Metabolic Systems and the Inherited Diseases of Man

J. B. STANBURY, MD, Unit of Experimental Medicine and the Clinical Research Center, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass., USA

In 1949, an attempt to summarise the existing knowledge of the inborn errors of metabolism would probably have been confined to albinism, pentosuria, cystinuria, and alkaptonuria: to exactly the diseases that Sir Archibald Garrod (1908) considered in his lectures on that subject. By 1949, Beadle and his colleagues had done their classic experiments on the inheritance of eye colour in the fruit fly (Ephrussi, 1942), and on the metabolism of the mould Neurospora (Beadle and Tatum, 1941), experiments which were fundamental in the development of medical genetics. Beadle (1945) first clearly developed the one gene-one enzyme concept in his monumental paper on biochemical genetics, but not one new disease was added to Garrod's original four, except for his later suggestion that porphyria might also be included.

In 1949, Pauling and his associates published their studies on the electrophoretic mobility of sickle cell haemoglobin. This paper, perhaps more than any other, inaugurated the modern period of medical genetics, for it provided a basis for theory and for hypothesis. Within two years, Gerty Cori (1952) had demonstrated the absence of a specific enzyme from the liver in one type of glycogen deposition disease, for the first time relating experimentally a disease with a deficiency of enzyme activity. Definition of inborn errors of thyroid function and of galactose metabolism were not far behind. Since then, there has been an astonishing increase in the number of human inherited diseases of which there is substantial biochemical understanding.

This intemperate growth has been bewildering. Almost every week some disease is given a new definition in terms of altered enzyme function or protein structure. The result is that in the minds of many these diseases constitute a confusing group, blurred in their interrelationships and lacking systemisation.

It is possible to bring a degree of order into this field. To do so it is necessary to remember that, for the most part, metabolic pathways consist of a series of discrete and simple biochemical reactions, and that each is controlled by its

own enzyme. Since the inborn errors of metabolism occur because a single enzyme (or protein) is defective or missing, one can systemise many of these diseases according to specific errors at particular steps in the appropriate metabolic pathways. Although it is not possible at present to incorporate all the inborn metabolic errors of man into a series of simple schemes, it is possible to do so in many instances. This way brings order and sense, and a method for linking biochemical information with human disease, with enhanced understanding of both. In the following sections several familiar metabolic systems are briefly described, and an attempt is made to show how lesions in these systems cause inherited disease.

THE GLUCOSE-GLYCOGEN CYCLE AND ITS DISEASES

In order to meet the intermittent energy demands for physical or metabolic work, many cells store glucose in the form of a highly arborised macromolecular polymer of glucose known as glycogen. The glucose residues are linked between the carbon in position-1- and that in position-4- of the adjacent molecule, while at the branch points additional linkages exist between carbons -1- and -6- of neighbouring glucose residues. Approximately one out of every

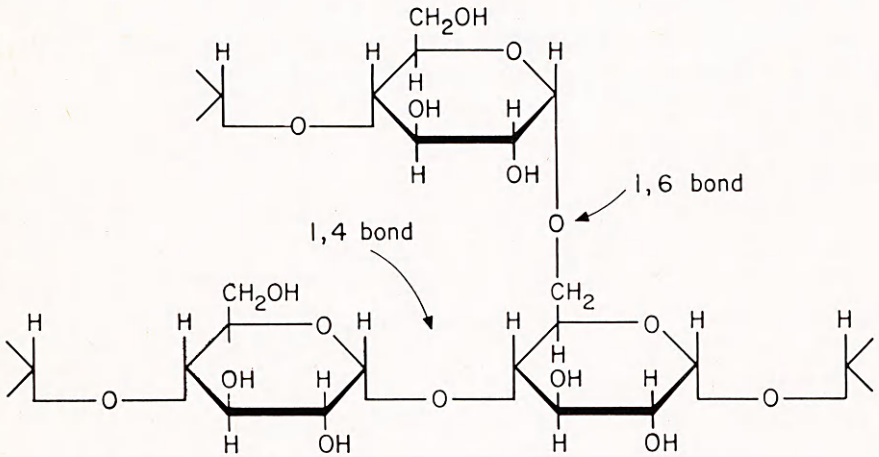

Fig. 1. The internal structure of glycogen, showing the branch points

eight glucose residues is at a branch point. This complex molecule must be formed, stored, and its constituent glucose residues regained in free form as needed. As in most reaction pathways, phosphate plays a key role, but it appears neither in the finished macromolecule, nor in the final product, glucose (Fig. 1).

Entry of plasma glucose into the muscle or fat cell requires insulin; as soon as glucose diffuses into the cytoplasm it is phosphorylated by hexokinase to glucose 6-phosphate. The enzyme phosphoglucomutase converts this reversibly to glucose 1-phosphate. The enzyme UDPG-pyrophosphorylase then forms uridine diphosphate glucose (UDP glucose, active glucose) from glucose 1-phosphate and uridine triphosphate, an ubiquitous nucleotide. The glucose moiety of UDP glucose may then be transferred to the 4-position of a terminal glucose in a glycogen molecule to lengthen the chain by one unit. The enzyme responsible for this is glycogen synthetase. It exists in two forms. In the phosphorylated form (D-form), the enzyme depends on the presence of glucose-6-phosphate (G-6-P) for activity; the dephosphorylated enzyme (I-form) is independent of the presence of G-6-P, and is therefore in a more active state. The liver and other cells contain a kinase for phosphorylation, activated by cyclic adenosine monophosphate (AMP) and a phosphatase for dephosphorylation of glycogen synthetase. The balance between these two forms of the enzyme governs the level of its activity. Since cyclic AMP levels are increased by such agents as epinephrine and glucagon, but decreased by insulin, hormonal control of glucose conversion to glycogen is governed in part by shifts of glycogen synthetase from the D- to the I-forms, and vice versa.

The glycogen molecule is in a continuous process of remodelling. When a side-chain becomes longer than approximately eight residues, it may be shifted from a 1-4 to a 1-6 linkage. The enzyme which does this is the so-called branching enzyme, or glycogen (1-4, 1-6) glucan transferase. Extensive redistribution of residues also takes place by transfer of short chains from one terminal glucose residue to another.

Mobilisation of glucose from glycogen requires the action of several enzyme systems. Hepatic phosphorylase splits a terminal glucose from 1-4 linkage to yield glucose 1-phosphate. This enzyme also exists in phosphorylated (phosphorylase a) and dephosphorylated (phosphorylase b) forms, and a specific phosphatase and a kinase, activated again by cyclic AMP, dephosphorylates and phosphorylates the enzyme. In contrast to glycogen synthetase, the phosphorylase is active when in its phosphorylated form and inactive when dephosphorylated.

When glucose bonded in 1-6 linkage has been reached in the deramification of a terminal chain, a debrancher enzyme, glucan 1,6 glucosidase, cleaves the 1-6 linkage, yielding free glucose. This glucose may escape from the cell for use elsewhere. There is also a maltase in many cells capable of splitting off the terminal glucose residues of glycogen to yield free glucose, but it is not very active. Glucose 1-phosphate, the principal product of glycogen breakdown, is isomerised to G-6-P, which may enter the Embden-Myerhof

pathway or the hexose phosphate shunt to meet local energy requirements, but cannot cross the cell wall and escape into the blood for use elsewhere until glucose 6-phosphatase cleaves it to free glucose.

In giving biochemical definition to one of the glycogen deposition diseases, Gerty Cori (1952) showed for the first time that an inborn error of metabolism

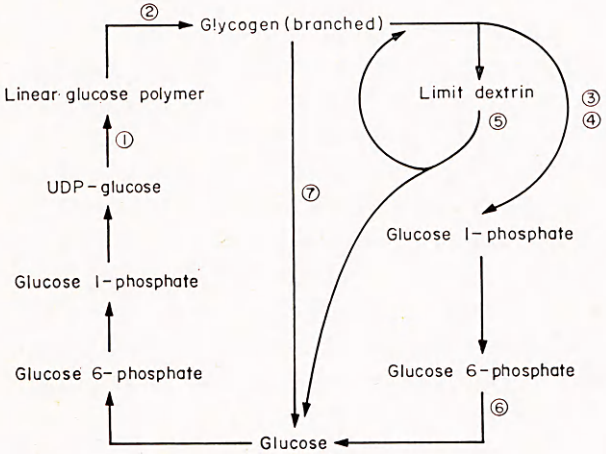

Fig. 2. The glucose-glycogen cycle and its diseases

1=glycogen synthetase deficiency GDD, 2=brancher deficiency GDD (Anderson's disease), 3=hepatic phosphorylase deficiency GDD (Hers' disease), 4=muscle phosphorylase deficiency GDD (McArdle's disease), 5=debrancher deficiency GDD (Forbes' disease), 6=glucose 6-phosphatase deficiency GDD (von Gierke's disease), 7=α-glycosidase deficiency GDD (Pompe's disease).

was due to the lack of a specific enzyme. This was glucose 6-phosphatase, the enzyme necessary for the dephosphorylation of G-6-P. Patients who have this disorder (usually known as von Gierke's disease), are severely handicapped. Growth is slowed. They have episodes of hypoglycaemia, chronic acidosis, and oftentimes severe hyperlipaemia. The liver is large and filled with normally structured glycogen. They are intolerant of glucose. Administration of glucagon, which activates phosphorylase, fails to elevate the blood sugar because mobilisation is blocked at the G-6-P stage.

At least six or seven other glycogen deposition diseases have now been defined (Stanbury *et al.*, 1966; Lewis *et al.*, 1963) (Fig. 2). Identical twins have been described who evidently lacked glycogen synthetase. They exhibited hypoglycaemic episodes, mental retardation, and seizures, and a greatly reduced rise in blood glucose after administration of glucagon. A liver biopsy from one patient had a glycogen content approximately one tenth of normal. While no other disease of glycogen synthesis is known, an error

in glycogen modelling has been well described in two patients. Their hepatic glycogen had unusually long external chains. This presumably was a result of an absence of the enzyme required for transferring lengthened chains in the glycogen molecule from a 1-4 linkage to a 1-6 linkage. Such new branch points are created by the brancher enzyme. Its absence would lead to the formation of glycogen with an altered structure containing long unbranched chains. This molecule may be difficult to degrade by phosphorolysis and lead to its destructive accumulation in the liver cell. At least, the patient with this disorder had hepatic failure.

Two varieties of phosphorylase deficiency are known. One of these is due to a lack of muscle phosphorylase activity and the other to a lack of liver phosphorylase activity. They may be distinguished on the basis of the response to epinephrine and glucagon. The blood glucose of the patient with hepatic phosphorylase deficiency rises poorly or not at all to either substance, whereas the glucose rises normally in those with muscle phosphorylase deficiency (the McArdle syndrome). Patients with McArdle's syndrome have abundant deposits of glycogen in their muscles but are unable to mobilise this on metabolic demand. Their muscles tire quickly and fail to secrete lactate into the venous blood during exercise. Patients with hepatic phosphorylase deficiency have retarded growth, and often show hypoglycaemia and ketosis; the liver is large and filled with glycogen. Variants of hepatic phosphorylase glycogen deposition disease have been described in both man and rat in which the deficiency is not of the phosphorylase itself, but of the kinase which normally changes it into its active form by adding phosphate to the dephosphorylated enzyme (Lyon *et al.*, 1967; Hug *et al.*, 1966).

In contrast to the brancher deficiency syndrome, there is also a glycogen deposition disease which is the result of an absence of the debrancher enzyme. These patients are able to degrade glycogen back to the 1-6 branch points but no farther. As might be expected, their disease is mild since they have access to a significant fraction of their glucose stores before reaching the blocked 1-6 branch points. They have mild hypoglycaemic episodes. Excessive glycogen is found in muscle, liver, and heart, and analysis discloses a molecular structure with an abnormally large number of branch points.

Still another glycogen storage disease seems to be due to an absence of an α-glycosidase. Glycogen deposition is generalised. Deposits in cardiac muscle cause a characteristic enlargement of the heart. Diagnosis may be established by demonstrating glycogen in excessive concentration in the blood leucocytes. Since absence of this enzyme would seemingly have little effect on total glycogen metabolism, it is by no means clear why this disease is so severe.

Many patients with glycogen deposition disease have been described who

do not fit easily into any of the categories which have been described (Hens, 1965). Some may be examples of abnormalities of glycogen metabolism which have not been elucidated. Others may have defied classification because of technical limitations. Still others may have been genetic variants which differed sufficiently from typical instances to have obscured accurate diagnosis. This might happen when an enzyme absent in one patient is present but in reduced activity in another. Most difficult to understand are those patients in whom two separate and distinct enzyme defects have been identified, and those instances in which siblings seem to have had different species of glycogen deposition disease. The problems offered by these difficult cases are certain to be resolved as we acquire more information about the pathways of the glucose-glycogen cycle and more technical skill in measuring them.

DISEASES OF THE PHENYLALANINE-TYROSINE PATHWAY

In addition to participating in protein synthesis, the amino-acids that gain entry into the body undergo a wide variety of metabolic transformations. A

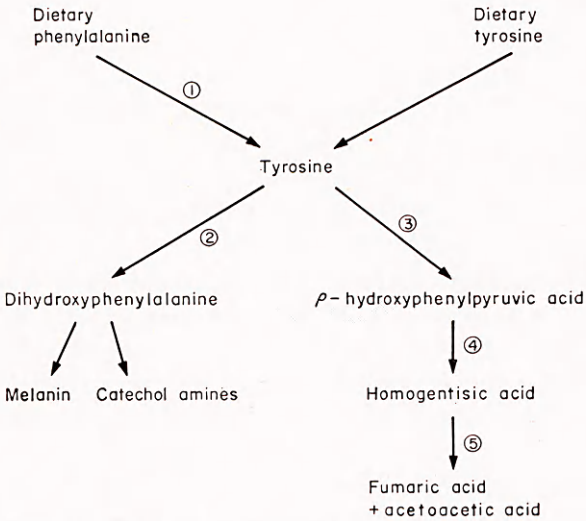

Fig. 3. The phenylalanine-tyrosine pathway and its diseases
1=phenylketonuria, 2=albinism, 3=tyrosinosis, 4=tyrosinaemia, 5=alkaptonuria.

large number of inborn errors of metabolism are now known to be a consequence of specific blocks in a number of these pathways. For example, there are at least five diseases involving the metabolism and degradation of phenylalanine and tyrosine, in addition to those involving the specific

metabolism of the iodinated derivatives of tyrosine which will be discussed in the next section.

Phenylalanine is essential for growth and development. One of its principal routes of metabolism is into tyrosine by hydroxylation in the *para*-position of the phenol ring. Of course, tyrosine is also a constituent of the diet, but it is not transformed to phenylalanine through the reverse reaction. The principal fates of these amino-acids appear in Fig. 3. Both may lose their amino groups through transamination or deamination to yield the pyruvate analogues, and then may be reduced to the corresponding lactates. Decarboxylation gives the acetic acid derivatives. An alternative and minor pathway of phenylalanine metabolism is hydroxylation in the *ortho*- instead of the *para*-position to form *ortho*-tyrosine, and this substance may undergo further degradation to *ortho*-tyramine, and so on.

An important route of tyrosine metabolism develops from the action of tyrosinase, a copper-containing enzyme. This enzyme converts tyrosine to dihydroxyphenylalanine (DOPA), and, in turn, converts this to a quinone derivative. Ring closure and polymerisation yields the important pigment melanin. Alternatively, the side-chain of DOPA may undergo a series of changes to yield the important catecholamines, adrenalin and *nor*-adrenalin.

A major pathway of tyrosine metabolism is transamination to yield the pyruvate analogue. This is oxidised by a specific enzyme to homogentisic acid. A further complex oxidation opens the phenolic ring to give fumaric and aceto-acetic acids.

Phenylketonuria is the result of impairment at the initial metabolic step, the hydroxylation of phenylalanine to tyrosine (Anderson and Swaiman, 1965; Weber and Zannoni, 1966). Since, normally, this is a major pathway of phenylalanine metabolism, phenylalanine accumulates and leads to a secondary increase in the quantity of metabolites such as phenylpyruvic acid, phenyllactic acid, and phenylacetic acid. Inhibition of tyrosinase by the high blood concentration of phenylalanine accounts for the fact that these patients are usually less pigmented than their normal siblings. Why mental deficiency occurs in this disease has not been ascertained. It is thought to be the result of some product of phenylalanine that accumulates to a toxic level because of the blocked pathway of phenylalanine disposal. Retardation of learning ability can be induced in rats by feeding a diet high in phenylalanine (Lipton *et al.*, 1967). If the disease is recognised early enough in infancy and the patient given a diet low in phenylalanine, there seems to be substantial improvement in mental development. On the other hand, if phenylalanine intake is too severely restricted, disturbances attributable to phenylalanine deprivation may occur.

Recently, it has become clear that phenylketonuria is not a single entity. Patients are recognised now who learn to dispose of phenylalanine through pathways other than tyrosine to a degree sufficient to permit a normal blood concentration of phenylalanine, although they may show an abnormal response to a phenylalanine loading test. The pathway of disposal of phenylalanine in these patients has not yet been ascertained. Other patients are found who have an increase in phenylalanine concentration in the plasma comparable to that seen in phenylketonuria without any evidence of mental retardation (Auerbach *et al.*, 1967). In some instances, this may be due to delayed maturation of the hydroxylase or tyrosinase.

Tyrosinosis was described in only one patient in 1927 (Medes, 1932). Fortunately, careful metabolic data were obtained and are available for analysis. The patient was little affected by the elevated blood concentration of tyrosine. The principal metabolic product in the urine was the pyruvic acid analogue of tyrosine. It now seems probable that the defect was a failure in the specific transaminase which transfers the amino group to α-ketoglutarate (La Du, 1966). The appearance of the pyruvic acid analogue in the urine is thought to have been due to deamination of tyrosine in the kidney. Recently, a number of patients have been described, principally from Scandinavia, who had a disorder of tyrosine metabolism that has been called tyrosinaemia (Halvorsen and Gjessing, 1964; Shear *et al.*, 1967). These patients had enlargement of the spleen and liver, with nodular cirrhosis. There was severe amino-aciduria with large amounts of tyrosine and tyrosyl derivatives in the urine. Evidence from *in vivo* studies and from observations on liver biopsies indicates that this disease results from a lack of the enzyme that oxidises the pyruvate derivative of tyrosine to homogentisic acid. Thus, the flow in this pathway is backed up, causing accumulation of tyrosine and its pyruvate and lactate analogues in the blood and their loss in the urine. The clinical differences between tyrosinosis and tyrosinaemia suggest that it is not the accumulation of tyrosine that is harmful, but rather the accumulation of the derivatives. By analogy, one may wonder whether patients with hyperphenylalaninaemia without mental retardation lack the transaminase acting on this amino-acid, while those with the disease phenylketonuria are able to metabolise phenylalanine by way of deamination to toxic products. Indeed, evidence has been presented for deficient phenylalanine transaminase in two patients with hyperphenylalaninaemia (Auerbach *et al.*, 1967).

Alkaptonuria is the prototype of the inborn errors of metabolism. Garrod's early interest in joint disease led to studies of alkaptonuria because affected patients often develop arthritis in later years. Although Garrod clearly recognised that this disease was an inherited error of metabolism, it was not

until 1958 that La Du and his colleagues demonstrated the absence of homogentisic acid oxidase from the liver and kidneys of patients with alkaptonuria (La Du *et al.*, 1958). Thus, fifty years intervened between the realisation that the metabolic defect was a block in the oxidation of homogentisic acid, and the demonstration that these patients actually lack the enzyme which catalyses the reaction. The block results in excretion of homogentisic acid in the urine and its deposition in cartilage. Polymerisation in cartilage and in urine accounts for the characteristic blue-black coloration.

Albinism was among the four diseases originally recognised as inborn metabolic errors. While this disease takes several forms, the common denominator in the depigmented areas is an insufficient quantity of tyrosinase (Fitzpatrick and Quevedo, 1966), which is absent from the melanocytes but not from other tissues such as those responsible for the formation of catecholamines. In the albino, the subcellular units of melanogenesis are normal and so is the melanocyte, except that it fails to carry forward the hydroxylation of tyrosine and oxidation of DOPA into the pigment pathway.

From the foregoing, one can see that at least five diseases are defined as errors in the metabolism of phenylalanine and tyrosine, in addition to those involving the thyroid hormones. These diseases differ markedly in their manifestations and the degree of disability that they induce. Unquestionably, the categories will be subdivided in the future, and familiar entities may be recognised as fitting the scheme at some point or other when new biochemical evidence accumulates.

THE THYROID HORMONE CYCLE

Strictly speaking, most of the diseases of the thyroid hormone cycle belong to the phenylalanine-tyrosine system, but it is convenient to consider them separately because of their special features. The principal pathways involved in iodine metabolism and thyroid hormone synthesis and secretion are illustrated in Fig. 4. Iodine from the gastro-intestinal tract is absorbed as iodide, and either excreted by the kidney or taken up into the 'iodine space' of the thyroid gland. This accumulation requires thermodynamic work by the thyroid. The iodide is oxidised to iodine and displaces protons from the 3-positions of the aromatic rings of tyrosyl residues to form mono- and di-iodotyrosine, while in peptide linkage these residues become coupled through a complex interaction to form tri-iodothyronine and thyroxine, the definitive hormones of the thyroid gland. These are stored until needed in the colloid of the thyroid follicle as thyroglobulin. The free hormones are cleaved from thyroglobulin by proteolytic digestion. Iodotyrosyl residues liberated at the same time are de-iodinated by a dehalogenase and the iodide re-utilised in

the thyroid, whereas the thyroxine and tri-iodothyronine are secreted into the blood. After their characteristic metabolic impact on the cells of the body the hormones are degraded and the iodine is released to re-enter the pool of iodine in the plasma.

Each step in this complex series of events is governed by one or more enzymes with cofactors and sources of energy, and at each step the possibility of one or more diseases exists. Thus, a block at any step in thyroid hormone

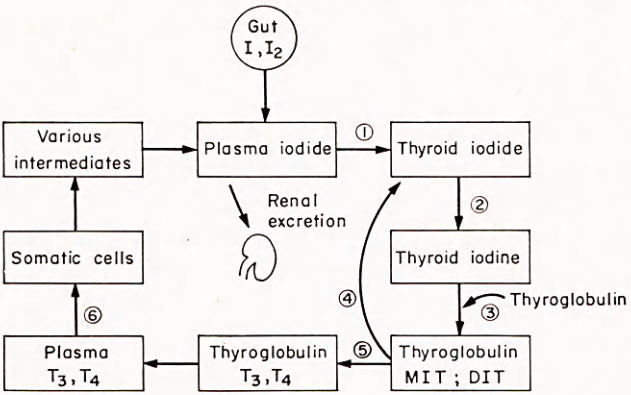

Fig. 4. The thyroid hormone cycle and some of its diseases

1=iodide transport defect, 2a=iodide organification defect, 2b=Pendred's syndrome, 3=thyroglobulin synthesis defect, 4=dehalogenase defect, 5='coupling' defect, 6=thyroid hormone resistance.

biosynthesis, secretion, or utilisation will result in a tendency towards a hypothyroid state and a compensatory effort on the part of the body to restore conditions to normal. The goitre, which often results, may or may not effect full compensation for hormone deficit.

A number of diseases of the thyroid have been more or less convincingly attributed to blocks in the pathways indicated in the figure (Stanbury, 1966). A few patients have been described who are unable to transport iodide into the thyroid (iodide transport defect). Since the enzymatic machinery coupled to this essential process is not known, and no carrier protein for iodide has been definitely identified, no molecular basis for this disease can be given at present although there is every reason to believe that an inborn error of protein synthesis is the source of this disease.

Many patients are now recognised who have an impairment of the cellular chemical apparatus for oxidising iodide to iodine (iodide organification defect and Pendred's syndrome). When severe, this block may result in complete failure of thyroid hormone biosynthesis. Hopeless cretinism is the outcome, unless treatment is instituted early.

Some patients may have severe hypothyroidism and goitre because they are unable to recover the iodine that is dissociated from mono- and di-iodotyrosine when thyroglobulin is degraded (dehalogenase defect). Loss of the iodinated amino-acids from the thyroid and their excretion in the urine, constitute a continuous and disastrous drain of iodine. The enzyme responsible for the salvage of iodine is a dehalogenase which is widely distributed, but is especially prominent in the thyroid. Its absence is one of the clear-cut inborn errors of thyroid metabolism.

Much less definite is a hypothetical defect of the cellular processes whereby tyrosine precursors are coupled in a complex sequence of biochemical events to form the finished thyroid hormones (coupling defect). The enzymes involved have not been identified and the mechanism of the reaction *in vivo* has not been defined. Nevertheless, there is indirect and circumstantial evidence for the existence of such an enzyme or complex of enzymes, and metabolic data from these patients clearly pinpoint the defect at the coupling step (Stanbury, 1957).

Other errors in the thyroid hormone pathway include an unresponsiveness of peripheral tissues to thyroid hormone (Refetoff *et al.*, 1967), an intrinsic failure of the thyroid cells to divide and to make thyroglobulin when stimulated by thyrotrophin (Stanbury *et al.*, to be published), and a disease in sheep involving failure of synthesis of thyroglobulin (Falconer, 1967). Still other errors are characterised by the appearance of several varieties of abnormal

TABLE 1. Inherited Metabolic Defects of the Thyroid

1.	Iodide transport defect
2.	'Peroxidase' defect
3.	Pendred's syndrome
4.	Dehalogenase defect
5.	Syndrome of iodothyronine unresponsiveness
6.	Iodotyrosyl 'coupling' defect
7.	Syndrome of thyrotropin unresponsiveness
8.	Failure of thyroglobulin synthesis in man and sheep
9.	Syndromes of abnormal plasma iodoproteins
10.	Syndromes of abnormal thyroglobulins

iodinated proteins in the peripheral blood, but these have not been given further definition at the molecular level. Thus there are at least two disorders of the thyroid for which there is convincing evidence for lack of a specific enzyme as the operating factor in the pathogenesis of the disease. There are six or eight others where the evidence permits the inference that a comparable

defect is responsible for impaired hormone synthesis. The principal inborn errors of the thyroid appear in Table 1.

Perhaps there are two reasons for so many errors in the thyroid hormone cycle. First, the metabolic processes of the gland involve many steps, at each of which the possibility of error exists. Second, the thyroid is not an organ absolutely essential for survival: it is possible for many errors to exist and generate clinical disease without destroying the subject or even seriously limiting his reproductive capacity. It seems certain that many inborn errors of the thyroid hormone cycle remain to be described, and that some of these may possibly be responsible for some of the frequently encountered diseases of the thyroid.

DISEASES OF THE UREA CYCLE

Ammonia in blood is derived from bacterial action in the gut, deamination of amino-acids, dehydrase reactions which remove the amino group from certain amino-acids, from the action of glutaminase on glutamine (a reaction especially active in the kidney), and from degradation of nucleic acids by adenase, guanase, etc. A major pathway of ammonia transport, but a minor route of ammonia disposal, is through coupling with glutamic acid and ATP to form glutamine.

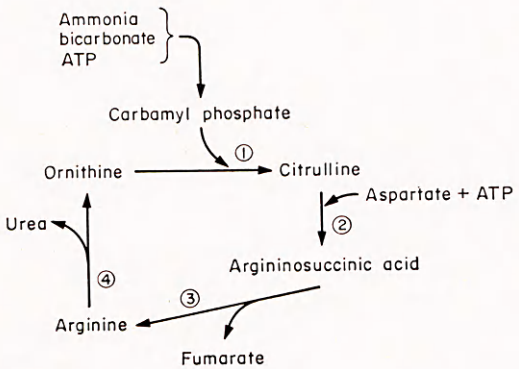

Fig. 5. The urea cycle
1 = hyperammonaemia, 2 = citrullinaemia, 3 = argininosuccinic aciduria, 4 = lysine intolerance.

The major route of detoxification of ammonia is a reaction with ATP, carbon dioxide, and water to form carbamyl phosphate (Fig. 5). Ornithine transcarbamylase transfers the amidino group from carbamyl phosphate to ornithine to form citrulline. Citrulline in turn condenses with aspartic acid to argininosuccinic acid in a reaction involving ATP, which is catalysed by the

enzyme argininosuccinic acid synthetase. In the succeeding reaction argininosuccinic acid is cleaved to arginine and fumaric acid. Finally, arginase, which is a widely distributed enzyme, hydrolyses arginine to urea and regenerates ornithine, which is then available for another turn of the cycle.

Chronic ammonia intoxication has been observed in two first cousins with severe mental retardation and cerebral atrophy. A severe deficiency of ornithine transcarbamylase was demonstrated in a liver biopsy of one of the patients. Inability to utilise carbamyl phosphate in the transcarbamylase reaction with ornithine evidently caused an accumulation of ammonia to toxic levels in these patients. One had a normal twin who appeared to be monozygotic, but this was not established by formal testing. Interpretation has been complicated by the demonstration of a marked reduction in the activity of the carbamyl transferase as well as in the carbamyl synthetase in a biopsy from the liver of the second patient (Ghadimi and Zischka, 1967). Further observations are needed before this conflict can be resolved.

Post-prandial elevation of the concentrations of citrulline and ammonia in the blood, spinal fluid, and urine has been observed in only two patients. The first was the child of first cousins. Mental retardation and seizures were characteristics of both patients. Studies on a liver biopsy disclosed a deficiency of argininosuccinic acid synthetase, and the activity of this enzyme was abnormally low in fibroblast cultures from the other (Mohyuddin *et al.*, 1967).

A number of patients are now known who lack the enzyme for cleaving argininosuccinic acid. These patients have usually been mentally retarded and have had seizures, disturbances of gait, and liver disease. In addition some have friable hair. Argininosuccinic acid is excreted in the urine and may accumulate in the cerebrospinal fluid. Blood ammonia concentration is elevated on fasting and rises dramatically after protein feeding. Deficiency of the cleaving enzyme has been demonstrated in blood cells as well as in the liver.

Still another occurrence of an accumulation of ammonia in the plasma appears to have been a result of inability to metabolise lysine (Colombo *et al.*, 1964), which is a potent inhibitor of arginase. A deficiency of lysine dehydrogenase was demonstrated in this patient but other patients with hyperlysinaemia have not had ammonia intoxication. Hyperammonaemia has also been reported in a patient with hyperglycinaemia; the reason for the elevated blood ammonia is not known (Freeman *et al.*, 1964). Obviously, many problems remain to be clarified in the relationships between the metabolism of amino-acids and accumulation of ammonia in the blood.

PATHWAYS OF THE SULPHUR-CONTAINING AMINO-ACIDS

The dietary sulphur-containing amino-acids are cysteine (and its disulfide form cystine) and methionine. Both are essential for protein synthesis. While methionine is synthesised by certain mammals, such as the cat, it is not synthesised by man. Its principal metabolic pathway, apart from incorporation into protein, begins with a reaction with ATP to release pyrophosphate

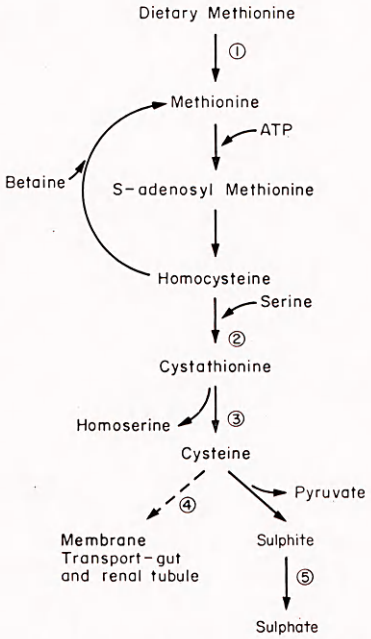

Fig. 6. Diagram of the metabolism of the sulphur-containing amino-acids, and some of the related diseases

1 = methionine malabsorption, 2 = homocysteinuria, 3 = cystathioninuria, 4 = cystinuria, 5 = sulphite oxidase deficiency.

and form S-adenosylmethionine (Fig. 6). This is one of the important methyl donors in the body. It is involved in the formation of adrenalin, creatine, choline, lecithin, and other important substances. Transfer of the methyl group leaves S-adenosylhomocysteine, which is quickly hydrolysed to homocysteine. The latter substance reacts with serine through the action of cystathionine synthetase to yield the mixed disulphide cystathionine. Cystathionine may then be cleaved to cysteine and homoserine by cystathionase in the presence of its cofactor pyridoxal phosphate. A minute amount of cysteine is excreted in the urine but the bulk of it is metabolised further to taurine and to smaller carbon fragments. Much of the sulphur appears in the urine as

sulphate and thiosulphate after oxidation of sulphite by sulphite oxidase. An additional complication in this pathway is a recovery route in which homocysteine reacts with betaine, an oxidation product of choline or with tetrahydrofolate to regenerate methionine.

A number of diseases involve the transport and metabolism of the sulphur-containing amino-acids. One of these, described in a single patient, appeared to be the result of the failure of the infant to absorb methionine (Hooft *et al.*, 1964). There is no known disease characterised by a deficiency in the formation of S-adenosylmethionine from methionine. One might assume that such a defect, blocking important methylation reactions, would be incompatible with life.

The disease homocystinuria, the consequence of a block one step further down the pathway, is well described. These patients vary widely in the severity of the clinical manifestations. Some have dislocated lenses, zonular cataracts, hernias, and hair that is fine and sparse; some have a tendency to form thrombi in the great vessels (McKusick, 1966). They have a characteristic malar flush and disorder of gait. Normal or near normal development is possible if the disease is recognised early and a diet low in methionine, i.e. largely vegetarian, with cystine supplements is adhered to. Some of these patients have a remarkable resemblance to those with Marfan's syndrome. Large amounts of homocystine appear in the urine and can be easily identified. Certain unusual sulphur-containing amino-acids are also found in the urine. These presumably derive from the homocysteine which is available as substrate for reactions that normally occur only to a negligible extent. Deficiency of the enzyme cystathionine synthetase has been demonstrated in a liver biopsy from one of these patients (Mudd *et al.*, 1964).

A diagnosis of cystathioninuria has been established with certainty in four patients, and probably in two others. Half have been severely retarded, and others have had no mental defect. There has been no consistent pattern of clinical abnormality. Because cystathionine is found normally in the brain, the mental retardation exhibited by some of these patients and by those with homocystinuria has been of interest. So far, there has been no correlation between cystathionine levels in the brain and the degree of retardation. Since cystathionase requires vitamin B_6 for its activity, patients have been treated with large doses of the vitamin. This has resulted in a fall in cystathionine levels, both in the blood and in the urine. The suggestion has been made that the defect in this disease is at the binding site for vitamin B_6, rather than at the active site of the enzyme (Shaw *et al.*, 1967).

Cystinuria is a disorder of transport rather than of metabolism in the strict sense. What was, until recently, thought to be a simple disease of renal

tubular transport of four related amino-acids has, on detailed analysis, proved to be a heterogeneous group of at least three separate disorders of amino-acid transport, with defects varying between renal tubule and gut epithelium, and with different patterns of inheritance (Rosenberg, 1966). The disease only creates a problem because of the unusual insolubility of cystine which causes stones to form in the urinary tract.

Sulphite oxidase deficiency has been described in a severely retarded child who had seven siblings, three of whom died in infancy with pathological changes in the central nervous system (Mudd *et al.*, 1967). The patient maintained an opisthotonic muscle rigidity until death. Why failure to oxidise sulphite to sulphate should result in such severe symptoms is not clear. It could be that a toxic metabolite accumulates along the pathway between cysteine and sulphite, or that there is an insufficient amount of sulphate available for synthesis of chondroitin sulphate, certain complex lipids, or for detoxification.

Other diseases involving the metabolism of the sulphur-containing amino-acids are less well defined. Deposits of cystine may occur in patients with a familial form of the Fanconi syndrome. A patient has been described with severe mental retardation, the child of a sibling union, who excreted a mixed disulphide of cysteine and β-mercaptolactate (Crawhall *et al.*, 1968). In view of the complexity of the pathways open to methionine and cysteine it is no surprise to find so many related diseases. Others certainly remain to be identified.

OTHER PATHWAYS AND SYSTEMS

The five metabolic systems already described were chosen for detailed description because the biochemical pathways are relatively simple and well known, and because a number of diseases fit nicely into the schemes. Other systems might just as well have been considered. For example, the pathways involved in the metabolism of the hexoses and its diseases might have been described. Into that scheme would fit galactosaemia, familial fructose intolerance, and, possibly, diabetes mellitus. In the hexose monophosphate shunt, one would find the haemolytic anaemias of G-6-P dehydrogenase deficiency and the closely related phosphogluconate dehydrogenase deficiency. At the 3-carbon level one would find pyruvate kinase and phosphoglycerate kinase haemolytic anaemias (Valentine *et al.*, 1968; Valentine and Tanaka, 1966).

There have been startling developments in the understanding of the biochemistry of the sphingolipids (Brady *et al.*, 1967). These complex substances are composed of a long chain amino alcohol, sphingosine, which is

acetylated on the nitrogen atom with a fatty acid. This basic structure is called a ceramide, and various components may be attached, such as sulphate, hexoses, N-acetyl neuraminic acid, and phosphorylcholine. The sphingolipids are important parts of cell membranes. Evidence to date suggests that turnover of some of the sphingolipids may be blocked at specific steps in degradation resulting in accumulation of the immediate precursor of the blocked reaction. For example, tissues of patients with typical infantile Niemann-Pick's disease fail to cleave phosphorylcholine from sphingomyelin, a ceramide (Brady, 1966). This finding is consistent with the widespread accumulation of sphingomyelin in the tissues of affected patients. Gaucher's disease and metachromatic leucodystrophy have been given analogous interpretations based on the nature of the accumulated sphingolipid and the demonstration of a low level of the specific cleaving enzyme. There are good reasons for believing that Farber's disease, Fabrey's disease, and Tay-Sach's disease will ultimately be given similar precise biochemical definitions as inborn errors in sphingolipid catabolism.

The branched chain amino-acids leucine, isoleucine, and valine form an interesting group with analogous catabolic pathways. Failure of transamination of valine results in the disease hypervalinaemia (Tada *et al.*, 1967) Failure of oxidative decarboxylation of the pyruvate analogues of the three amino-acids is found in maple syrup urine disease (Mackenzie and Woolf, 1959). There appear to be three separate and distinct enzymes, each specific to its own keto acid, yet oxidation of all three keto acids is depressed in the disease (Goeddle and Keller, 1967). One would not expect that all three enzyme activities would be depressed in the same individual, unless the appearance of all three is under the control of a regulator gene. Isovaleric acid is derived from the keto acid analogue of leucine, and is further oxidised to β-methylcrotonylic acid. Failure of this oxidation to take place results in isovaleric acidaemia, a disease that has been described in two children with slight mental retardation, intermittent acidosis, and coma, and a strong characteristic cheesy or sweaty odour (Budd *et al.*, 1961).

The complex pathways of synthesis, transformation, and degradation of the steroids offer a variety of possibilities for metabolic error. Among these are the several forms of the adrenogenital syndrome (Stempfel and Tomkins, 1966). After oxidation at C_3 and isomerisation, pregnenolone is hydroxylated at C_{11}, C_{17}, and C_{21}, to form cortisol. Each hydroxylation is controlled by a separate enzyme. When a block occurs, cortisol formation is impaired, and an increase in ACTH production follows. This stimulates the adrenal which then produces excessive amounts of androgens which give rise to the adrenogenital syndrome. The clinical pattern depends upon the degree and the location of

the blocked hydroxylation. For example, a complete block at C_{21} impairs aldosterone synthesis and causes salt wasting, whereas a block at C_{11} causes salt retention and hypertension.

There is a general class of inborn errors of metabolism that does not lend itself to the kind of schematisation described above because the altered proteins are not involved in metabolic processes in the same sense. These are the inborn errors of carrier or transport proteins. Some of these are highly visible, as in the case of the haemoglobins, transferins, caeruloplasmin, thyroid carrier proteins, plasma lipoproteins, and so on. Others can only be surmised, as in the conditions in which there is failure of transport across a membrane or cell barrier. Hartnup disease, fructosuria, renal glucosuria, and, possibly, cystic fibrosis of the pancreas belong to this group. As an alternative to metabolic maps it is no great chore to construct schemes for mnemonic convenience in which these diseases can be easily accommodated.

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Ex Libris . . .

Bernardino Rocca's *De discorsi di guerra libri quattro* (Venice, 1582) is not a book of great significance in the literature of military art and science, though another edition was published at Bologna in 1573. Nor is much known about the author except that he wrote two other books in the same field, and was nicknamed 'the little shrimp' (il Gamberello). The signatures in the book and the arms on the cover provide the interest. One is 'W. Ralegh' and the other is 'George Carew', whose arms are stamped on the cover. It was Carew who in 1618 unsuccessfully sought to dissuade James I from carrying out the sentence of execution pronounced on Sir Walter Raleigh; while Lady Carew proved a kind friend of the family after Raleigh's death. Apart from the fact that Raleigh's signature has been verified against another elsewhere, the occurrence of the two signatures together, and the coat of arms on the cover leave little doubt about its authority.

During his twelve years' imprisonment in the Tower in the early part of the reign of James I, Raleigh was allowed a room in which he fitted up a laboratory. This fact, coupled with the belief that he had brought with him from Guiana some wonderful curative balsam, ensured a lively public interest in his 'Great Cordial'. No authentic formula of it exists, but Charles II became curious about it and commissioned Lefebure, his French apothecary, to make up, test, and report on it. It was in 1665 that his *Discours sur le grand cordial de Sir Walter Rawleigh* was published in London. It is tempting to speculate on a possible connection between the 'Ch Massonet' who signed our copy and Peter Massonet who was created Doctor of Medicine at Oxford in 1646 and became a tutor to James, Duke of York, future King of England.

The original formula for Raleigh's 'Cordial' is said to have consisted of forty roots, seeds and herbs, macerated in spirit of wine and then distilled. It first appeared in the *Pharmacopoeia Londinensis* in 1721 as *Confectio Raleighana*. The name was later changed to *Confectio Cardiaca* and the formula simplified. A vestigial remnant has persisted in the pharmacopoeias of our own day under the name of Aromatic Chalk Powder. Aubrey in his *Brief Lives* mentions this 'excellent cordiall, good in feavers. Mr Robert Boyle has the recipe and does great cures by it.'