with a standard deviation of 18. This of course must be an approximation, even if the frequency of deaths amongst red cells were to follow exactly a normal distribution curve. Since the isotopically labelled cells were not all formed at the same instant, a distribution derived directly from the data of Fig. 1 cannot be exactly normal. However, if the approximation is accepted, it would follow that about 68 % of all cells die between 112 and 142 days after their appearance in the circulation. London, Shemin, West & Rittenberg (1949) calculated from the rate of decrease of isotope in the haemin in two different subjects that about 50 % of the cells died between the 106th and 141st day and 91st and 123rd day respectively.

The incorporation of ^{15}N into faecal stercobilin of porphyric

The results obtained with the porphyric are shown in Fig. 2. Comparison with the results obtained in the normal (Fig. 1) show that the first

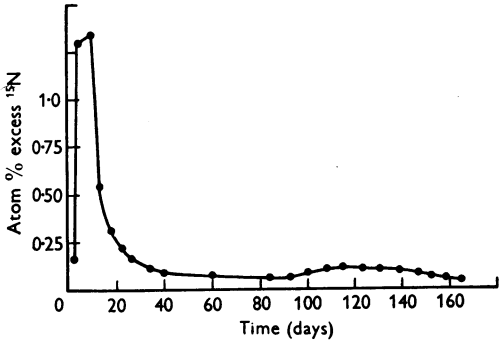

Fig. 2. ^{15}N contents of stercobilin hydrochloride samples obtained at various times after administration to a congenital porphyric of glycine (12 g.) containing 31·65 atom % excess ^{15}N. Experimental points represent 4-day collections. Glycine was fed over days 1–4.

peak is greatly raised, while the second peak is barely noticeable. Comparison of the isotope contents of the stercobilin with those of faecal coproporphyrin for the same periods (Gray & Neuberger, 1950) shows that for the first 8 days the coproporphyrin figures are somewhat higher than those of stercobilin, but subsequently the reverse is the case. In the preceding paper, evidence was presented to show that the isotope contents of the excreted porphyrins are only slightly lower than those of the newly formed haem. The high ^{15}N content of the stercobilin during the initial period would therefore indicate that, in contrast to the normal, almost all of the stercobilin excreted by the porphyric must be derived from some source other than the red cells of normal life span. This is also shown by the fact that at the period 110–150 days when the ^{15}N content of the circulating haem decreases rapidly, the isotope content of the stercobilin rises only very slightly. The possibility that this high ^{15}N content in the initial part of the experiment may be caused by destruction of red cells of abnormally short life span was considered especially in view of the fact that evidence was obtained that a haemolytic episode was taking place during the first glycine experiment. However, in the second experiment where no evidence of a gross haemolytic process was present and the haem curve was apparently normal, the isotope content of the stercobilin collected between the 3rd and 8th day of the experiment was 1·24 atom % excess, almost identical with that found in the first experiment. It therefore appears that the high ^{15}N content of the stercobilin cannot be due to any large extent to the breakdown of red cells of extremely short life span.

Another less striking difference between the normal curve and that of the porphyric is the relatively high ^{15}N content of the stercobilin sample collected between the 30th and 90th day. This may possibly be related to the smaller glycine 'pool' in the porphyric (see Gray & Neuberger, 1950).

It is of interest to note that the initial part of the stercobilin curve after the 4th day of the experiment cannot be fitted to an exponential equation. The curve approximates more closely to a hyperbola, but the physical significance of this is obscure.

Comparison of stercobilin obtained from porphyric with that obtained from non-porphyric patients

The unexpected results described above of the high ^{15}N content of the stercobilin of the porphyric subject during the first days of the experiment suggested the possibility that the stercobilin excreted by the porphyric might be derived from the type I porphyrin or might contain more than two carboxyl groups. A comparison was therefore carried out between the stercobilin excreted by the porphyric and samples obtained either from a normal subject or from patients suffering from haemolytic anaemia (the latter presumably excrete stercobilin of normal structure).

Melting points. Stercobilin samples were dried first over paraffin wax and then over P_2O_5 *in vacuo* at 65°. All samples of stercobilin hydrochloride started sintering at about 120° and melting appeared to be complete at 140°. This agrees with the observations of Watson (1934).

Analyses. Elementary analyses of stercobilin hydrochloride, as shown by Fischer & Halbach (1936), give results too low for C and H, but too high for Cl. This was explained as being due to the retention by the crystals of small amounts of $CHCl_3$ which could not be removed by prolonged drying. Similar results were obtained in the present work; no differences were found between 'normal' and 'porphyric' stercobilin. (Found for sample 1 ('normal'): C, 59·9; H, 7·1; N, 9·0; Cl, 7·0. Found for sample 2 ('porphyric'): C, 60·0; H, 7·2; N, 8·7; Cl, 6·8. Calc. for $C_{33}H_{46}O_6N_4HCl$: C, 62·8;

H, 7·5; N, 8·9; Cl, 5·6%.) These analytical figures are almost identical with those obtained by Fischer & Halbach (1936).

Specific rotations. Sample 1 ('normal') had $[\alpha]_D^{27°} - 3770 \pm 70°$ in $CHCl_3$ (c, 0·1); sample 2 ('porphyric') had $[\alpha]_D^{27°} - 3670 \pm 70°$ in $CHCl_3$ (c, 0·09).

Visible and ultraviolet absorption. In 0·1 N-HCl both samples possessed identical absorption curves showing maxima at 490 mμ. ($\epsilon = 9·3 \times 10^4$) and at 364 mμ. ($\epsilon = 0·79 \times 10^4$). There was no significant difference in the spectral absorption curve in the visible or near ultraviolet regions.

Infrared absorption. Two specimens of stercobilin, one presumed to be normal and the other obtained from the porphyric, were examined by Dr N. Sheppard of the Department of Colloid Science, University of Cambridge. Spectra

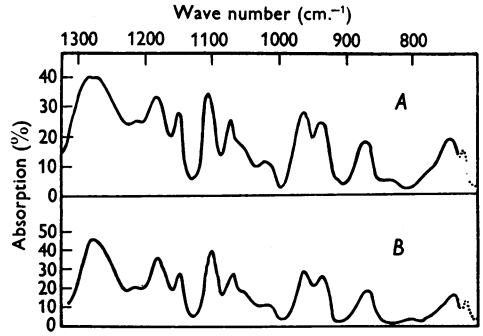

Fig. 3. Comparison of the infrared spectrum of stercobilin hydrochloride obtained from a normal subject (curve *A*) with that obtained from a porphyric subject (curve *B*). The samples were in Nujol suspension; the normal stercobilin was a thick suspension whilst the other was a medium suspension.

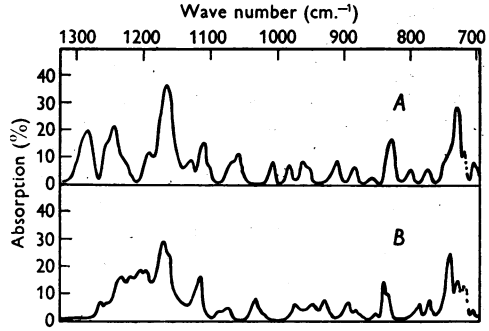

Fig. 4. Comparison of the infrared spectra of coproporphyrin I methyl ester (curve *A*) and of coproporphyrin III methyl ester (curve *B*). The samples were in Nujol suspension. (The spectra of the two esters were identical between 3500 and 1300 cm.$^{-1}$, with the exception that the coproporphyrin III ester had an extra weak band at about 1350 cm.$^{-1}$.)

were taken between 3000 and 700 cm.$^{-1}$, only the region between 700 and 1300 cm.$^{-1}$ being shown on Fig. 3. No differences between the two specimens were observed in the region above 1300 cm.$^{-1}$, but there were very slight differences near 830 and 800 cm.$^{-1}$. In Dr Sheppard's opinion, these very slight differences might be related to 'slight overall changes in molecular structure' or be caused by the presence of small amounts of impurities. The latter explanation appears more probable since the spectra of coproporphyrin methyl esters of series I and III which differ in structure in the same way as normal and the possible 'abnormal' stercobilin differed considerably in the region 1500–700 cm.$^{-1}$ (Fig. 4). The weight of evidence obtained from the infrared spectra therefore favours the assumption that these two stercobilin samples have identical structures. The elementary analysis proves that the stercobilin obtained from the porphyric does not contain more than two carboxyl groups.

DISCUSSION

The origin of stercobilin in the normal subject

Evidence for three metabolic fractions of stercobilin. The results obtained with ^{15}N suggest that stercobilin consists of three separate fractions of different metabolic origin.

The third and largest fraction is derived from the breakdown of red cells of normal life span and is represented in Fig. 1 by the peak which has a maximum at or near the 138th day.

The first peak has a maximum during the second 4-day period, when the isotope content of the glycine 'pool' of the body is also highest. The slope of that part of the curve (Fig. 1) which extends from the time of the second faeces collection (5th–8th day of the experiment) to the 20th day corresponds in shape very closely to a curve calculated for the decrease of ^{15}N in the glycine 'pool' of the body. The latter is obtained from the haem results on the same subject (Gray & Neuberger, 1950) assuming that the ^{15}N content of newly formed circulating haem reflects approximately the isotope content of the glycine 'pool'. This correspondence between the two curves and the relatively high isotope content of the stercobilin collected during the first 4 days of the experiment, when the circulating haem is devoid of isotope, proves that this first stercobilin fraction cannot be derived from circulating haemoglobin, but must originate from some other precursor by a very fast reaction. The time taken for the transformation of the nitrogen of glycine to that of this first stercobilin fraction cannot be accurately assessed, but must be less than 4 days. The excretion of bile, the transformation of bilirubin to stercobilin and the elimination of the latter in the faeces may occupy 1–2 days.

London, West, Shemin & Rittenberg (1948) have also briefly reported the incorporation of ^{15}N in the stercobilin shortly after administration of isotopic glycine to a normal subject.

The continuous excretion of stercobilin with a small, but apparently constant, ^{15}N content between 20 and 80 days suggests that there is a second stercobilin fraction, the metabolic origin of which may differ from that of the first and the third fractions. The haem results suggest that no normal red cells

are destroyed to any significant extent until they reach the age of about 80 days. This second fraction cannot therefore be derived from circulating haemoglobin. It is also unlikely that the first and second fractions are formed by the same mechanism. The isotope content of stercobilin collected near the 60th day is 0·021 atom % excess as compared with a value of 0·161 atom % excess for the second 4-day period. The assumption that the two fractions are formed by the same reaction would imply that the isotope content of the glycine 'pool' had only decreased to about one-eighth in this intervening period. This is obviously not the case. Some bile pigment is stored in the liver and in the gall bladder, and this may lead to a reduction of isotope content of the stercobilin in the beginning of the experiment and an increase in the later stages. However, on this basis we should expect a tailing off of the ^{15}N content of the stercobilin between the 20th and 80th day. This is not the case, and it seems, therefore, probable that the second stercobilin fraction is formed by a separate mechanism.

The relative proportions of the three fractions. The relative amounts of the three stercobilin fractions can be assessed by a graphical integration of the areas enclosed by the extrapolated curves (Fig. 1, broken lines). Such an estimate can only be approximate, since the degree of overlapping cannot be ascertained. However, it appears that about 70 % of the excreted stercobilin originates from the breakdown of circulating haemoglobin (fraction 3), 15–20 % is formed by a fast reaction (fraction 1) and the residual 10–15 % by a much slower reaction (fraction 2). This estimate is supported by other data. At a time when the breakdown of haemoglobin is at its maximum, the rate of decrease of ^{15}N in the total circulating haem is about 0·002 atom % excess/day. Since a fraction corresponding to $1/\bar{T}$ (where $\bar{T}$ is the average life span) is broken down each day, the ^{15}N content of the haem which is converted to bile pigment during that period should have an isotope content of $0{\cdot}002 \times 130$ which is 0·260 atom % excess. The experimental value for the stercobilin excreted at this time was 0·210 atom % excess, suggesting that at least 20 % of the stercobilin was derived from a precursor which contains little or no label. The average isotope content of porphyrins formed during the second 4-day period has been assumed on the basis of the haem results to be about 0·8 atom % excess. The stercobilin excreted during that time had 0·16 atom % excess ^{15}N. It would thus appear that the stercobilin which forms fraction 1 comprises about 20 % of the total.

Metabolic sources of the stercobilin. There are three possible sources of the two fractions of the stercobilin which give rise in the isotope experiments to a relatively high ^{15}N content in the beginning and to a fairly constant, but low, ^{15}N content in the middle of the experiment. It is possible that porphyrins or haems are not involved as intermediates and the bile pigment may be formed directly by condensation of pyrrolic or dipyrryl compounds. This is unlikely, since the arrangement of the side chains in bilirubin and stercobilin resembles completely that found in protoporphyrin IX. There is no reason to expect the same arrangement of side chains in stercobilin, if it were formed by direct condensation of monocyclic or dicyclic compounds. If, however, a linear tetrapyrrolic compound were an intermediate in porphyrin synthesis, such a precursor might be directly converted to biliverdin or bilirubin and thence to stercobilin. The second possibility is that a part of the stercobilin is associated with porphyrin formation outside the erythropoietic system. Cytochromes and catalase are present in almost all animal cells and we may assume that these haem proteins are formed *in situ*. The concentrations of these proteins in the human body are very low compared with that of haemoglobin (Drabkin, 1948), but the turnover rates of such porphyrins may be high. Catalase and cytochrome *c* contain protoporphyrin IX and a modified protoporphyrin respectively, and these proteins may therefore give rise to bilirubin. The oxidation of some of the other cytochromes is likely to produce bilirubin of abnormal structure. Quantitatively the most important haem protein other than haemoglobin is myoglobin, which in man accounts for about 5 % of the total porphyrin (Drabkin, 1948). Its turnover rate in animals, however, appears to be low, as judged from experiments with isotopic iron (Theorell, communication at the First International Congress of Biochemistry, Cambridge 1949). It is suggested, therefore, that the metabolism of porphyrins derived from myoglobin, catalase and possibly cytochrome *c* may account for the ^{15}N content of the stercobilin excreted between the 30th and 80th day, but only for a small proportion of the high isotope content found in the initial period of the experiment.

Finally, it is possible that the first fraction of the stercobilin may in fact be associated with the process of red-cell formation. It has been suggested (Gray *et al.* 1949) that normal red cells have a certain 'infantile mortality', i.e. a certain proportion of cells is normally destroyed before being released into the circulation. There is no evidence, so far as we are aware, for such a hypothesis, but it cannot be excluded. A more probable explanation is as follows: as shown by Thorell (1947) the synthesis of protein in the red cell precedes that of porphyrins. However, there is likely to be a stage during which the red cell behaves like any normal growing cell with respect to porphyrins or haems. During this phase of development both synthesis and destruction of porphyrins will take place, the rate of the latter being smaller than that of the former with the net

result that the total amount of porphyrin increases. The discarded haem or porphyrin will be transformed to bilirubin and stercobilin. On the basis of this hypothesis bile-pigment formation is normally associated with erythropoiesis and an increased excretion of stercobilin may indicate either an increased rate of destruction of red cells or an increased rate of formation. The speed with which the labelled glycine is converted to stercobilin agrees with this explanation.

Stercobilin formation in the porphyric

From the evidence produced it appears almost certain that the stercobilin excreted by the porphyric has a normal structure. The results obtained with ^{15}N indicate that the fraction of the stercobilin which in the normal represents about 70% of the total, and is derived from the breakdown of circulating haem, is only a small fraction (possibly about 10–15%) of the stercobilin excreted by the porphyric. Most of the stercobilin is derived from a precursor which is itself formed from glycine at a very fast rate. It is reasonable to assume that this precursor is identical with that of fraction 1 in the normal and that one of the features of congenital porphyria is a greatly increased rate of formation of this as yet unknown precursor. With respect to the origin of the main fraction of the stercobilin there are the same three possibilities which are discussed above in connexion with the source of fraction 1 of stercobilin in the normal. Haematological findings and other isotope results will be presented later, and the stercobilin figures obtained with the porphyric will be discussed in some detail.

Grinstein, Aldrich, Hawkinson & Watson (1949) in a preliminary note have also reported a high rate of incorporation of ^{15}N into the coproporphyrin, uroporphyrin and stercobilin during the first few days after feeding labelled glycine to a congenital porphyric.

SUMMARY

1. The ^{15}N contents of stercobilin samples collected over a period of 180 days after feeding labelled glycine to a normal subject have been determined. The highest ^{15}N contents were observed in samples obtained between the 120th and 150th day of the experiment, at a time when the rate of breakdown of the labelled circulating haem was highest.

2. A second peak was observed in the first few days of the experiment; this is assumed to be caused by a breakdown of haemoglobin during the maturation of the red cell. Other explanations for this phenomenon are discussed.

3. The stercobilin samples obtained between the 30th and 100th day of the experiment contained small amounts of ^{15}N. It is suggested that this fraction may largely represent bile pigment formed from haem proteins other than haemoglobin.

4. In a similar experiment with a congenital porphyric it was found that the isotope content of the stercobilin was very high in the first 2–3 weeks of the experiment; the peak corresponding to the breakdown of circulating haemoglobin was not very marked.

5. Evidence is presented to indicate that the stercobilin excreted by the porphyric has the same structure as that obtained from normal subjects.

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REFERENCES

Drabkin, D. L. (1948). *Fed. Proc.* **7**, 483.

Fischer, H. & Halbach, H. (1936). *Hoppe-Seyl. Z.* **238**, 59.

Gray, C. H. & Neuberger, A. (1950). *Biochem. J.* **47**, 81.

Gray, C. H., Neuberger, A. & Sneath, P. H. A. (1949). *Biochem. J.* **45**, x.

Grinstein, M., Aldrich, A. R., Hawkinson, V. & Watson, C. J. (1949). *J. biol. Chem.* **179**, 983.

London, I. M., Shemin, D., West, R. & Rittenberg, D. (1949). *J. biol. Chem.* **179**, 463.

London, I. M., West, R., Shemin, D. & Rittenberg, D. (1948). *Fed. Proc.* **7**, 169.

Shemin, D. & Rittenberg, D. (1946). *J. biol. Chem.* **166**, 621.

Thorell, B. (1947). *Studies on the Formation of Cellular Substances During Blood Cell Production*, London: Henry Kimpton.

Watson, C. J. (1934). *J. biol. Chem.* **105**, 469.