THE BREAKDOWN OF HAEMOGLOBIN AND FORMATION OF BILE PIGMENTS

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During the past 25 years, Lemberg and his colleagues in Australia have made a systematic study of the chemical structure of bile pigments and of their formation *in vitro* from haemoglobin.[1,2] Haemoglobin incubated with ascorbic acid in air at pH 7·4 and 37°C. was shown to give rise to biliverdin.

On the results of spectroscopic observations, the Australian workers based a new concept of haemoglobin breakdown, as depicted in the following scheme:

New concept

Haemoglobin
↓ + O_2
Choleglobin
↓ + O_2
Verdoglobin
↓ — Fe and protein
Biliverdin
↓ + H_2
Bilirubin ——+ H_2→Sterco (Uro)bilinogen.

Old concept

↑ + O_2
Porphyrin
↑ — Fe
Haematin
↑ —protein
Haemoglobin

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Changes in the haem prosthetic group (Fig. 1) were thought to occur, while the globin remains attached and unchanged. They envisaged that at first the α-methine bridge is oxidized, but the ring remains carbon-closed: the compound formed is choleglobin. Later the carbon is replaced by oxygen and the protein is then verdoglobin. Only now is the central iron atom removed and biliverdin detached from globin. Neither haematin nor porphyrin is an intermediate in this chain of events.

Independent support for this hypothesis came from Japan,[3] and from Scandinavia, where Sjöstrand[4] detected carbon monoxide arising during the coupled oxidation of haemoglobin and ascorbic acid. Using Lemberg's conditions, sometimes modified with regard to oxygen concentration, Kench[5] has shown that, of a number of haem compounds tested, only cytochrome c did not provide a source of bile pigments *in vitro*. Horse-liver catalase and a plant peroxidase were good sources of bile pigment, and metmyoglobin prepared from horse myocardium gave most bile pigment of any haem derivative examined. Protoporphyrin was not disrupted, but haematin[6] gave yields of biliverdin equal to, and methaemalbumin more than twice, those derived from haemoglobin. On these results it was possible to argue that biliverdin could arise from breakdown of haematin after the prosthetic group had become detached from globin, and such a process was proposed as a valid alternative to the choleglobin hypothesis as the *in vivo* metabolic pathway. Isotopically-labelled haematin and protoporphyrin, injected intravenously, have been shown by London and his co-workers[7,8] to be converted into bile pigments, but this does not prove that the former

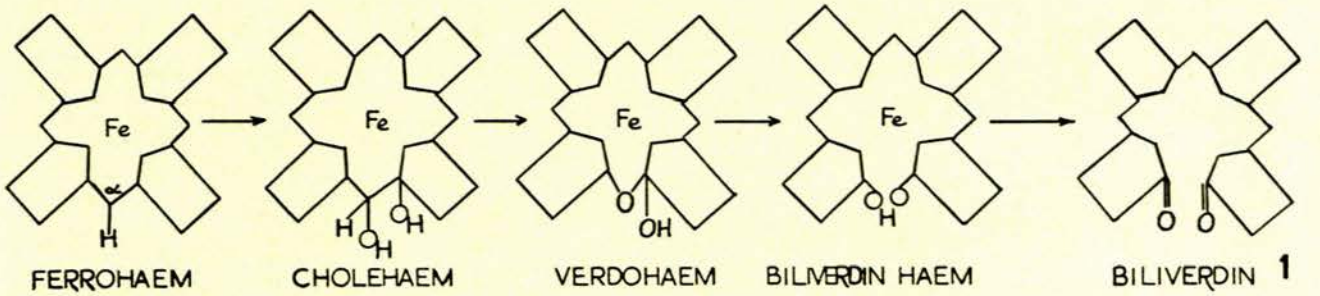


Fig. 1. Changes in the prosthetic group.

compounds are intermediates in the catabolic pathway normally followed. Methaemalbumin or some other haemoprotein could readily be formed and degraded by an unusual route.

So far the green haemoproteins, choleglobin and verdoglobin, have defied all attempts at isolation, and it may yet prove that the peroxidative system is not a reliable guide to *in vivo* conditions, which are probably enzymically controlled.

In an attempt to resolve some of the anomalies, we have carried out experiments, here described, to determine whether appropriate tissues, added to the peroxidative system — haemoglobin, ascorbic acid, oxygen — could augment the yield of biliverdin. By these means we hoped to ascertain, if possible, the importance of enzymic and non-enzymic factors in the formation of bile pigments.

EXPERIMENTS AND RESULTS

The Peroxidative System

A concentrated haemoglobin solution was prepared from human blood according to the method of Drabkin,[9] which involved washing of packed red blood cells with sodium-chloride solution, haemolysis with distilled water and precipitation of the cellular stroma with toluene. The haemoglobin content of the solution was determined from its optical density at 578 mμ (after suitable dilution) and that of a standard solution of crystalline human haemoglobin.[10]

In the degradation experiments, haemoglobin (500 mg.; 50 ml. of 1% w/v) and L-ascorbic acid (50 mg. of 0·1% w/v) were incubated together at 37°C. in Petri dishes open to the air for variable periods, with or without shaking. The mixture was prepared by dissolving 1 G. of crystalline human haemoglobin in approximately 20 ml. of water, the pH adjusted to 7·3 by addition of Sörensen's phosphate buffer (M/15, pH 7·5) and the volume brought to 100 ml. with distilled water. 50 mg. of L-ascorbic acid were then dissolved in 50 ml. of the haemoglobin solution. As the reaction proceeded, the spectral absorption of oxyhaemoglobin in the green and Soret regions, and of ascorbic acid at 265 mμ, fell progressively. Plateaux appeared at 630 mμ after 2 hours' incubation, and at 670 mμ somewhat later, both attaining maximum extinction at 6 hours. After 24 hours all specific absorption had virtually disappeared throughout the optical range 400 - 700 mμ (Fig. 2).

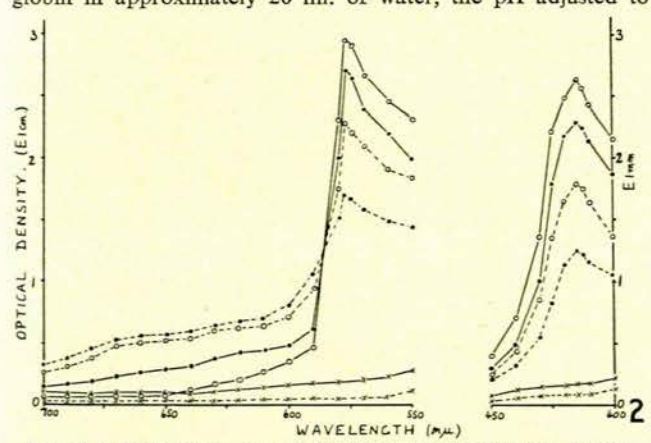


Fig. 2. Changes in the absorption spectra accompanying the coupled oxidation of haemoglobin and ascorbic acid — 500 mg. of haemoglobin, 50 mg. of L-ascorbic acid in 50 ml. of diluted phosphate buffer, pH 7·3, incubated in air at 37°C.
O——O = 0 hr.; ●——● = 2 hr.; O— — —O = 4 hr.;
●— — —● = 6 hr.; X——X = 24 hr.; X— — —X = 48 hr.

Bile pigments (biliverdin and bilipurpurin), either free or in acetic acid-labile combination, were extracted following incubation by the method of Lemberg, Lockwood and Legge,[11] with modifications.[6] Peroxide-free ethyl ether was the solvent, from which biliverdin and bilipurpurin were removed successively by 1·37 N, 2·74 N and 5·48 N hydrochloric acid. The concentrations of the two pigments were calculated from the optical densities measured at 675 mμ [maximum optical density (MOD), biliverdin] and 595 mμ (MOD, bilipurpurin).

Liberation of Amino Acids

Experiments were made to discover if the formation of bile-pigment intermediates was accompanied or preceded by liberation of amino acids from the globin part of the haemoglobin molecule. Haemoglobin was incubated with ascorbic acid, as already described, for periods of 0 - 6 hours. One portion was extracted for biliverdin, and another for measurement of the free amino acids. For this, a protein-free filtrate was obtained by shaking the reaction products with ice-cold ethanol (9 vols. of 85% v/v brought to pH 8·0 by addition of solid sodium bicarbonate), the resultant mixture being allowed to stand at 4°C. for 2 hours. It was then filtered and a measured volume evaporated to dryness *in vacuo* and the residue dissolved in 2 ml. of aqueous isopropanol (10% v/v). This solution was heated with the ninhydrin (indanetrione hydrate) reagent of Cocking and Yemm[12] and the optical density of the violet-coloured product was measured at 570 mμ. A standard curve was prepared, using glycine.

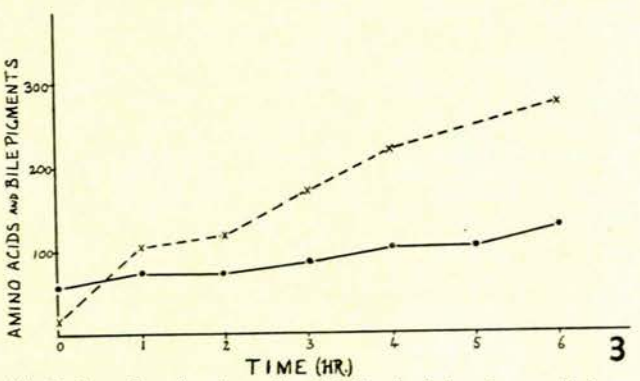


Fig. 3. Formation of amino acids and biliverdin during the peroxidative breakdown of haemoglobin.
●——● = amino-acid nitrogen.
X— — —X = biliverdin and bilipurpurin.
Results are expressed as μg./100 mg. of haemoglobin.

The observations are presented in Fig. 3. A measurable quantity of amino acids was present before the addition of ascorbic acid, and the concentration could not be significantly reduced by overnight dialysis against distilled water (4°C.) or running tap water. It is clear from Fig. 3 that an 18-fold increase of biliverdin took place while the free amino-acid nitrogen was doubling itself. The yield of biliverdin was 7·5% of the theoretical value, that of amino acids 0·47%. Evidently, therefore, opening of the haem ring was not dependent on an antecedent, fine-fragmentary degradation of the globin molecule, but breaking up of the molecule into polypeptides too large to be soluble in 77% v/v ethanol could not be discounted by this procedure.

Size of the Bile-pigment Intermediates

Haemoglobin (100 mg. of 1% w/v solution, pH 7·3) was incubated with L-ascorbic acid (10 mg.) for 5 hours at 37°C. The mixture was then transferred to a cellophane sac which was suspended in running tap water for 4 hours. The non-diffusible contents of the sac were extracted for biliverdin, in parallel with a non-dialysed control.

The quantities of biliverdin found were: dialysed 158 μg., control 185 μg. It appears that the bile pigments originate almost entirely from compounds of molecular weight greater than 5,000.[13] This is the approximate lower limit of molecules which would not diffuse through this membrane. The yield of biliverdin was not improved when activated papain was added to the reaction mixture to see if proteolysis of the globin would facilitate formation of bile pigments.

Foetal Haemoglobin

Foetal haemoglobin was prepared from cord blood in a like manner to adult haemoglobin. Its concentration was determined by the one-minute denaturation method,[14] which is based on the fact that HbF is much more stable in N/12 sodium-hydroxide solution than is HbA.

The maximal production of biliverdin from the two haemoglobins was similar, but the concentration was maintained for a longer period in the case of HbF. This difference was apparently due to denaturation and precipitation of the reaction products from adult haemoglobin.

Effects of Tissues on the Peroxidative Breakdown of Haemoglobin

These experiments were carried out to test the influence of cellular components which might participate in the degradation of the erythrocyte, and its contents, in the reticulo-endothelial system.

1. *Cellular stroma.* Red-cell stroma was prepared as described by Ponder.[15] Packed red corpuscles of freshly-withdrawn human blood were washed with isotonic sodium-chloride solution, and then haemolysed with distilled water. The haemolysate was saturated with carbon dioxide, the stroma centrifuged down and the supernatant discarded. The stroma, washed repeatedly with 0·1 % w/v sodium-chloride solution saturated with CO_2, was lyophilized and stored at 4°C. Addition of this stromal preparation to the standard peroxidative system did not measurably alter the course of bile-pigment formation. The cellular membranes, which together are the stroma, are composed of lipoprotein complexes. It seemed possible that one or more of the degradative products of the cell membrane might function as catalysts of haemoglobin catabolism. To test this hypothesis, the stromal preparation was subjected to the action of activated papain and lecithinase C (*Cl. welchii* type A, dried filtrate, B 563 H. kindly supplied by Dr. G. H. Warrack, Wellcome Research Laboratories), either singly or together. In no instance was the bile-pigment yield improved (Fig. 4).

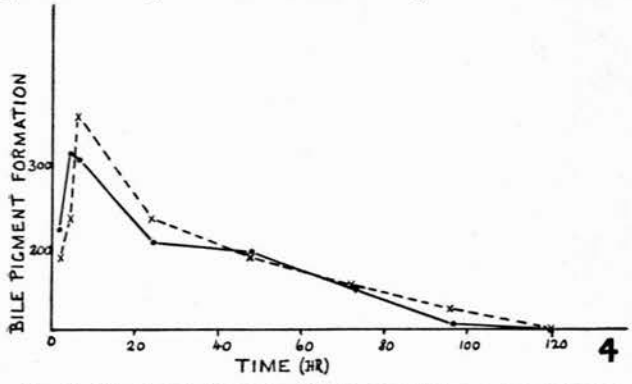


Fig. 4. Bile-pigment formation in the peroxidative system following addition of cellular stroma previously treated with papain and lecithinase.
X— — —X = haemoglobin-ascorbic acid alone.
●———● = haemoglobin-ascorbic acid and treated cellular stroma.

2. *Leucocytes.* In response to subcutaneous injections of oleum terebintheae, sterile abscesses were formed in rabbits at the site of the injections. The pus contained numerous neutrophilic polymorphonuclear leucocytes and no detectable micro-organisms. 2 ml. of the freshly-withdrawn, pooled material were added to the standard haemoglobin-ascorbic acid system, when it was observed that the formation of bile pigment was remarkably inhibited (Fig. 5). Since the Soret absorption did not decline during 24 hours of incubation (Fig. 6), no perceptible decomposition of haemoglobin had occurred. A possible explanation is that leucocytes are rich in myeloperoxidase and

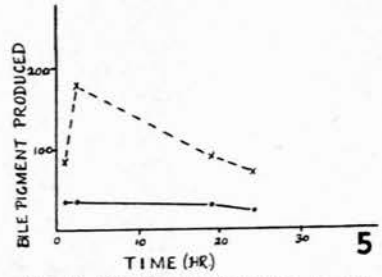


Fig. 5. Bile-pigment formation in the peroxidative system following addition of leucocytes.
X— — —X = haemoglobin-ascorbic acid alone.
●———● = haemoglobin-ascorbic acid + leucocytes.

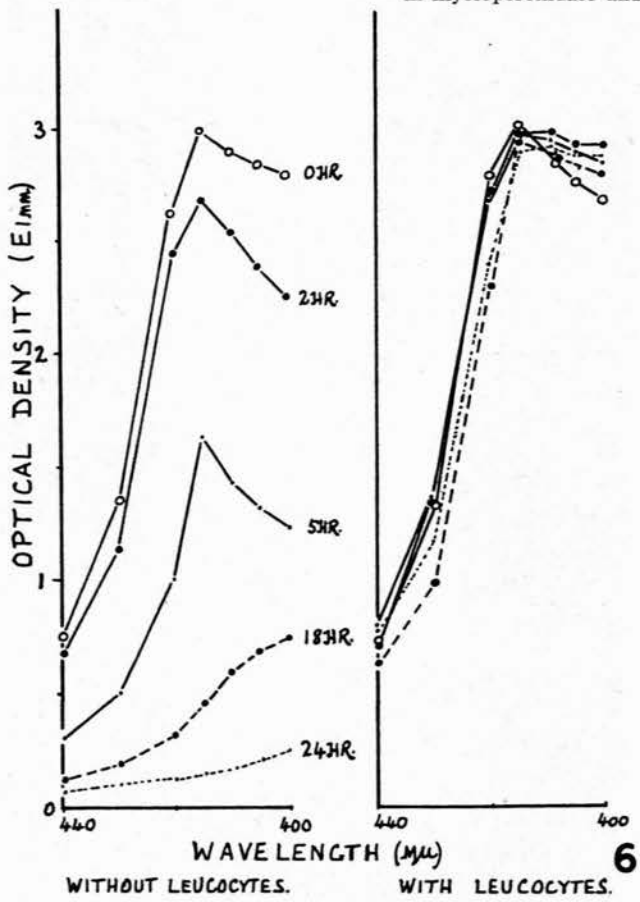


Fig. 6. Changes in the absorption spectra of the peroxidative system following addition of leucocytes.

this enzyme may decompose the hydrogen peroxide, the active agent which arises in the peroxidative system, before it can react with the haem prosthetic group.

3. *Reticulo-endothelial cells.* The principal sites of haemoglobin catabolism are bone marrow, liver and spleen.[16,17] No degradative changes in haemoglobin within circulating erythrocytes have been detected.[18] To test the effects of tissues, rich in reticulo-endothelial cells, as adjuvants in the peroxidative system, they were homogenized (5 G., except in the case of neonatal bone-marrow) in Sörensen's phosphate buffer, M/15, pH 7·4, at 0°C. by the procedure of Schwartz and Watson,[19] and the homogenate was added immediately to the haemoglobin-ascorbic acid reaction mixture. Since such tissues may contain free bile pigments initially, biliverdin and bilipurpurin were extracted and determined before and following incubation. To do this, the solution or tissue homogenate was shaken thoroughly with diethyl ether (2 vols. freed from peroxides over sodium) and biliverdin was transferred from the ethereal phase into small volumes of 1·37 N hydrochloric acid. The concentration of biliverdin in the acid extract was measured spectrophotometrically at 675 mμ.

(*a*) *Adult tissues:*

Liver

This tissue, obtained 2 hours after death, had no detectable influence on bile-pigment formation.

Spleen

Spleen 1. This tissue was homogenized immediately following splenectomy of a patient suffering from essential thrombocytopenia. The splenic homogenate contained

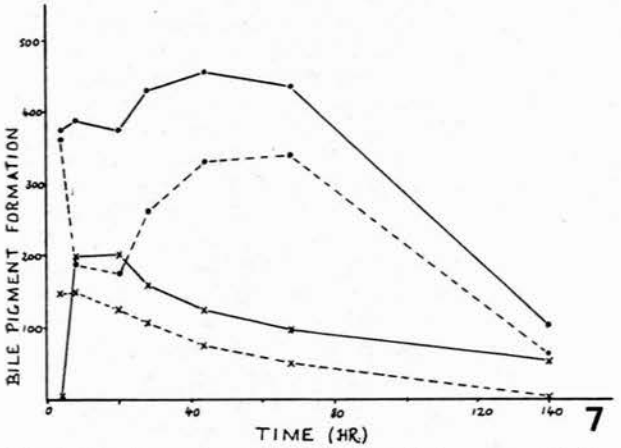


Fig. 7. Bile-pigment formation in the peroxidative system following addition of the splenic pulp of a patient with haemolytic anaemia.
X— — —X = haemoglobin-ascorbic acid alone.
X————X = splenic pulp-ascorbic acid.
●————● = haemoglobin-ascorbic acid + splenic pulp.
●— — —● = corrected curve : (haemoglobin-ascorbic acid + splenic pulp) − (splenic pulp-ascorbic acid).

4 μg. of bilirubin, but no biliverdin, and an increase of 10 μg. of bilirubin took place during the course of the experiment. There was no significant net accumulation of bile-pigment precursors.

Spleen 2. This organ was that of a patient suffering from haemolytic anaemia and was removed surgically. 4·3 μg. of bile pigments (biliverdin and bilipurpurin) were found in the splenic pulp itself, i.e. 0·86 μg/G. of fresh spleen. It effected a remarkable enhancement of haemoglobin conversion to bile-pigment intermediates (Fig. 7), only part of which arose from haemoglobin in the tissue. The optical density in the Soret region of splenic pulp mixed with ascorbic acid (Fig. 8) is equivalent to approximately 100 mg. of haemoglobin. When a correction has been applied to allow for this extra haemoglobin, it is clear that the splenic pulp definitely raised the output of bile pigment (Fig. 7). Apart from doubling of the

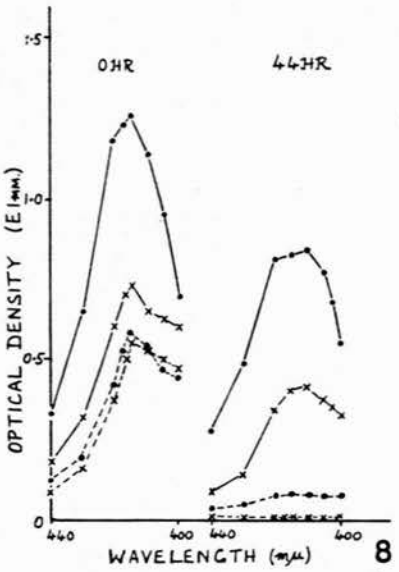


Fig. 8. Changes in the absorption spectra in the peroxidative system following addition of the splenic pulp of a patient with haemolytic anaemia.
X— — —X = haemoglobin-ascorbic acid alone.
X————X = splenic pulp-ascorbic acid.
●————● = haemoglobin-ascorbic acid + splenic pulp.
●— — —● = haemoglobin-ascorbic acid + heated splenic pulp.

yield at 4 hours, bile-pigment compounds remained at high concentration in the mixture for at least 70 hours.

Similar experiments were performed on splenic homogenate after it had been heated for 30 minutes in boiling water to inactivate the enzymes present. The adjuvant effect of the spleen was thereby considerably diminished (Fig. 9) although much activator still remained following heat treatment. Heating completely denatured the haemoglobin present initially in the spleen (Fig. 8). As judged by the fall in optical density at 415 mμ at 44 hours, the quantity of haemoglobin degraded was little changed by the addition of fresh or heated spleen. It may be concluded, therefore, that the spleen of this haemolytic anaemic patient contained substances, some heat-stable and others heat-labile, whose presence was associated with accumulation of bile-pigment proteins. The yields, however, were never greater than 10% of that theoretically possible.

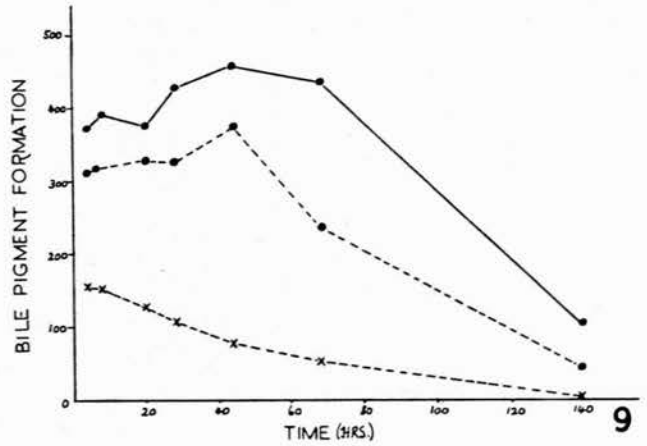


Fig. 9. Bile-pigment formation in the peroxidative system following addition of freshly-homogenized spleen or heated splenic pulp.
X— — —X = haemoglobin-ascorbic acid alone.
●————● = haemoglobin-ascorbic acid + fresh spleen.
●— — —● = haemoglobin-ascorbic acid + heated spleen.

(*b*) *Neonatal tissues:*

The source of these was a 30-week-old foetus who died 2 minutes following forceps delivery, and homogenates of liver, spleen and bone marrow were prepared. All the tissues simulated the action of haemolytic spleen in bringing about the conversion of haemoglobin to bile-

pigment proteins (Fig. 10). A number of further experiments were performed with neonatal liver to analyse this catalytic process. The observations can be summarized as follows:

1. Bile-pigment proteins were present only in traces in neonatal liver: mean, 6 neonatal livers, 1·2 μg. bile pigment per G. of tissue.

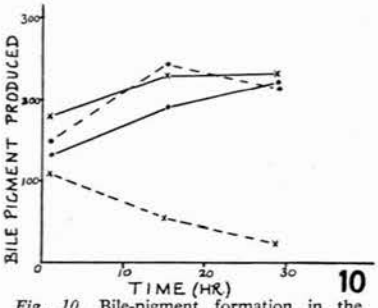


Fig. 10. Bile-pigment formation in the peroxidative system following addition of neonatal tissues.
X— — —X = haemoglobin-ascorbic acid alone.
●——● = haemoglobin-ascorbic acid + spleen.
X——X = haemoglobin-ascorbic acid + bone marrow.
●— — —● = haemoglobin-ascorbic acid + liver.

2. There was activation of the haemoglobin-ascorbic acid system towards bile-pigment protein formation, additional to that arising from haemoproteins in the liver itself.

3. Approximately one-third of the activation was abolished by heating for 30 minutes at 100°C.

4. Diffusible and non-diffusible activators present in the neonatal liver made approximately equal contributions to the reaction.

5. Addition of glutathione, a heat-stable component of cells which Lemberg *et al.*[20] found to promote the coupled oxidation of haemoglobin and ascorbic acid, did not raise the maximal concentration of bile-pigment proteins further at 28 hours.

DISCUSSION

The catalytic influence of certain tissue preparations on the conversion of haemoglobin to bile-pigment proteins has been demonstrated. In each instance, both heat-stable and heat-labile components were contributory, and in neonatal liver some were diffusible, others non-diffusible. The precise nature of the acid-labile proteins, the source of the liberated bile pigments, is as yet unknown, but the maximum conversion of haemoglobin to such products never exceeded 10% of that theoretically possible.

Cellular envelopes apparently had no effect on *in vitro* degradation of haemoglobin, while leucocytes were markedly inhibitory.

The observations on free amino acids appearing in the peroxidative system during incubation led to the conclusion that haem disruption may proceed without proportional proteolysis of globin, and there was no evidence spectroscopically of the appearance of haematin as an intermediate (absorption band of haematin at 390 - 400 mμ). It is conceivable that haematin did arise and was decomposed equally rapidly, but previous work[5] has shown that a much higher oxygen pressure would have been needed to achieve such a high catabolic rate. Prior attack on the globin molecule by papain did not apparently expedite the appearance and accumulation of bile-pigment proteins.

Two aspects of the observations are of special interest from the viewpoint of the *in vivo* process of bile-pigment formation:

1. The overall yield of bile pigment, either free or combined in acid-labile compounds, was consistently 10% or less of that theoretically possible, compared with about 80% *in vivo*, as far as one can judge from daily excretion of urobilinogen.

2. The quantities of free bile pigment (biliverdin and bilipurpurin) which arose were always relatively very small.

These findings may provide a useful guide to future exploration in this very difficult field. A number of possible explanations come to mind with regard to these phenomena, discussion of which may prove most worth while. As a general observation, bile pigments are found widespread in nature[21] and may be as ubiquitous as haems and haemoproteins. Fresh human and ox bile contains only bilirubin, the bile of some other species only biliverdin, e.g. that of amphibia and of birds. Biliverdin is the pigment responsible for the blue-green colour of birds' eggs. It is found in many insects, as butterfly-wing pigments, the green integument of *Mantis religiosa*, and in the gut of blood-suckers such as the cannibal bug Rhodnius and the louse.

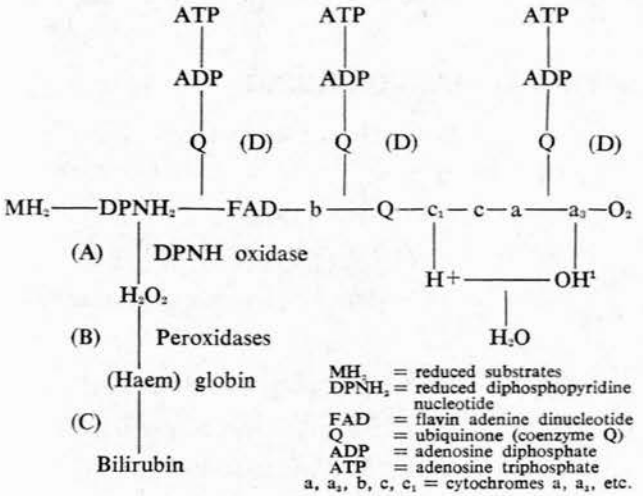
Bilirubin, -verdin and -purpurin are responsible for much of the pigmentation of crustacea, molluscs, the sea anemones, polychaete worms and sea snails, and they add their beautiful hues to the plant kingdom as well. In the seaweeds, bile pigment is the prosthetic group of the chromoproteins phycoerythrin and phycocyanin, the red and blue pigments of these algae, which may be photoabsorptive. Even more surprising is the oft-quoted discovery of Virtanen and Laine[22] of the bile-pigment intermediate, choleglobin, in the root nodules of leguminous plants. Whether haems or haemoproteins are the source of the bile pigments in every instance has not been established, but it is probably so. Just as, firstly, haem and later haemoproteins evolved early in biological time as compounds fitted to fulfil the functions of oxygen transport and intracellular respiratory catalysts, it appears, from the general distribution of bile pigments in nature, that breakdown of haems to the open-tetrapyrrolic chain was from the first, and has remained, the highly favoured catabolic pathway.

This is by no means the only possible chemical process by which the closed haem molecule can be destroyed, as was well demonstrated many years ago.[23] When haemoglobin forms a coupled oxidation system with the unsaturated fatty acids (linoleic and linolenic) the molecule is thoroughly degraded, no bile pigments or dipyrromethenes being detectable among the products. The clear inference from this is that this chosen bile-pigment pathway must confer some biological advantage and fitness for survival, a mechanism which has been retained by genetic and enzymic means. It is inconceivable, on other grounds, that a process as important as the degradation of a compound as vitally essential as haemoglobin should be a chemical autoxidative one, the rate of which varies relatively little with temperature as compared with enzymic processes elsewhere in the body.

Working Hypothesis

On the contrary, it would be more in keeping with modern concepts of integrated metabolic pathways in living cells to envisage a link between the electron transport system, from oxygen to substrates, and the peroxidative disruption of haemoglobin itself. Such a link would only become manifest within the reticulo-endothelial cells

where the requisite enzyme systems are present, presumably in mitochondria and microsomes, bile-pigment intermediates being absent in the circulating erythrocytes.[18] In common with other metabolic processes sharing precursors (eg. cholesterol biosynthesis), the first enzymic step in haemoglobin catabolism would constitute an important control point, susceptible to a number of factors relating to respiration, general metabolism and the maintenance of adequate concentration of haemoglobin for oxygen transport. These and other considerations we have attempted to reconcile with our experimental data in a very tentative working hypothesis embodied in the following scheme:



The first stage of catabolism (A) follows the release from inhibition of the enzyme DPNH oxidase located within the reticulo-endothelial cells. It may be that the activity of this enzyme is dependent in some fashion on the interaction of the whole red blood corpuscle with the reticulo-endothelial cell. Some change in the ageing corpuscle may activate a reticulo-endothelial lipoprotein lipase to bring about dissolution of the corpuscular stroma, and during this process a compound is formed which triggers DPNH oxidase to bring about the oxidation of the reduced coenzyme $DPNH_2 + O_2 \rightarrow DPN + H_2O_2$.

Hydrogen peroxide will then couple with the methine group of the haemoglobin haem causing oxidative removal of the bridge carbon (B). This peroxidative step and others in the pathway will be under the influence of the appropriate enzymes. Diversion of O_2 directly into the bile-pigment route will be additionally facilitated by the fact that bilirubin, and perhaps other bile-pigment intermediates, are uncouplers of oxidative phosphorylation on the routes marked (D). Utilization of DPNH and O_2 and uncoupling of oxidative-phosphorylation together will impair the aerobic respiration of the reticulo-endothelial cell, perhaps to such an extent that, for a period, biochemical mechanisms for interaction with further red blood corpuscles (as, for example, lipoprotein lipase) will be in abeyance until the intracellular bilirubin has fallen to a permissive concentration. In such a manner, the oxygen requirements for aerobic respiration of the tissues could be brought into line with the rate of dissolution of corpuscles and catabolism of haemoglobin to bile pigments: indeed, a positive feed-back mechanism might operate from this chain of events to divert succinyl coenzyme A from the tricarboxylic-acid cycle into biosynthesis of haem.

We have allowed ourselves much liberty in the development of this theme, since there appears at present a great need for some unifying concept on bile-pigment formation. A satisfactory answer to many of the questions raised in this paper must await the isolation of reticulo-endothelial cells and, since cellular membranes may even then present impermeable barriers, the removal of such obstacles by separation and study of mitochondria and other intracellular organelles.

The early stages in the catabolism of haemoglobin to biliverdin will, perhaps, eventually prove equal in interest to those changes undergone by bilirubin in its passage through the liver and the gastro-intestinal tract.

SUMMARY

This paper describes experiments designed to discover whether appropriate tissues added to a peroxidative system — haemoglobin, ascorbic acid and oxygen — could augment the yield of biliverdin-containing compounds.

The results were, briefly, as follows:

1. Opening of the haem ring was not dependent on an antecedent, fine-fragmentary degradation of the globin molecule.

2. Intermediary compounds, from which the bile pigments were derived, were almost entirely of a molecular weight greater than 5,000.

3. The yield of biliverdin was not improved when haemoglobin was first treated with papain.

4. The maximal production of biliverdin from adult and foetal haemoglobins was similar, but the concentration was maintained for a longer period in the case of HbF.

5. Red-blood corpuscular stroma had no measurable effect on the course of haemoglobin breakdown to biliverdin.

6. The formation of bile pigment was markedly inhibited by the addition of rabbit leucocytes, which impeded the peroxidative decomposition of haemoglobin.

7. Adult liver and spleen of an essential thrombocytopenic patient were ineffective adjuvants, but a spleen from a patient suffering from haemolytic anaemia was markedly stimulatory to the formation of bile-pigment compounds.

8. Some activators were proved to be heat-stable, others heat-labile.

9. Neonatal liver, spleen and bone marrow all facilitated the conversion of haemoglobin to bile-pigment proteins.

10. About one-third of the activation was due to heat-labile compounds.

11. Diffusible and non-diffusible activators made approximately equal contributions to the catalysis.

12. Added glutathione did not promote the coupled oxidation of haemoglobin and ascorbic acid.

13. The results are discussed and a tentative hypothesis proposed which it is hoped may stimulate further attempts

to elucidate the way in which haemoglobin is catabolized to biliverdin.

This work formed part of an investigation described in a thesis presented by one of us (S.N.V.) to the University of Manchester for the M.Sc. degree. We gratefully acknowledge the generous help of Dr. V. Schwarz in the preparation of the thesis.

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