THE
HÆMOLYTIC ANÆMIAS
Congenital and Acquired

PART I

THE CONGENITAL ANÆMIAS

By

J. V. DACIE

M.D.(Lond.), F.R.C.P.(Lond.)

Professor of Hæmatology, University of London
(Postgraduate Medical School of London)

SECOND EDITION

With 118 Illustrations

Knowledge of the blood and its diseases continues to advance at a tremendous rate, and in particular that concerning abnormal haemoglobins and syndromes to which they give rise.

The incorporation of new information into the framework of the first edition has been a formidable task and has led to an almost complete re-writing of the book, but the original aim has been before the author, namely, to provide an up-to-date and reasonably complete reference book useful to physicians and pathologists. In order to keep the work up to date it has been necessary to issue it in two separate parts. Part II will deal with the Acquired Anaemias.

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Part I: The Congenital Anæmias

PREFACE TO THE SECOND EDITION

In the preparation of a new edition of this book I have been confronted with some difficult problems. Our knowledge of the blood and its diseases continues to advance at a tremendous rate and the number of new papers which have been published in the last five years dealing with the hæmolytic anæmias and allied fields must run into thousands. In particular, the pace of advance in knowledge of the abnormal hæmoglobins and the syndromes to which they give rise has been breathtaking. The incorporation of even the more important parts of this new information into the framework of the old has been a formidable task and has led to an almost complete re-writing of the book. The size of the task and the ever-diminishing time that I have been able to devote to it explains why the book is appearing in two parts. To complete the whole would have meant postponement of publication for at least another year, perhaps longer.

The present volume (Part I) includes the introductory Chapter and the five chapters devoted to the congenital hæmolytic anæmias and the hæmoglobinopathies. Part II, when completed, will deal with the acquired and secondary hæmolytic anæmias, the drug-induced hæmolytic anæmias, paroxysmal nocturnal hæmoglobinuria and hæmolytic disease of the newborn.

I have kept to the aim I had in writing the first edition: that is to say, I have attempted to provide an up-to-date and reasonably complete reference book useful to Physicians and Pathologists interested in blood diseases. To cover adequately and completely the literature of the whole world would be an almost superhuman task and I certainly have not succeeded in doing this. However, I hope that I have not overlooked too many papers of major importance and that I have given due credit to the pioneer workers in the various fields touched upon. I have omitted, for reasons of space, the few case reports which were to be found in the corresponding chapters of the first edition of this book.

Facts or opinions which are generally accepted and which are not controversial have usually been set out without any specific references, except where reference is made to the original author(s) or where "key" references have been given. The authors of relatively new or less well substantiated information have, on the other hand, generally been named. In the absence of any reference,

a statement or opinion may be assumed to be in line with my own view of the subject.

As before, I have not hesitated to include observations made on patients I have studied personally, and I have been greatly helped by my colleagues Drs. J. C. White, D. L. Mollin and S. M. Lewis, and other past and present members of the medical and technical staff and students of the Postgraduate Medical School, who have allowed me to quote observations they themselves have made. I am also exceedingly grateful to many friends and colleagues in other hospitals who have been so good as to refer patients to me.

I should like to record my gratitude to Dr. H. Lehmann for allowing me to reproduce ten diagrams in Chapters 5 and 6. Some of the diagrams have appeared in a slightly different form in *The Abnormal Hæmoglobins: A Symposium*, recently published by Blackwell Scientific Publications. I am grateful for the publisher's permission to reproduce them, and also for allowing me to include the three illustrations which appear as Figs, 17, 89 and 90, which were originally published in the *British Journal of Hæmatology*. I should also like to thank Dr. P. L. Mollison and Blackwell Scientific Publications for permission to reproduce two figures from *Blood Transfusion in Clinical Medicine*, 2nd edition, 1956. Mrs. P. A. Benyon and Mr. F. Saunders have finished the illustrations for me and Mr. W. H. Brackenbury has taken the photomicrographs which are new to this edition. I am greatly indebted to them and also to Dr. S. M. Lewis for his valued help in proof-reading. As always, I have greatly appreciated the co-operation and understanding of the publishers in the preparation of this edition.

J. V. DACIE

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CHAPTER 1

GENERAL FEATURES OF INCREASED HÆMOLYSIS. BLOOD PICTURE AND METHODS OF INVESTIGATION OF THE HÆMOLYTIC ANÆMIAS

THE essential feature of a hæmolytic anæmia is a reduction of the life-span of the patient's erythrocytes. As will be shown later, this may be due to many different causes. This chapter is concerned with the ways in which an increased rate of erythrocyte destruction *in vivo* may be recognized and with the methods which may be used to measure the intensity of increased hæmolysis. The clinical effect on the patient and the characteristic changes in the blood picture are also described in general terms and brief reference is made to the structure and metabolism of the erythrocytes and the processes which bring about their destruction in health. Finally, the significance and importance of certain laboratory tests will be discussed in connection with the diagnosis of hæmolytic anæmia.

Certain points of definition need consideration. Crosby (1955) pointed out that the term "hæmolytic anæmia" is often used incorrectly, inasmuch as anæmia may be absent although the erythrocytes are being destroyed at an increased rate, and that the same is true of the use of the term "hæmolytic jaundice" for jaundice likewise may be absent. He himself uses "hæmolytic disease" as the generic term and refers to cases without anæmia as suffering from "compensated hæmolytic disease." Although Crosby is undoubtedly correct in what he says, the fact remains that the great majority of patients with hæmolytic disease *are* anæmic; it is for this reason that the term "hæmolytic anæmia" is retained in this book.

Another term which needs careful definition is the word "hæmolysis." It is convenient to refer to "increased hæmolysis" as indicating simply that erythrocyte destruction is proceeding in the patient at a rate in excess of normal. It does not specify the way in which the destruction is brought about, and, in particular, it does not necessarily mean that the cells are being lysed *in vivo* by complement as is usually meant when hæmolysis is used to describe the results of tests *in vitro*.

According to Crosby (1952), William Hunter, of London, was the first to coin and to use the term "hæmolytic." In his book *Pernicious Anæmia*, published in 1901, Hunter referred to the presence of yellow[1] spherical microcytes (Eichhorst's corpuscles) in pernicious anæmia. He wrote (p. 67): "My experiments shew that similar bodies can be produced artificially by action of destructive agents; that they mark the anæmia as due to excessive destruction of blood and not to deficient formation, that they denote the anæmia to be *hæmolytic*, not hæmogenic, in its origin" [my italics].

Classification. The classification of the hæmolytic anæmias presents a number of difficulties, and no new or elaborate system will be presented in this work.

A clinical classification into acute or chronic cases, primary or secondary cases, or into cases with or without hæmoglobinuria, is of limited usefulness. Classifications according to ætiology or pathogenesis are also unsatisfactory, because often one or both are unknown or incompletely known.

The classification adhered to in this book, in which a distinction is drawn between congenital and acquired cases, is orthodox: separation of the congenital hæmolytic anæmias from the rest is based not only on the concept of a genetic cause but also on a distinct type of pathogenesis, the congenital hæmolytic anæmias being the result of various intrinsic abnormalities of the erythrocyte, or of its hæmoglobin, or are secondary to a definite genetically-controlled disorder of hæmopoiesis.

The acquired hæmolytic anæmias are an even more mixed collection of anæmias of varied, and often unknown, causation. Some of them may have an as yet undetermined genetic basis, as has recently been established in certain drug-induced "acquired" hæmolytic anæmias (see Chapter 15). Most of them, nevertheless, certainly depend upon pathological processes which affect normal erythrocytes as well as the patient's corpuscles (extrinsic mechanism of hæmolysis). Paroxysmal nocturnal hæmoglobinuria appears to be unique in that there is no clear evidence for a genetic basis, yet the abnormality is one which seems to be intrinsic to the patient's erythrocytes. This fascinating disease is dealt with in the penultimate chapter; it is followed by hæmolytic disease of the newborn which is unique in a different way, being congenital and "acquired" (from the mother), yet not hereditary.

The separation of the hæmolytic anæmias into two main groups—those due to intrinsic defects of the erythrocyte and those due to mechanisms extrinsic to the cell—has been widely adopted,

[1] As viewed unstained.

and has been used both by Dausset (1953) and Dameshek (1955) in their classifications.

DESTRUCTION OF ERYTHROCYTES IN HEALTH

The Erythrocyte and its Metabolism

It is reasonable to hope that the progress made in recent years in the understanding of the structure and metabolism of the erythrocyte will help in the elucidation of hæmolytic mechanisms. The erythrocyte, although non-nucleated, is alive: after water, its main constituent is hæmoglobin; its framework of stroma consists mainly of protein and lipid, the latter being concentrated at the surface. In addition to water, protein and lipids the erythrocyte contains numerous electrolytes and enzymes. Many of these constituents are in a constant state of replacement and exchange with the surrounding plasma. Hæmoglobin, however, is an exception. The stroma appears to be in a state of flux, particularly with respect to the lipids (London and Schwarz, 1953; James, Lovelock and Webb, 1957). Glycolysis continually occurs and is the main energy-producing mechanism of the cell. Details as to structure and metabolism are given in the recent reviews of Ponder (1954), Prankerd (1955, 1956, 1959), Crosby (1957a) and Jacobs (1958).

The metabolic activity of young cells (reticulocytes) differs substantially from that of mature cells. Reticulocytes retain the ability to synthesize hæmoglobin (London, Shemin and Rittenberg, 1950), and there is an appreciable aerobic respiration; glycolysis, too, is increased in rate. As the reticulocyte matures it diminishes in volume and surface area, and apparently loses lipid as it does so (Crosby, 1952; Prankerd, 1958). Enzymic activity lessens (Allison and Burn, 1955).

Life-Span of Erythrocytes in Health. As indicated above, there is evidence of lessening in the metabolic activity of the erythrocyte as it matures, and it is now generally agreed that it is the exhaustion of enzyme systems which control metabolic activities essential for the integrity of the cell as a whole or of its surface which determines its finite life-span (Granick, 1949; Ponder, 1951; Prankerd, 1955; Mollison, 1956b). Approximately 1/120th of the total number of circulating erythrocytes of a healthy adult is destroyed, and replaced, daily. This figure is based upon estimates of the average life-span of the normal erythrocyte which have been derived from several different types of experiment (Callender, Powell and Witts, 1945, 1947; Jope, 1946; Shemin and Rittenburg, 1947; Mollison, 1956a).

It is generally agreed that the erythrocytes of both men and women survive for approximately the same length of time. However, whereas the curve of elimination in men is normally a straight line, it is often slightly curvilinear in women. It is doubtful whether this can be accounted for entirely by loss of cells at menstruation and there is a suspicion that a certain amount of random destruction takes place. It is highly probable that even in health not every cell survives for exactly the same time in the circulation, *i.e.*, there is some scatter around the mean cell life-span, but the extent of this scatter is not exactly known. It is possible, too, in health that a small proportion of very short-lived cells are also produced (see Mollison, 1956a).

The exact survival time of fœtal (*e.g.*, cord-blood) erythrocytes has been the subject of controversy. However, their life-span appears to be shorter than that of adult cells, but to what degree is uncertain (Hollingsworth, 1955a; Mollison, 1956a; Gilardi and Miescher, 1957).

It seems probable that the normal mechanism of elimination of erythrocytes from the blood stream is by the removal of fragments of cells or of effete intact cells by reticulo-endothelial cells in the spleen and elsewhere (Rous and Robertson, 1917; Rous, 1923). The reticulo-endothelial cells probably act passively and remove the cells or fragments in the same way as they remove foreign particles (Miescher, 1956, 1957). Indirect evidence in favour of the fragmentation hypothesis was provided by Stewart, Stewart, Izzo and Young (1950) who, making use of corpuscles labelled with ^{59}Fe, showed that the mechanical fragility of the oldest cells increased before they were eliminated from the circulation. Recent work has demonstrated in guinea-pigs and rabbits, at least, that the bone marrow, by virtue of its size and rich content of phagocytic cells, is the most important organ in physiological erythroclasis (Miescher, 1956; Ehrenstein and Lockner, 1958). Certainly, the normal spleen can be removed without any great effect on the rate of destruction.

The changes in the erythrocyte or at its surface which cause the fragmentation or increased sensitivity to mechanical trauma are obscure. Presumably, actual loss of substance or increased rigidity are the consequence of metabolic failure.

It is possible, but unproved, that influences outside the cell play a part in bringing about the cumulative damage which limits the life of the normal erythrocyte. It has been suggested, for instance, that stagnation of the blood stream, particularly in the spleen, might be deleterious (Fåhraeus, 1939; Ham and Castle, 1940), and it is conceivable

that tissue lysins normally inhibited by plasma may play a part under conditions of stasis (Ponder, 1951). Normal plasma, too, is known to contain potential auto-agglutinins and lysins, active at 37° C. against erythrocytes damaged by enzymes such as trypsin (Rosenthal and Schwartz, 1951), or against defective erythrocytes like those of paroxysmal nocturnal hæmoglobinuria. It is conceivable that normal corpuscles, although apparently insensitive to these agglutinins and lysins in crude tests *in vitro*, are significantly affected *in vivo* where they are exposed to the action of these factors for much longer periods of time.

The mechanism of hæmolysis where there is a pathological increased rate of cell destruction, and the sites in the body where this takes place, are considered in later chapters in relation to the different hæmolytic syndromes.

Catabolism of Hæmoglobin. There seem to be two main channels for the disposal of hæmoglobin liberated by erythrocyte destruction. If a cell or a fragment of a cell is taken up by an erythrophage in the bone marrow or spleen or elsewhere in the body (extravascular lysis), the hæm of the hæmoglobin molecule becomes transformed to bilirubin which is eventually eliminated from the circulation by the liver and finally forms a major part of the stercobilinogen of the fæces. The iron and protein part of hæmoglobin are retained in the body. This is probably the main method by which hæmoglobin is disposed of in health. If, on the other hand, as in certain hæmolytic anæmias, the erythrocyte breaks up or is lysed in the blood stream (intravascular lysis), the liberated hæmoglobin is disposed of in two ways: if in low or moderate concentration it is retained in the plasma in the form of a haptoglobin-hæmoglobin complex; if in high concentration part will pass through the renal glomeruli and appear in the urine as hæmoglobinuria and part is quickly broken down in the plasma liberating hæm groups which are oxidized and unite with albumin to form the brown pigment methæmalbumin (Fairley, 1941; Allison and ap Rees, 1957). The pigment moiety of methæmalbumin is probably excreted by the liver as bilirubin (Pass, Schwartz and Watson, 1945; London, 1950). The haptoglobin-hæmoglobin complex is eliminated slowly, according to Laurell and Nyman (1957) at a constant rate of about 13 mg. per 100 ml. per hour. It is thought that the complex is taken up by reticulo-endothelial cells, the end-product also being bilirubin (see also p. 12).

A detailed consideration of the complicated steps in the breakdown of bilirubin to fæcal stercobilin is beyond the scope of this book. Recent views are to be found in the reviews of Watson (1957) and Billing and Lathe (1958) (see, however, p. 8).

The distinction between an extravascular and an intravascular mechanism of hæmolysis was made as early as 1901 by Hunter. Referring to "*chronic hæmatocytolysis*" (p. 363), he said: "They [the red corpuscles] become spherical, deeper in colour, and retain their hæmoglobin to the last. In this form they continue to circulate until finally they are enclosed within the active cells of the spleen, or leucocytes of the blood, and are stored up within the *spleen* or in the *capillaries of the liver*" [author's italics]. He then went on to refer to "*acute hæmocytolysis*" (p. 364), saying: "The second process is marked by a different series of phenomena. The first of these is a liberation of hæmoglobin from the corpuscle. It escapes from the corpuscle, either alone, or in combination with the albuminous stroma. Its fate is not, as in the former case, to be taken up by splenic cells or leucocytes within the blood, but it is carried to the liver in the portal blood, where it is taken and broken up by the liver cells."

EVIDENCE FOR AN INCREASED RATE OF HÆMOLYSIS

As the bile and fæcal pigments are largely derived from the catabolism of hæmoglobin, it is natural to expect increased production and elimination of these substances whenever the rate of erythrocyte destruction is increased.

Hyperbilirubinæmia. In hæmolytic anæmia the plasma bilirubin concentration usually lies between 1 and 3 mg. per 100 ml. Occasionally, it is within the normal range; it is rarely above 5 mg. per 100 ml. The direct Hijmans van den Bergh reaction is usually negative or delayed positive in uncomplicated cases. The bilirubin concentration, however, is an unreliable measure of hæmolysis, as it depends not only on the amount of pigment produced, but also on the efficiency of the liver in excreting it. Moreover, the total amount produced depends not only on the rate of hæmolysis but also upon the total number of erythrocytes present. For instance, the same amount of bilirubin might be expected to be produced per day by the destruction of 5% of a patient's erythrocytes when the total count was 5,000,000 per cu. mm. as by the destruction of 25% of the erythrocytes when the count was 1,000,000 per cu. mm. Other things being equal, therefore, the highest bilirubin levels might be expected in patients with the highest erythrocyte counts. In practice, however, this expected correlation is seldom found, as the patients with the highest counts are usually those in whom the rate of hæmolysis is not great, *i.e.*, they are patients in whom compensation for hæmolysis is possible (see p. 26).

It is probable that in those patients in whom the plasma-bilirubin level is normal despite evidence of increased hæmolysis, the normal levels are maintained by the ability of the healthy liver

to excrete far more bilirubin than it is normally called upon to do.

The relationship one to the other of the bile pigments giving rise, respectively, to direct and indirect Hijmans van den Bergh reactions has recently been clarified. Cole and Lathe (1953) were able to separate by reverse phase chromatography two types of bile pigment, neither of which was bound to protein; the slow-moving less soluble fraction was the normal indirect-reacting pigment of the blood stream, and the faster-moving more soluble fraction was the direct-reacting pigment.

In a recent review Billing and Lathe (1958) summarize much recent progress. The faster-moving pigment consists of two fractions, I and II, both of which predominate in bile and in the blood in obstructive jaundice. These fractions are the mono- and di-glucuronides of prehepatic bilirubin and it is this conjugation which allows the direct Hijmans van den Bergh reaction to proceed. Billing and Lathe make the point that the terms "indirect bilirubin" and "direct bilirubin" have no real chemical or physiological significance and should be abandoned in favour of "bilirubin" and "conjugated bilirubin."

In hæmolytic jaundice, bilirubin predominates and only small quantities of pigments I and II are present. In hæmolytic disease of the newborn the pigment is entirely composed of bilirubin, and it is the inability of the neonatal liver to conjugate bilirubin that is responsible for the physiological jaundice of the newborn and for the very high plasma levels which are reached in hæmolytic disease at that time.

The ability of the healthy liver to conjugate and excrete bilirubin is very great. According to Billing and Lathe (1958), if all the hæmoglobin in the body were transformed to bilirubin, this could be excreted in 10–12 hours. It is thus easy to see why patients with hæmolytic anæmia rarely become markedly jaundiced and may in fact not be jaundiced at all. According to Crosby (1955) it nevertheless takes about $1\frac{1}{2}$ hours for plasma to be completely cleared of bilirubin present at a normal concentration. This figure is arrived at in the following way: at a concentration of 0·5 mg. per 100 ml. there would be 15 mg. of bilirubin circulating in a plasma volume of 3,000 ml.; if the total amount of bilirubin leaving the blood stream in 24 hours was 250 mg., then each 15 mg. would be excreted in $\frac{24 \times 15}{250}$ hours $= 1\frac{1}{2}$ hours, approximately.

Excretion of Urobilinogen

"Urobilinogen" is the name given to the fæcal pigments derived from bilirubin which, when reduced, give coloured compounds with Ehrlich's reagent, dimethylaminobenzaldehyde. In the

fæces they exist as a series of colourless compounds, *d*-urobilinogen, *i*-urobilinogen (mesobilirubinogen) and *l*-urobilinogen (stercobilinogen), and as orange-yellow derivatives formed as the result of loss of two hydrogen atoms, *d*-urobilin, *i*-urobilin and *l*-urobilin (stercobilin) (Watson, 1957).

In hæmolytic anæmia the excretion of fæcal urobilinogen is often far in excess of normal and the quantitative estimation of the pigments has often been used as a measure of the degree of increased hæmolysis (Watson, 1938; Crosby and Akeroyd, 1952). In the following section the accuracy of such estimations, their interpretation and the figures obtained in health will be briefly considered.

Normal Urobilinogen Excretion. One gram of hæmoglobin theoretically should give rise on degradation to approximately 35 mg. of urobilinogen. This relationship is based on the ratio of the molecular weights of hæmoglobin (68,000) and four molecules of hæm (2,000) from which the bilirubin and urobilinogen are derived. If, therefore, in a normal adult, 6 g. of hæmoglobin are catabolized daily, this should give rise to 210 mg. of urobilinogen.

The figure of 6 g. is arrived at as follows: A 70-kg. man, with an erythrocyte volume of 30 ml. per kg., will have a total circulating erythrocyte volume of 2,100 ml. Assuming the MCHC to be 33%, this means that the total circulating hæmoglobin is about 700 g. Dividing this figure by 120, on the assumption that 1/120th of the hæmoglobin is catabolized daily, gives a figure of approximately 6 g.

Not all the fæcal urobilinogen comes from catabolized hæmoglobin. Studies with ^{15}N-labelled glycine have shown that a significant proportion of the fæcal hæm pigment is derived from sources other than hæmoglobin (London, West, Shemin and Rittenburg, 1950; Gray, Neuberger and Sneath, 1950; Watson, 1957). In health, this proportion may be as high as 10–20%; in pernicious anæmia it is higher and may reach 40% (London and West, 1950). It is thought that the non-hæmoglobin-derived pigment comes from several sources: (*a*) from tetrapyrrol (hæm or porphyrin) pigments formed but not utilized for hæmoglobin formation; (*b*) from hæmoglobin-containing cells destroyed in the bone marrow before delivery into the blood stream; (*c*) from defective very short-lived cells, possibly trapped and destroyed in the spleen; or (*d*) from myoglobin and other hæm pigments such as catalase. (*a*), (*b*) and (*c*) represent "early-appearing" urobilinogen.

Returning to the urobilinogen excretion of the healthy 70-kg. adult referred to above, 30 mg. of pigment should be added to the 210 mg. of hæmoglobin-derived pigment to allow for the early-appearing pigment. This gives a total of 240 mg. of pigment excreted per day.

Study of published data on urobilinogen excretion in health shows that figures as high as this are rarely attained and that the recorded normal ranges are very wide. Greppi (1926) gave a range of 90–150 mg. per day, Watson and Bilden (1941), 40–280 mg. per day, Watson (1942), 40–280 mg. per day and Maclagan (1946), 22–121 mg. per day. Sparkman (1939) recorded figures varying from 76–520 mg. per 100 g. of fæces as the daily excretion in 100 normal adults. Mills and Mason (1952) gave normal values for children.

It is obvious that the daily urobilinogen excretion of a healthy child will be less than that of an adult because his total erythrocyte volume is much less, and the same is true of an anæmic adult, if the rate of hæmoglobin breakdown is normal. The only satisfactory way to get round this difficulty is to relate the pigment excretion to the total circulating hæmoglobin (Greppi, 1926; Watson, 1938).

Greppi used a hæmolytic index in which he related the patient's weight, hæmoglobin percentage and urobilinogen excretion to normal values of 70 kg., 100% and 120 mg. per day, respectively. This index, normally 1·0, gave values as high as 10–15 in acute hæmolytic states. Miller, Singer and Dameshek (1942) reported urobilinogen excretion in relation to the total circulating hæmoglobin and gave values of 11–21 mg. per 100 g. of hæmoglobin in health, while Giblett and her co-workers (1956), using a similar index, reported an excretion of 0·14–0·48 mg. per g. of hæmoglobin (mean 0·248 mg.) in 18 healthy young men. Again, the excretion figures vary widely, even when based on the total circulating hæmoglobin, and there is a disquieting discrepancy between the last two sets of data quoted, the mean of those of Giblett and her co-workers exceeding the upper limit of normal given by Miller, Singer and Dameshek.

It is obvious that it is difficult to estimate fæcal urobilinogen with any degree of accuracy. The technical difficulty of the collection of 24-hour or 96-hour samples of fæces, difficulties in obtaining representative samples of the specimens, and the use of an arbitrary colour standard in the actual estimation, all combine to reduce the reliability of the figures obtained. Furthermore, constipation, diarrhœa and antibiotics reduce the amount of pigment that can be estimated.

As already mentioned, not all the fæcal urobilinogen is derived from the hæmoglobin of effete erythrocytes; contrariwise, not all the catabolized hæmoglobin can be accounted for as fæcal or urinary urobilinogen. Although in dogs with artificial biliary fistulæ

given acetylphenylhydrazine, 88% of the hæm liberated from the breakdown of hæmoglobin could be recovered as bilirubin in the bile (Cruz, Hawkins and Whipple, 1942), the proportion recoverable in man is unknown. Moreover, it is known that the amount of pigment that can be estimated as fæcal urobilinogen is considerably less than the bilirubin excretion. This suggests that either the conversion of bilirubin to urobilinogen is not quantitative, or else that the urobilinogen is altered in part to other substances which are not readily estimated, or that some of the pigment is reabsorbed (Watson, 1942; Crosby and Akeroyd, 1952; Gray, 1953).

The discrepancy between hæmoglobin destruction and urobilinogen excretion is considered in some detail by Watson James (1955), who studied the excretion of two normal males over many months, and Watson (1957).

In Watson's view the discrepancy does not seem to be accountable by the formation of dipyrrolmethenes such as mesobilirubifuscin, which, although a normal constituent of the fæces, he considers to be a by-product of hæm synthesis. Watson referred to a patient with refractory anæmia whose average urobilinogen excretion was 30 mg. daily, although a figure of 133 mg. would be expected on the basis of hæmoglobin catabolism. An increase in mesobilirubifuscin or failure of conjugation of bilirubin was ruled out as causes, and conservation of pigment and its re-utilization for hæm synthesis also seemed improbable. He concluded that reabsorption of pigment to an abnormal degree without re-utilization might have been the explanation. That some urobilinogen is normally reabsorbed is demonstrated by the presence of the pigment in the urine. The amount, however, excreted daily in health is small, $<3{\cdot}5$ mg. (Watson, 1942), and the greater part of that absorbed is thought to be re-excreted by the liver.

Urobilinogen Excretion in the Fæces in Hæmolytic Anæmia. That the fæces of patients with hæmolytic anæmia are often dark in colour and their content of urobilinogen unusually high have been known for many years (see Watson, 1937, 1938). Crosby and Akeroyd (1952) and Crosby (1955) have brought the subject up to date. In an adult the fæcal urobilinogen excretion often exceeds 500 mg. per day and occasionally rises to within the 1,000–1,500 mg. range. A patient with a chronic hæmolytic anæmia, whose erythrocyte count is in equilibrium as the result of the maximum possible hyperplasia of the bone marrow, can synthesize 6–8 times the normal amount of hæmoglobin (Crosby and Akeroyd, 1952), namely, about 50 g. daily, compared with the normal synthesis of 6–7 g. in a 70-kg. man. A daily breakdown of 50 g. of hæmoglobin would theoretically be expected to yield 1,750 mg. of urobilinogen. However, as has been explained above, this amount of pigment is not likely to be estimable.

Crosby (1955) has given details of two patients intensively studied over long periods of time. In one of these patients, who had a hereditary non-spherocytic hæmolytic anæmia, the output of pigment, based on