HÆMOGLOBIN METABOLISM

A SYMPOSIUM on hæmoglobin metabolism, under the chairmanship of Prof. G. Payling Wright, was held in London on the evening of February 17 by the Section of Pathology of the Royal Society of Medicine. Dr. A. Neuberger was prevented by illness from giving the opening paper, which was on hæm biosynthesis, and his place was therefore taken by Mr. John Scott. Mr. Scott referred to the important work of Shemin and Rittenburg, who were the first to show that glycine is the specific precursor of porphyrin nitrogen. They also showed that the incorporation of nitrogen-15 into hæmoglobin after feeding such nitrogen-labelled glycine to a man revealed that the hæmoglobin of red cells is not continuously degraded and synthesized like other proteins, but has a life-span of about a hundred and thirty days. Certain limitations, inherent in isotope experiments, together with the delay in the delivery into the circulation of hæmoglobin after synthesis of the porphyrin in man, render difficult the interpretation of the persistence of small quantities of isotope in the red cells even two hundred and fifty days after the feeding of glycine. In contrast, the Ashby agglutination technique appears to show that no appreciable number of red cells survive for more than a hundred and thirty days. While this might be due to the synthesis of porphyrin from glycine retaining a low but definite isotope-content for as long as a hundred days, it is not impossible that there is some re-utilization of hæmoglobin or hæm from the cells breaking down. Experiments by Shemin *et al.*, and by Muir and Neuberger, using glycine and acetate labelled in various ways by nitrogen-15 and carbon-14, have shown that all the atoms of the protoporphyrin molecule can be accounted for in terms of glycine and a four-carbon compound, such as succinic acid, derived from acetate by the Krebs tricarboxylic acid cycle. The experimental evidence is in accord with the initial synthesis of a monopyrrolic unit bearing a carboxyethyl and a carboxymethyl group in the β positions. Condensation of four of these units under the restricted conditions postulated by Neuberger, Muir and Gray would lead to the formation of uroporphyrins I and III, the latter in excess. Decarboxylation of the carboxymethyl group of these would lead to coproporphyrins I and III; an oxidative decarboxylation of two of the carbethoxy groups of this last porphyrin would lead to protoporphyrin IX.

Prof. C. Rimington emphasized the enormous synthetic capacity of the hæmopoietic system. Whipple has stressed the great importance of the liver and labile protein reserve in meeting emergencies for the production of fresh red cells and hæmoglobin by the hæmopoietic organs. Reference was made to the stimulating effect of amino-acids in hæmoglobin regeneration after hæmorrhage. Borsook has observed that the incorporation of some amino-acids labelled with carbon-14 into the hæmoglobin and stroma of reticulocytes from phenylhydrazine-treated rabbits is always greater when the maturation is studied in a plasma medium rather than in saline. This effect is in part due to some unidentified factor present in plasma. Thorell has shown that the total protein in bone marrow cells has reached a maximum, and the ribonucleic acid content, which is always correlated with protein synthesis, has fallen to a very low level by the time hæm production is occurring. Although this suggests that globin is formed before the hæm, the microspectrographic method used does not discriminate between globin and other proteins. *In vivo* and *in vitro* exposure to X-rays affects the synthesis of hæm and globin to a different extent, and also suggests that the two processes are independent. Muir, Neuberger and Peronne have shown that, when glycine labelled with carbon-14 is fed to the rabbit, the glycine of the globin and of the hæm are of comparable radioactivity only after twenty-four hours. Before this time, more activity was in the globin glycine. This could be due to globin formation preceding hæm formation or to the relative sizes of the globin and porphyrin pools and other factors. Hæmoglobin synthesis may also be studied in hæmolysates of avian nucleated red cells, and much valuable information may in future be obtained from such a system.

Dr. W. M. Davidson described the structure of the red cell with its preferential barrier to diffusion, and was of the opinion that, at the end of its life-span, certain enzymes become used up so that the cells are destroyed by the reticulo-endothelial system. Lytic processes play a major part in certain disorders, but probably not in normal red cell destruction. Lysis can be effected by certain chemicals, antibodies, hypotonic solutions, heat, freezing, buffeting, alteration in *p*H and the effects of light especially in the presence of photosensitizing agents, such as porphyrins, Rose Bengal, etc. Cells which have been partly damaged by some lysins or are congenitally fragile lyse in slightly hypotonic solution. Ionic surface-active lysins penetrate monolayers of cholesterol and lecithin, substances known to be important constituents of the cell structure, and raise the surface pressure until collapse occurs. The relationship of non-ionic agents to hæmolysis is less clear. Sphering appears to be an important but not essential preliminary to lysis, and the process, though reversible in its early stage, soon becomes irreversible. Serial estimations of the diameters of red cells show the process of development and disappearance of the spherocytes. Lysis is sometimes preceded by agglutination, the formation of sidero-cytes, Heinz bodies or basophil stippling. It is probable that the hæmolytic activity of bile salts, which are present in circulating blood, is normally prevented by certain inhibitors. In most immune hæmolytic reactions, a factor sensitizes the red cells for the lytic agent which is usually complement. Some hæmolysins cause only agglutination *in vitro*, but hæmolysis *in vivo*; and some incomplete antibodies have no apparent effect on red cells *in vitro*, but cause hæmolysis *in vivo*. Human hæmolytic disorders are classified according to whether the cells of the affected person have, or have not, a shortened life after transfer into normal individuals. There is a definite difference between *in vitro* and *in vivo* effects of the immune hæmolysins; *in vivo*, some factor, possibly a tissue lysin, greatly exaggerates the reaction.

According to Prof. C. H. Gray, the mechanism of breakdown of hæmoglobin to bile pigment is obscure. The original view was that the globin is split from the hæmoglobin molecule to give hæmatin, which is then broken down through protoporphyrin

to bile pigment. Earlier workers who attempted to demonstrate the conversion of hæmatin to bile pigment obtained discordant results, but in 1950 London showed by isotope experiments that at least 20 per cent of administered hæmatin is rapidly metabolized to bile pigment. Although the experiments of Grinstein and his colleagues indicate that part of the fæcal protoporphyrin in the dog may be formed from breakdown of hæmoglobin, in man porphyrin excretion can contribute no more than an insignificant amount to the total breakdown products of hæmoglobin. Lemberg has shown that hæmoglobin is oxidized by oxygen in the presence of ascorbic acid to choleglobin, a complex of globin and the iron derivative of a bile pigment. The proportion of the total hæmoglobin broken down by intra-corpuscular degradation of this kind is quite unknown. It seems likely that the dipyrrolic compounds, such as propentdyopents and bilifuscins, are derived from bile pigments subsequent to their excretion by the liver. Isotope experiments have shown that bile pigment can arise not only from cells at the end of a normal life-span, but also by a very rapid mechanism as well as by a slower mechanism not from cells at the end of a normal life-span. In the small intestine, the bilirubin excreted in the bile is reduced to 'fæcal urobilinogen', a mixture of colourless precursors of the pigments stercobilin, urobilin IXα and *d*-urobilin. Baumgartel believes that mesobilirubinogen, the precursor of the optically inactive urobilin IXα, is formed only in the liver, but Watson has contested this view. The determination of fæcal urobilinogen has been much used as a rough measure of hæmoglobin breakdown. Interpretation of the results obtained must, however, always take into account certain limitations: urobilin and urobilinogen are converted into other substances not estimated by the Ehrlich reaction; and hæmoglobin may break down directly into dipyrrolic compounds, or other unknown substances. The very high proportion of bile pigment not formed from old red cells may occur in other diseases as well as in pernicious anæmia and congenital porphyria.

Dr. J. C. Houston outlined the view that the body is unable to excrete iron, and that the amount of this element within the body is normally regulated by its absorption, which is strictly limited, according to the needs of the tissues. Apoferritin of the duodenal and intestinal mucosa appears to act as an acceptor. When all the apoferritin has combined with iron to give ferritin, no more iron can be absorbed until some has been given up to the plasma. The factors controlling the rate of release of iron from the mucosal ferritin into the plasma are obscure. The total iron-binding capacity of plasma is normally only about one-third saturated. In hæmochromatosis, serum iron is always above normal, and there is complete or almost complete saturation. The serum iron of hæmochromatosis patients does not increase after oral ferrous sulphate as does that of a normal person. Attempts using radioactive iron to determine the absorption of iron and the amount laid down in the tissue stores have given discordant results, either because of technical difficulties in measuring fæcal radioactivity or because patients have been investigated at different stages of the disease. Very little isotope passed into the blood, presumably because of dilution by a pool of physiologically available iron that is larger than normal. That some of these enormous deposits of iron in hæmochromatosis are available for regeneration of hæmoglobin has been shown by the failure of these patients to become anæmic even after repeated large bleedings. Patients with hæmochromatosis can thus regenerate hæmoglobin at a great rate, and it seems likely that it is the small reserve of available iron which limits the rate of hæmoglobin regeneration after hæmorrhage.

In the subsequent discussion, Dr. W. W. Payne emphasized the importance of the mucosal block in the absorption of iron by reporting a case of fersolate poisoning in which the serum iron concentration reached 20 mgm./100 ml., consequent to the destruction of the intestinal mucosa. In reply to a question from Prof. N. F. Maclagan, Prof. Gray observed that 'fæcal urobilinogen' determinations are of some value in the diagnosis of hæmolytic disease. Prof. H. Munro Fox described how hæmoglobin synthesis in the crustacean *Daphnia* is greatly stimulated by oxygen lack and how, on re-oxygenation, the extra hæmoglobin rapidly disappears, apparently without bile pigment formation. Dr. J. C. White referred to the observation of Haurowitz, that the oxidation of fatty acids is coupled with the breakdown of hæmoglobin to colourless products. Dr. T. With remarked that in the horse the serum bilirubin is much greater than in man, yet in the horse the bile contains very little bilirubin. Dr. M. G. Good was of the opinion that the thyroid influences hæmopoiesis.

EDUCATION IN CHEMISTRY

A DISCUSSION on education in chemistry was held on February 18, under the joint auspices of the London Sections of the British Association of Chemists and the Royal Institute of Chemistry. This attracted an audience of about two hundred people, not only from the London area, but also from various other parts of Britain. Introductory talks were followed by an animated discussion, and altogether twenty-one people spoke in the course of just under three hours. They were representative of the University of London, the Royal Institute of Chemistry, technical colleges, technical schools, the Ministry of Education and industry.

The five introductory speakers dealt with distinct facets of the subject. Prof. C. K. Ingold (University College, London) spoke about the aim of the revised regulations for the Special degree in chemistry of the University of London. A specialist in chemistry, he said, should reach a standard at which he has studied some aspects in sufficient detail, in order to have the right to form an independent judgment. At the same time, the conventional barriers between chemistry and other sciences are breaking down, and it is necessary for chemistry specialists to have a broad background in other sciences. Prof. Ingold thought that sufficient experience has been gained with the Internal degree of the University to be sure that the students trained under the revised regulations are better developed than those trained under the old regulations.

Dr. J. H. Skellon (Acton Technical College) spoke about External degrees in chemistry of the University of London, mainly from the point of view of the technical colleges. He described ways in which these colleges are adjusting their arrangements to fulfil the additional requirements of the new regulations, and thought that there is a danger of the main