Section of Experimental Medicine and Therapeutics

President Professor M D Milne MD

Meeting May 10 1966

President's Address

Lessons from Inborn Errors of Metabolism

by Professor M D Milne MD
(*Westminster Medical School, London*)

Mendel's (1865) fundamental work on the inheritance of plant characters remained generally unrecognized for many years until it was resurrected at the beginning of this century. Bateson (1902) played a major role in popularizing and interpreting Mendel's theory and had a great influence on the work of Sir Archibald Garrod, particularly in relation to the genetic aspects of the hereditary disease alkaptonuria (Garrod 1902). Garrod realized that excretion of homogentisic acid was due to an abnormality typical of recessive mendelian heredity. Some of the siblings of the patients were affected, whereas other relatives were usually normal. Affected individuals were often derived from consanguineous unions, and he postulated that the condition was due to a 'metabolic block' from a failure to convert homogentisic acid to other compounds. In his classical Croonian lectures Garrod (1908) added pentosuria, cystinuria and albinism as further 'inborn errors of metabolism,' and in the second edition of his monograph (1923) also included congenital erythropoietic porphyria and 'congenital steatorrhœa.' The latter was presumably mucoviscidosis.

Garrod's views were considerably in advance of his time. He almost reached the fundamental concept of modern biochemical genetics, the 'one gene, one enzyme' theory first clearly postulated by Beadle & Tatum (1941). The importance of inborn errors of metabolism was in the meantime largely disregarded by medical scientists, and even in 1940 Garrod's fundamental pioneer work on alkaptonuria remained unrecorded in most standard texts of medical genetics. However, important advances in the field were being made by botanists. Variations in flower colours were shown to be due to modification in the anthocyanin pigment molecules, usually by methylation or hydroxylation. These chemical steps were found to be mediated by single specific enzymes whose presence and activity was determined by mendelian principles (Wheldale 1909, Lawrence & Price 1940).

Beadle & Tatum's classical work (1941) emphasized the fundamental importance of inborn errors of metabolism in the elucidation of problems in biochemistry, metabolism, genetics, and molecular biology. Numerous major advances have been made in the last twenty years, but knowledge and understanding in this field is still fragmentary and inconclusive. Many of the views in this lecture are therefore speculative, and may well be proved incorrect by future research.

Recessive and Sex-linked Hereditary Metabolic Disorders

The six diseases described by Garrod (1923) are all inherited as mendelian recessive conditions, and many other such disorders have since been described. Probably the single most basic fact in the elucidation of these conditions is the characterization of the 'missing enzyme' responsible for the biochemical anomaly. Approximately fifty separate disorders have now been described where there is either certain or at least probable absence of a single enzyme protein whose synthesis is under genetic control. All of these conditions are inherited either by recessive or by sex-linked mendelian principles. The homozygote is easily recognizable as abnormal, whereas the heterozygote, with only one abnormal gene and usually capable of synthesizing the affected enzyme in amounts of about 50% of the normal, is either apparently identical to healthy controls or shows clinically unimportant biochemical abnormalities after administration of large amounts of the enzyme substrate.

These diseases have proved invaluable in the elucidation of major and minor biochemical pathways in man, e.g. in phenylalanine and tyrosine

metabolism, and in the steps of the Krebs-Henseleit cycle (1932) in conversion of ammonia to urea. In addition, such diseases provide important evidence relating to the specificity of human enzymes, where classical biochemical techniques are often hampered by ethical considerations. In maple-syrup urine disease (Menkes *et al.* 1954) there is accumulation of the three branched chain amino acids, valine, leucine and isoleucine, as well as the corresponding keto acids. This implies that the oxidative decarboxylation in the further metabolism of the keto acids is mediated by an enzyme common to all three. By contrast, in hypervalinæmia (Wada *et al.* 1963) there is increase only of plasma valine, indicating that valine transaminase is an enzyme distinct from any transaminase involved in leucine and isoleucine metabolism. Classical biochemical techniques suggested that proline and hydroxyproline were metabolized by a common oxidase. The recognition of the distinct hereditary disorders prolinæmia (Scriver *et al.* 1961) and hydroxyprolinæmia (Efron *et al.* 1965), with absence of the corresponding oxidase in each, shows that this supposition is incorrect, and that there are in fact two specific oxidases for these two related amino acids.

An important problem related to enzyme deficiency is still largely unanswered in many inherited recessive and sex-linked disorders. Is there complete failure to synthesize the affected enzyme protein, or is there a defect at an active part of the protein molecule which renders the protein impotent from an enzymatic point of view? Analbuminæmia (Bennhold & Kallee 1959) is a classical example of a genetic defect involving complete inability of the body to synthesize a quantitatively important protein. In many cases technical difficulties make the solution of this question very uncertain in most inherited conditions. In the Swiss type of acatalasia (Aebi *et al.* 1964) it was possible to isolate a small amount of normal catalase from erythrocytes obtained from cases of the disease. This shows that the defect is probably due to abnormalities of a regulator or operator gene controlling the rate of catalase synthesis, rather than of a structural gene defining its chemical structure. Pseudocholinesterase deficiency has been shown to be of at least two types. In one variety the plasma enzyme is altered in structure but still retains some hydrolytic activity (Harris *et al.* 1960). In the second variety (Hodgkins *et al.* 1965) the enzyme protein is completely absent. A further interesting example occurs in the diseases which are relieved by administration of excess pyridoxine although there is no evidence of total body pyridoxine deficiency (Scriver 1966). These include the syndrome of pyridoxine-dependent infantile convulsions, cystathioninuria, some cases of pyridoxine-dependent sideroblastic anæmia, and xanthurenic aciduria. Scriver suggests that in all these conditions there is a structural abnormality of various apoenzymes in the affected organ or tissue, which reduces its enzymatic activity unless there is excess concentration of the co-enzyme, pyridoxine. Normal metabolic processes can only proceed in presence of an abnormally high content of pyridoxine in body fluids.

Recessive hereditary diseases of especial interest are those in which the primary abnormality seems to be one of transport rather than of metabolism (Table 1). Most of these conditions involve disordered transport of essential metabolites in the kidney, the small intestine, or in both situations, where the epithelial cells are especially concerned in these specialized functions. There may be minor similar transport defects elsewhere in the body, but these are difficult to demonstrate with present relatively insensitive techniques. Conclusions derived from the functional defects in these diseases are as follows:

(*a*) Amino acids and probably glucose are transported by similar mechanisms in the renal tubules and the jejunal epithelium, or at least one essential step in transport is identical in both sites.

(*b*) Amino acids can be divided into separate transport groups (Milne 1964) which are transported across cells by an identical process for each member of the group, and there is mutual competition of available members for the transporting system. Thus the large group of mono-amino monocarboxylic acids are involved in Hartnup disease, and the smaller group of di-basic amino acids in cystinuria.

(*c*) Minor defects of amino-acid transport can occur in the gut without necessarily being present in the kidney.

Some hereditary defects of proximal tubular transport e.g. Lignac-Fanconi disease, are due to nonspecific renal damage rather than to isolated innate functional defects as in cystinuria and Hartnup disease. In some of these conditions the toxic agent causing the renal damage is known either with certainty or strong probability, e.g. cystine deposits in Lignac-Fanconi disease, galactose-1-phosphate in hereditary galactosæmia, and copper in hepatolenticular degeneration. The primary metabolic cause of the condition is, however, known only in the case of galactosæmia. The nonspecific functional defects of the renal tubules in these diseases can be closely simulated by the effects of exogenous poisons or toxins, e.g. by lead, cadmium, uranium, or maleic acid. By contrast, the specific disorder of amino-acid transport in cystinuria and Hartnup disease is a unique experiment of nature, and cannot be exactly simulated by any known method.

Table 1
Hereditary metabolic disease principally characterized by defects of transport

Disease	*Type of heredity* ●	*Compounds involved*	*Known sites of transport defect*	*Reference*
Cystinuria	A R	Cystine, lysine, arginine, ornithine	Proximal renal tubule. Jejunum	Dent & Rose (1951) Milne *et al.* (1961)
Hartnup disease	A R	Many mono-amino-mono-carboxylic amino acids	Proximal renal tubule. Jejunum	Baron *et al.* (1956) Milne *et al.* (1960)
'Blue diaper syndrome'	A R	Tryptophan. Calcium	Jejunum	Drummond *et al.* (1964)
'Oast-house syndrome'	A R	Methionine	Jejunum	Smith & Strang (1958) Hooft *et al.* (1964)
Lignac-Fanconi disease	A R	Most amino acids. Glucose. Phosphate. Urate	Proximal renal tubule	Bickel *et al.* (1952)
Oculo-cerebro-renal dystrophy	S R	As in Lignac-Fanconi disease	Proximal renal tubule	Lowe *et al.* (1952)
Fanconi syndrome without cystinosis	A D	As in Lignac-Fanconi disease	Proximal renal tubule	Hunt *et al.* (1966)
Glycinuria with urolithiasis	A D	Glycine	Proximal renal tubule	de Vries *et al.* (1957)
Renal glycosuria	A D	Glucose	Proximal renal tubule	Marble (1932)
Gluco-glycinuria	A D	Glycine, and glucose	Proximal renal tubule	Kaser *et al.* (1962)
Hereditary renal tubular acidosis	A D	Hydrogen ion	Distal renal tubule	Seldin & Wilson (1966)

● A D, autosomal dominant; A R, autosomal recessive; S R, sex-linked recessive types of heredity

Apparent exceptions to the one gene, one enzyme theory: Auerbach & DiGeorge (1965) have reviewed ways in which there is an apparent exception to the rule of 'one gene, one enzyme' in recessive metabolic conditions. They classify these into three groups: (1) Defects in two related enzymes in the same individual appearing to be due to a single gene defect. (2) Different but related enzyme defects in siblings. (3) Distribution anomalies of the affected enzyme in various tissues and organs.

Several instances have been recorded of children suffering from hereditary glycogenosis in which two separate enzymes are deficient in the same individual, and similarly families are reported where two of the six known types of glycogenosis have been present in siblings. Double defects have likewise been recognized in inborn errors of thyroxine and hydrocortisone synthesis (Gandy *et al.* 1960, DiGeorge 1961, Bongiovanni 1962). These double defects occur only in a small minority of affected patients, and are therefore unlike the situation in hereditary orotic aciduria where there is always a deficiency both of orotidylic pyrophosphorylase and orotidylic decarboxylase (Becroft & Phillips 1965, Smith *et al.* 1961). Similar double defects have been described in lower organisms, e.g. neurospora (Catcheside 1965), where they are usually explained on the basis of operator gene mutations rather than defects in a structural gene.

Distribution anomalies in which there is variation in content of the defective enzyme in different tissues can at least partly be explained by the increasing knowledge of iso-enzymes, recently reviewed by Wilkinson (1965). Examples include hereditary galactosæmia where some individuals may have absence of the affected enzyme in erythrocytes, and yet metabolize galactose to CO_2 and water at a normal rate because the liver content of the enzyme is normal (Segal *et al.* 1965), and the so-called 'Duarte' variant of the disease where the homozygote retains about 50% of the normal enzyme content in erythrocytes (Beutler *et al.* 1965). Similarly the already somewhat complicated classification of the glycogenoses into six fundamentally different types can be made still more difficult by subdivisions based on variation of distribution of the affected enzyme within different organs and tissues (Hers 1963, 1964). In cystinosis, where the fundamental enzymatic defect is still unknown, distribution of the cystine deposits has a profound effect on the clinical severity of the disease. In the Lignac-Fanconi disease in childhood, cystine deposits in the renal parenchyma result in progressive kidney damage with death from uræmia before or during adolescence. By contrast, in the adult form of cystinosis (Cogan *et al.* 1957, Cogan *et al.* 1958, Lietman *et al.* 1966) the kidney remains unaffected, and the patient has only mild disability.

Auerbach & DiGeorge (1965) advance an ingenious theory based on possible differences in the transcription of deoxyribonucleic acid from the abnormal gene to messenger ribonucleic acid to

explain these apparent anomalous contradictions to the classical 'one gene, one enzyme' theory of modern biochemical genetics. Further investigation of these seeming exceptions to the general rule may prove of great future importance in the elucidation of the mechanisms of hereditary metabolic disease.

Dominant Hereditary Metabolic Disease

In the case of autosomal or sex-linked recessive hereditary metabolic disease the available evidence strongly suggests that all cases are due to lack or alteration of a specific protein which is usually an enzyme. In autosomal recessive conditions the heterozygote with a half content of the affected enzyme either shows no demonstrable abnormality, or the defect is mild and far less disabling than is the disease in the homozygote. Similarly, in sex-linked recessive conditions the heterozygote female is either completely normal or only mildly affected.

In dominant hereditary metabolic disease the heterozygote shows the full effects of the disorder. Homozygotes are in general unrecognized, and it may well be that here the disability is so extreme that the fœtus is nonviable and death occurs *in utero*. From the 'one gene, one protein' hypothesis the severely affected heterozygote must possess about equal amounts of a normal and an abnormal protein within affected cells. In over fifty separate recessive or sex-linked diseases the nature of the absent enzyme or protein has been characterized with certainty or near certainty. In no single case has this been achieved in dominant hereditary metabolic disease. Admittedly, recognition of a partial deficiency is more difficult than in cases where it is complete or virtually complete. The great discrepancy between these two groups of hereditary disorders may possibly be explained by the argument that at least some of the dominant conditions are not due to a similar mechanism as occurs in recessive disease, and that some other defect than deficiency of a specific enzyme is the primary cause.

Sickle-cell anæmia is an example of a recessive hereditary disease in which there is an unusual degree of disability in the heterozygote, e.g. sickling of red cells, an undue tendency to vascular thrombosis, and inability to form a normally concentrated urine. In this condition, it is known that the disabilities shown by the heterozygote are not due to deficiency of the normal protein, hæmoglobin A, but to the actual presence of the abnormal protein, hæmoglobin S. A similar mechanism might well apply in dominant conditions, the disability of the heterozygote being due to the presence of an abnormal protein, rather than to a partial deficit of the normal one. This could conceivably result in structural defects of the affected cells, either of the protoplasmic contents or of the cell membrane.

Sweeney (1965) has developed these speculations in relation to the four dominant metabolic disorders, acute intermittent porphyria, variegate porphyria, familial periodic paralysis, and hereditary spherocytosis. He emphasizes that these diseases differ from recessive metabolic conditions in that their onset is always some years after birth, they may on occasions remain latent throughout the whole of the patient's life, and each has a peculiarly periodic character, exacerbations often being produced by known stimuli. He suggests that investigations of membrane permeability and of the structural components of the affected cells may well be more profitable than research to find an enzymatic deficiency which may well not in fact exist. The remainder of this Address extends these arguments to the known functional and structural defects of these and other dominant hereditary diseases.

Dominant hereditary types of porphyria: Erythropoietic protoporphyria (Magnus *et al.* 1961) is a dominant hereditary disease characterized by comparatively mild photosensitivity. The basic metabolic defect is an increased formation of protoporphyrin in developing erythrocytes, the pigment being excreted in excess in the fæces. In intermittent acute porphyria, most common in Sweden (Waldenström 1937) there is excess production of δ-aminolævulinic acid (ALA) within liver cells, leading to increased excretion of this acid and of porphobilinogen, formed by condensation of two molecules of ALA. Nakao *et al.* (1966) have recently shown that there is excess of ALA synthetase in liver cells obtained at biopsy from a case of the disease. In variegate porphyria, most common in South Africa (Barnes 1951, Dean 1953) there is increased fæcal output of coproporphyrin and protoporphyrin, with excess urinary uroporphyrin and coproporphyrin and their precursors during clinical exacerbations. The excretory pattern is similar to that produced by injections of large amounts of ALA (Berlin *et al.* 1956). All these disorders may be clinically latent throughout life, or may show sudden acute exacerbations which may be triggered by known stimuli. Possibly the basic disorder is excess production of enzymes of the porphyrin biosynthetic pathway, and this may well be due to abnormalities of operator or control genes, rather than of structural genes primarily concerned with specific protein synthesis.

Hereditary hyperbilirubinæmias: The Crigler-Najjar (1952) type of hereditary jaundice is a recessive disease due to absence of the enzyme glucuronyl transferase in liver cells. By contrast, the hereditary hyperbilirubinæmias described by Gilbert *et al.* (1907), Dubin & Johnson (1954),

and Rotor *et al.* (1948) are dominant hereditary conditions in which no enzymatic defect has been found. The basic abnormality in the Gilbert type is a deficiency of transfer of unconjugated bilirubin from blood into liver cells, whereas in the Dubin-Johnson and Rotor types there is a corresponding transport defect between the liver cells and the bile canaliculi. The abnormalities could conceivably be due to structural anomalies of the liver cell membrane.

Periodic paralysis: The various types of hereditary periodic paralysis are dominant conditions which usually remain asymptomatic for some years after birth. The modern classification into the hypokalæmic (Aitken *et al.* 1937), normokalæmic (Poskanzer & Kerr 1961), and hyperkalæmic types (McArdle 1962) is due to interest in the serum potassium concentration during paralytic attacks. These three conditions are distinct entities as they breed true in separate affected families. There are defects of polarization of the muscle cells consequent to abnormalities of the rate of potassium flux between plasma and the affected cells. Structural lesions consisting of vacuoles within muscle fibres are found in all the three types during or preceding paralytic attacks. The primary abnormality may lie in the muscle cell membrane or sarcoplasm, and could as easily be due to a structural defect from the presence of an abnormal protein as to a specific enzyme deficiency.

Muscular dystrophies: The Duchenne type of muscular dystrophy is due to a sex-linked gene, but the facioscapulohumeral type (Landouzy & Déjèrine 1885) and myotonic dystrophy are dominant conditions. There is in all types a random progressive atrophy of muscle fibres, including cardiac muscle. The basic disorder seems to be an abnormal leak of intracellular constituents through the muscle cell membrane, accounting for creatinuria, reduction of myohæmoglobin and organic phosphate esters within the affected muscle fibres, and the appearance of many intracellular enzymes in excess in plasma, e.g. aldolase, lactic dehydrogenase, transaminases, phosphoglucomutase, glycerophosphate isomerase, and creatine phosphokinase. Again the primary abnormality may well be an abnormal permeability of the cell membrane due to a structural defect. A more extreme and obviously structural variety of dominant hereditary muscle disorder occurs in central core disease (Shy & Magee 1956). Here there is an absence or decrease of mitochondria and sarcoplasmic reticulum in the central portions of the muscle cell. Mitochondrial enzymes are obviously decreased or actually absent in the affected areas.

'Pseudo-pseudohypoparathyroidism': Most variants of this condition are transmitted as a sex-linked hereditary disease (Mann *et al.* 1962), but in some families (Hermans *et al.* 1964, Goeminne 1963, Minozzi *et al.* 1963, Arnstein *et al.* 1966) there is a dominant type of heredity. Cases of overt pseudohypoparathyroidism in other members of the family have not been recorded in the dominant type. The primary abnormality may be a disorder of calcium and phosphate transport in the affected bones, and in the blood vessels of the basal ganglia. This could equally well be due to a structural as to an enzymatic anomaly.

Hereditary amyloidosis: In all varieties of amyloidosis there is an abnormal deposition of a fibrous protein in the extracellular compartment of connective tissue. Of the five clinical variants of hereditary amyloidosis, the type causing urticaria, nerve deafness and nephropathy (Muckle & Wells 1962), and the two neuropathic types (Andrade 1952, Rukavina *et al.* 1956) are all dominant hereditary disorders. The presence of an abnormal protein in all these conditions is obvious, but there is no proof that this is the primary abnormality directly influenced by a single defective gene.

Hereditary spherocytosis: Much detailed investigation has been devoted to this important dominant hereditary metabolic disease. No constant enzymatic deficiency has been reported, despite the unusual availability of the affected cells. The present popular view of the disease is that there is a primary abnormality of the erythrocyte membrane, making the cells abnormally permeable to sodium. This may be partly compensated by increased activity of the ATP-ase system, with greater efficiency for extrusion of sodium from the cell interior. In the unfavourable environment of the splenic sinusoids, the presumably compensatory increase of sodium pump activity is impaired, and excess accumulation of sodium and water within the cell will cause lysis. The primary cell membrane abnormality may well be one of structure rather than of an enzymatic defect.

Hereditary dominant defects of renal tubular transport: Many varieties of renal tubular transport defect are inherited by a mendelian dominant type of transmission (Table 1). These include the Fanconi syndrome without cystinosis, where six affected families have been described (Hunt *et al.* 1966), hereditary renal glycosuria, familial glycinuria with urolithiasis (de Vries *et al.* 1957), glucoglycinuria (Kaser *et al.* 1962), and the hereditary type of renal tubular acidosis, where 14 affected families have been described. All these conditions except renal tubular acidosis show transport defects for glucose, amino acids, and sometimes phosphate and uric acid in the proximal renal tubular epithelial cells. In renal tubular acidosis the primary defect is an inability to produce or maintain a high gradient of hydrogen ion

between plasma and urine. The disorder may equally well be due to lack of concentrative ability for H^+ by the distal tubular epithelial cells, or alternatively to an excess passive back diffusion of H^+ from the lumen of the renal tubule distal to the site of concentration of H^+. The latter explanation would obviously conform better to the thesis that dominant hereditary disease may often be due to a protein structural defect affecting the basic architecture of the cell or cell membrane.

Many of the examples of dominant hereditary disease described above show abnormalities which could be better explained by the presence of an abnormal protein disturbing the normal structure of the cell contents or of the cell membrane rather than to the partial absence of a specific but unknown enzyme. Many of the basic functional defects are disturbances of transport of substances into or out of the affected cells, possibly conditioned by alterations of the permeability of the cell membrane. Studies of ultra-structure or more basic research into membrane permeability may be of greater future promise than the methods of more conventional biochemistry.

SUMMARY

The 'one gene, one enzyme' theory of hereditary metabolic disease has proved of inestimable value in the elucidation of recessive and sex-linked conditions. Many fundamental biochemical discoveries are either directly due to studies of these disorders, or have been greatly assisted by them. Apparent exceptions to the theory may assist future work on the fundamental mechanisms of cellular inheritance. By contrast, the theory has proved virtually useless in research on dominant metabolic hereditary disease, where the primary defect is in all cases still unknown. Arguments are advanced that these conditions may be due to abnormalities of structure of the cell contents or of the cell membrane due to the presence of an abnormal protein, rather than secondary to a partial enzymatic defect.

REFERENCES

Aebi H, Baggiolini M, Dewald B, Lauber E, Suter H, Micheli A & Frei J (1964) *Enzm. Biol. Clin. (Basel)* **4**, 121
Aitken R S, Allott E N, Castleden L I M & Walker M (1937) *Clin. Sci.* **3**, 47
Andrade C (1952) *Brain* **75**, 408
Arnstein A R, Frame B, Frost H M & Block M A (1966) *Ann. intern. Med.* **64**, 996
Auerbach V H & DiGeorge A M (1965) *Amer. J. med. Sci.* **249**, 718
Barnes H D (1951) *S. Afr. J. clin. Sci.* **2**, 117
Baron D N, Dent C E, Harris H, Hart E W & Jepson J B (1956) *Lancet* ii, 421
Bateson W (1902) Mendel's Principles of Heredity. Cambridge
Beadle G W & Tatum E L (1941) *Proc. nat. Acad. Sci., Wash.* **27**, 499
Becroft D M O & Phillips L L (1965) *Brit. med. J.* i, 547
Bennhold H & Kallee E (1959) *J. clin. Invest.* **38**, 863
Berlin N I, Neuberger A & Scott J J (1956) *Biochem. J.* **64**, 80, 90
Beutler E, Baluda M C, Sturgeon P & Day R (1965) *Lancet* i, 353
Bickel H, Smallwood W C, Smellie J M & Hickmans E M (1952) *Acta pædiat, Stockh.* **42**, Suppl. 90, p 27
Bongiovanni A M (1962) *J. clin. Invest.* **41**, 2086
Catcheside D G (1965) *Biochem. biophys. Res. Commun.* **18**, 648
Cogan D G, Kuwabara T, Hurlbut C S jr & McMurray V (1958) *J. Amer. med. Ass.* **166**, 1725
Cogan D G, Kuwabara T, Kinoshito J, Sheehan L & Merola L (1957) *J. Amer. med. Ass.* **164**, 394
Crigler J F & Najjar V A (1952) *Pediatrics, Springfield* **10**, 169
Dean G (1953) *Brit. med. J*, ii, 1291
Dent C E & Rose G A (1951) *Quart. J. Med.* **20**, 205
DiGeorge A M (1961) *Trans. Stud. Coll. Phycns, Philad.* **28**, 213
Drummond K N, Michael A F, Ulstrum R A & Good R A (1964) *Amer. J. Med.* **37**, 928
Dubin I N & Johnson F B (1954) *Medicine, Baltimore* **33**, 155
Efron M L, Bixby E M & Pryles C V (1965) *New Engl. J. Med.* **272**, 1299
Gandy H M, Keutmann E H & Izzo A J (1960) *J. clin. Invest.* **39**, 364
Garrod A E (1902) *Lancet* ii, 1616
(1908) *Lancet* ii, 142, 173, 214
(1923) Inborn Errors of Metabolism. 2nd ed. London
Gilbert A, Lereboullet P & Herscher M (1907) *Bull. Soc. méd. Hôp., Paris* **24**, 1203
Goeminne L (1963) Proc. XI International Congress of Genetics. New York
Harris H, Whittaker M, Lehmann H & Silk E (1960) *Acta genet. (Basel)* **10**, 1
Hermans P E, Gorman C A, Martin W J & Kelly P J (1964) *Proc. Mayo Clin.* **39**, 81
Hers H G (1963) *Biochem. J.* **86**, 11
(1964) Advances in Metabolic Disorders. Ed. R Levine & R. Luft New York
Hodgkins W E, Giblett E R, Levine G, Bauer W & Motulsky A G (1965) *J. clin. Invest.* **44**, 486
Hooft C, Timmermans J, Snoeck J, Antener I, Oyaert W & Hinde C van den (1964) *Lancet* ii, 20
Hunt D D, Stearns G, McKinley J B, Froning E, Hicks P & Bonfiglio M (1966) *Amer. J. Med.* **40**, 492
Kaser H, Cottier P & Antener I (1962) *J. Pediat.* **61**, 386
Krebs H A & Henseleit K (1932) *Hoppe-Sehl. Z.* **210**, 33
Landouzy L & Déjèrine J (1885) *Rev. méd. franç.* **5**, 81, 253
Lawrence W J C & Price J R (1940) *Biol. Rev.* **14**, 35
Lietman P S *et al.* (1966) *Amer. J. Med.* **40**, 511
Lowe C U, Terrey M & MacLachlan E A (1952) *Amer. J. Dis. Child.* **83**, 164
McArdle B (1962) *Brain* **85**, 121
Magnus I A *et al.* (1961) *Lancet* ii, 448
Mann J B, Alterman S & Hills A G (1962) *Ann. intern. Med.* **56**, 315
Marble A (1932) *Amer. J. med. Sci.* **183**, 811
Mendel G (1865) *Verh. naturf. Ver. Brünn* **4**, 1
(1901) Versuche über Pflanzhybriden. Leipzig
Menkes J H, Hurst P L & Craig J M (1954) *Pediatrics, Springfield* **14**, 462
Milne M D (1964) *Brit. med. J.* i, 327
Milne M D, Asatoor A M, Edwards K D G & Loughridge L W (1961) *Gut* **2**, 323
Milne M D, Crawford M A, Girao C B & Loughridge L W (1960) *Quart. J. Med.* **29**, 407
Minozzi M *et al.* (1963) *Folia Endocr. (Roma)* **16**, 168
Muckle T J & Wells M (1962) *Quart. J. Med.* **31**, 235
Nakao K *et al.* (1966) *Nature, Lond.* **210**, 838
Poskanzer D C & Kerr D N S (1961) *Amer. J. Med.* **31**, 328
Rotor A B, Manahan L & Florentin A (1948) *Acta med. philipp.* **5**, 37
Rukavina J C *et al.* (1956) *J. Lab. clin. Med.* **47**, 365
Scriver C R (1966) *Pediatrics, Springfield* **37**, 553
Scriver C R, Schafer I A & Efron M L (1961) *Nature, Lond.* **192**, 672
Segal S, Blair A & Roth H (1965) *Amer. J. Med.* **38**, 62
Seldin D W & Wilson J D (1966) In: The Metabolic Basis of Inherited Disease. 2nd ed. Ed. J B Stanbury, J B Wyngaarden & D S Fredrickson. New York; p 1230
Shy G M & Magee K R (1956) *Brain* **79**, 610
Smith A J & Strang L B (1958) *Arch. Dis. Childh.* **33**, 109
Smith L H jr, Sullivan M & Huguley C M jr (1961) *J. clin. Invest.* **40**, 656
Sweeney G D (1965) *S. Afr. med. J.* **39**, 1075
Vries A de, Kochwa S, Lazebnik J, Frank M & Djaldetti M (1957) *Amer. J. Med.* **23**, 408
Wada Y *et al.* (1963) *Tohoku J. exp. Med.* **51**, 46
Waldenström J (1937) *Acta med. scand.* Suppl. 82
Wheldale M (1909) *Proc. Camb. phil. Soc.* **15**, 137
Wilkinson J H (1965) Isoenzymes. London