Disorders of Amino-Acid Transport*

M. D. MILNE,† M.D., F.R.C.P.

Brit. med. J., 1964, 1, 327-336

Transport mechanisms of essential metabolites are now recognized as being of great importance in physiology and disease. There are three sites in the body which are of especial importance in this respect: the jejunum, where substances are transported from the intestinal lumen to the portal blood; the renal tubules, where transport mechanisms are essential in the reabsorption of substances filtered at the glomeruli; and the cellular membranes, which control the passage of metabolites between the extracellular and intracellular fluids. Historically, glucose was the substance in which transport mechanisms were first studied in detail because of the use of phlorhizin as a physiological tool. Phlorhizin was first isolated from apple-tree bark by de Koninck (1835), and its action in causing glycosuria was recognized by von Mering (1888). The investigations of Mayrs (1923) and Poulsson (1930) suggested that this action was due to failure of reabsorption of glucose from the glomerular filtrate, a view finally confirmed by Jolliffe *et al.* (1932), who showed that the glucose, xylose, sucrose, and raffinose clearances were identical in the phlorhizinized dog, although widely different in the normal animal.

These physiological results were rapidly applied to clinical medicine. Marble (1932) defined criteria for the certain diagnosis of renal glycosuria; criteria which are still used in practice to-day. Later it was recognized by application of clearance techniques to clinical patients that renal glycosuria was of two types (Govaerts and Lambert, 1949; Reubi, 1951). In the more severe varieties, occurring either as an isolated tubular defect or in association with generalized tubular disorder as in the Fanconi syndrome, there was a true abnormality of glucose transport, similar to that of the phlorhizinized animal. In most patients with renal glycosuria, however, no transport defect was found, the disorder being due to an unusual degree of heterogeneity of individual nephrons in respect of their ability to reabsorb glucose from the glomerular filtrate.

Similar studies of amino-acid transport were at first hampered by lack of suitable analytical methods. Dent (1950), applying the method of paper chromatographic separation devised by Consden *et al.* (1944) to biological fluids, showed that abnormal amounts of amino-acids in urine could be due either to an "overflow mechanism" similar to diabetes mellitus or to a "renal mechanism" resembling renal glycosuria. In the former type there was an increase of plasma concentration of one or several amino-acids, and the kidney was reacting normally to an abnormal environment. In the latter type the plasma concentration of amino-acid was either normal or somewhat reduced, and the basic abnormality lay in tubular amino-acid transport mechanisms. The situation is obviously more complex in relation to the 21 separate amino-acids derived from protein hydrolysis as compared with the single substance involved in renal glycosuria. Harris (1957) stated that a single disease

might show a dual abnormality, there being an "overflow mechanism" for some amino-acids and a "renal mechanism" for others.

Stanbury (1958) carried the analogy to renal glycosuria a stage further, and stated that there was no proof that all types of pathological renal amino-aciduria were, in fact, due to abnormalities of transport, and that some could conceivably be due to abnormal heterogeneity of nephrons in respect of amino-acid reabsorption. Later work has, however, made this view less likely, and the available evidence suggests that most examples of renal amino-aciduria can safely be considered under the heading of "disorders of amino-acid transport." Admittedly, however, the effect of heterogeneity of amino-acid reabsorption by individual nephrons has not been adequately investigated by modern clearance techniques and may well account for some minor variations in amino-acid excretion, and possibly for the physiological hyperamino-aciduria of the premature infant and the newly born child (Huisman, 1957; Jagenburg, 1959).

This lecture gives a more comprehensive classification of pathological amino-aciduria, discusses the diseases associated with amino-aciduria which provide clues to the physiological processes of amino-acid transport, and reviews an important modern therapeutic advance—that is, the use of D-penicillamine in the treatment of cystinosis (Clayton and Patrick, 1961) and of cystinuria (Crawhall *et al.*, 1963).

Physiological Aspects of Amino-acid Transport

Amino-acids are water-soluble substances which do not readily cross lipid-containing cellular membranes. Their passage may obviously be either by active transport or by simple diffusion. The differences between these two mechanisms are given in Table I. The everted intestinal sac (Wilson and Wiseman, 1954a), usually of the hamster or the rat, has proved the most convenient preparation for the study of amino-acid transport *in vitro*. It has been conclusively proved that most amino-acids derived from protein hydrolysis are conveyed across the intestinal membrane by active transport. In particular, they are carried against a concentration gradient, they may show mutual inhibition of transport, and the natural L-enantiomorph is transported more readily than the D-form (Elsden *et al.*, 1950; Gibson and Wiseman, 1951; Wiseman, 1953; Kuroda and Gimbel, 1954). Demonstration of active transport does not, however, exclude the coexistence of simple diffusion, and there has been recent discussion (Booth and Kanaghinis, 1963; Booth, 1963) of the possible importance of diffusion in amino-acid absorption from the gut.

All amino-acids derived from protein hydrolysis—with the exception of proline and hydroxyproline, more strictly termed "imino-acids"—can be regarded as substances of the chemical formula $R.CH(NH_2).COOH$, where R is an organic radical.

* Bradshaw Lecture delivered at the Royal College of Physicians of London on 4 November 1963.

† Department of Medicine, Westminster Hospital Medical School, London.

Obviously there are three possible alternatives regarding active transport mechanisms: (a) there could be a separate transport system for each individual amino-acid; (b) there could be a single system for all naturally occurring amino-acids; or (c) amino-acids could be divided into groups, each involving a separate transport system.

TABLE I.—Comparison of Active Transport and Passive Diffusion

	Active Transport	Passive Diffusion
Relation to concentration gradient	Transport from fluids of a lower to a higher concentration occurs	Movement only from fluids of a higher to a lower concentration
Relationship to apparent molecular volume in compounds of similar type	Speed of transport not necessarily related to apparent molecular volume	Smaller molecules in general diffuse at a greater rate
Stereo-specificity	Stereo-specific. Stereo-isomers in general are transported less readily than naturally occurring compounds	Rate of transfer of stereo-isomers identical
Saturation of system at high concentrations	Rate of transfer not proportional to concentration gradient, and approaches a limiting maximal value	Rate of transfer proportional to concentration gradient and no limiting maximal rate occurs
Competitive inhibition by related compounds	Rate of transfer reduced by simultaneous transport of a related compound sharing the same transport system	Rate of transfer unaffected by the presence of closely related compounds
Non-competitive inhibition	Transport reduced by metabolic poisons—e.g., dinitrophenol	Rate of transfer not affected unless obvious cellular damage occurs

The last alternative seems the most likely and is, in fact, that which actually occurs. Experiments involving transport across the everted hamster- or rat-intestinal sac (Wiseman, 1955; Hagihira *et al.*, 1961) or clearance tests for amino-acids after separate or combined infusions in both man and the experimental animal (Kamin and Handler, 1951; Robson and Rose, 1957; Brown *et al.*, 1961; Scriver *et al.*, 1961; Webber *et al.*, 1961; Webber, 1963) have shown that identical groups for active transport occur in both the intestine and the proximal renal tubules.

The four groups may be classified as follows:

1. Monoamino-monocarboxylic amino-acids: Alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, asparagine, glutamine, histidine, cysteine, methionine, and citrulline.
2. Dibasic amino-acids: Lysine, arginine, ornithine, and cystine.
3. Dicarboxylic amino-acids: Glutamic and aspartic acids.
4. The imino-acid and glycine group: Proline, hydroxyproline, and glycine.

The position of glycine is somewhat anomalous, as its transport depends on systems 1 and 4, but the latter is the more important.

The pattern of excretion of amino-acids in various diseases associated with increased urinary output of amino-acids strongly supports this division into four separate transport systems.

Classification of Disease Associated with Amino-aciduria

The older simple classification of amino-aciduria into the "overflow" and the "renal" types, although a valuable division, is not comprehensive enough to give an adequate classification of amino-aciduria in human disease. The following more adequate classification is proposed:

1. Pure "overflow" amino-aciduria: (a) generalized amino-acid loss; (b) specialized loss of amino-acids of the "threshold" type; (c) specialized loss of amino-acids of the "non-threshold" type.
- II. Mixed "overflow" and "renal" amino-aciduria with partial or complete saturation of an amino-acid transport system: (a) involving group 1 (monoamino-monocarboxylic amino-acids); (b) involving group 2 (dibasic amino-acids); (c) involving group 4 (imino-glycine group).
- III. Pure "renal" amino-aciduria involving a specific amino-acid transport system and without other evidence of disordered renal

tubular function: (a) involving group 1 (monoamino-monocarboxylic amino-acids); (b) involving group 2 (dibasic amino-acids).

IV. "Renal" amino-aciduria associated with generalized tubular damage: (a) known causation; (b) unknown causation.

I. Pure Overflow Amino-aciduria

The diseases in this group will not be considered in detail as they are disorders of metabolism and not of amino-acid transport. Generalized amino-aciduria of the overflow type occurs after infusion of protein hydrolysates, and in severe liver failure with inability of deamination of amino-acids to ammonia (Walshe, 1953). There is always a generalized increase of plasma amino-acids, with a gross rise of plasma alpha-amino-nitrogen.

There are four hereditary diseases which are examples of an overflow amino-aciduria involving amino-acids that are normally almost completely reabsorbed by the renal tubules and therefore have a low renal clearance: phenylketonuria-phenylalanine (Fölling, 1934; Knox, 1960); maple-syrup-urine disease—the branched-chain amino-acids valine, leucine, and isoleucine (Menkes *et al.*, 1954; Dancis and Levitz, 1960); histidinaemia-histidine (Ghadimi *et al.*, 1961; Auerbach *et al.*, 1962; Davies and Robinson, 1963; La Du *et al.*, 1963); and glycinaemia-glycine (Childs *et al.*, 1961). The first three have certain features in common (Table II). They are classical examples of a recessive gene causing the absence of a single known enzyme which is essential in the metabolism of the amino-acids involved. The increased tubular load of the

TABLE II.—Comparison of Classical Hereditary Overflow Amino-acidurias

	Phenylketonuria	Maple-Syrup-Urine Disease	Histidinaemia
Physical and mental effects	Severe	Extremely severe	Slight
Enzyme deficiency	Phenylalanine hydroxylase	Branched-chain keto-acid oxidative decarboxylase	Histidine β -deaminase (histidinase)
Location of enzyme deficiency	Liver only	General	General
Amino-acids in excess in body fluids	Phenylalanine	Valine, leucine, isoleucine, and allo-isoleucine	Histidine
Keto-acids in excess	Phenylpyruvic acid	α -Ketoisovaleric acid α -Ketoisocaproic acid α -Keto- β -methyl-valeric acid	Imidazol pyruvic acid
Hydroxy acids in excess	Various phenolic acids and indolyl lactic acid	α -Hydroxyisovaleric acid α -Hydroxyisocaproic acid α -Hydroxy- β -methyl-valeric acid Indolyl lactic acid Indolyl-acetic acid and its conjugate	Imidazol lactic acid
Other inorganic acids in excess	Phenylacetyl-glutamine, Indolyl-acetic acid and its conjugate		Imidazol acetic acid

affected amino-acids is insufficient to saturate the tubular transport of group 1 amino-acids, and therefore there is no secondary renal amino-aciduria. Similarly, in glycinaemia the increased load of glycine is insufficient to affect tubular reabsorption of the amino-acids proline and hydroxyproline. Experimentally this renal transport system is easily saturated by infusion of either proline or hydroxyproline, but not by infusion of glycine (Scriver *et al.*, 1961).

Overflow amino-aciduria affecting amino-acids of the "non-threshold" type include the harmless metabolic anomaly β -aminoisobutyric aciduria (Crumpler *et al.*, 1951; Armstrong *et al.*, 1963), and hereditary diseases causing severe mental deficiency such as arginino-succinuria (Allan *et al.*, 1958; Levin *et al.*, 1961) and cystathionuria (Harris *et al.*, 1959; Frimpter *et al.*, 1963). The amino-acids concerned are normally intracellular constituents, and are excreted in the urine at a high clearance, approximating to the glomerular filtration rate. They are not amino-acids derived from protein hydrolysis, and have no effect on amino-acid transport systems.

II. Mixed Overflow and Renal Amino-aciduria

The renal transport system of group 1 amino-acids is not easily saturated by infusion of any single amino-acid. Transport is unaffected by the increased tubular loads of phenylalanine, branched-chain amino-acids, or histidine in phenylketonuria, maple-syrup-urine disease, and histidinaemia respectively. The only amino-acids excreted in excess in the urine are those in which the metabolic defect has caused an increased plasma concentration. There is, however, suggestive evidence that saturation of the system occurs in the rare hereditary disease citrullinuria, which is another cause of mental deficiency (McMurray *et al.*, 1962). There is increase of citrulline both in plasma and in urine, the amino-aciduria being of the overflow type. In addition, however, there is increased excretion of alanine, aspartate, glycine, histidine, and serine (McMurray and Mohyuddin, 1962; McMurray *et al.*, 1963), although the plasma concentrations are normal.

The transport system for dibasic amino-acids is easily saturated experimentally. Thus infusions of lysine (Robson and Rose, 1957) or, better, of arginine (Ruszkowski *et al.*, 1960) cause excess excretion not only of the amino-acid infused but also of the other three members of the group, although their plasma levels, with one exception, remain normal. The single exception is that infused arginine is rapidly metabolized to ornithine (Webber *et al.*, 1961) and therefore there is increase of the plasma concentration of both amino-acids. No disease is known in which there is a block in metabolism of the amino-acids of this group, and therefore saturation of this particular transport system is only of physiological interest. Infusion of aspartate in the dog causes excess excretion of glutamate (Webber, 1963) as well as of the amino-acid given, but again no disease causing a specific elevation of plasma glutamate or aspartate has been described.

By contrast, the three amino-acids of the imino-glycine group have all been described as separately increased in plasma, the disorders being termed glycinaemia (Childs *et al.*, 1961), prolinaemia (Schafer *et al.*, 1962), and hydroxyprolinaemia (Efron *et al.*, 1962). In all three types there was an overflow excretion of the involved amino-acid, but only prolinaemia caused a secondary renal amino-aciduria of the other two amino-acids. Proline is experimentally the most effective amino-acid in saturation of the imino-glycine transport system (Scriver *et al.*, 1961).

III. Renal Amino-aciduria Involving a Specific Amino-acid Transport System

In Hartnup disease and cystinuria the primary defect is either absence or deficiency of active transport of amino-acids of group 1 and group 2 respectively. The defect is present both in the renal tubules (Dent and Rose, 1951; Baron *et al.*, 1956) and in the jejunum (Milne *et al.*, 1959, 1960, 1961; Shaw *et al.*, 1960; Asatoor *et al.*, 1962), but there is no evidence that it occurs elsewhere in the body. The diseases differ fundamentally from those previously described, because the amino-acids involved are in normal or reduced concentration in the plasma. They are pure disorders of amino-acid transport, and not metabolic disorders of a single amino-acid or of several amino-acids.

Cystinuria

Although this condition has been recognized for over 150 years (Wollaston, 1810), it is only in the last decade that it has been reasonably well understood. Except for the remarkable insolubility of cystine in water and urine, it would be a harmless anomaly of metabolism, similar to β -amino-isobutyric aciduria, and would probably not have been recognized before the development of chromatographic techniques in the last two

decades. In fact, it is the cause of about 1% of all cases of nephrolithiasis, and a considerably higher proportion of renal calculous disease in children. Dent and Rose (1951), using paper chromatographic methods, showed there was an isolated abnormality of proximal renal tubular function, as there was reduced reabsorption from the glomerular filtrate of the four amino-acids cystine, lysine, arginine, and ornithine. These results have since been confirmed by polarographic methods (Fowler *et al.*, 1952; Dent *et al.*, 1954b), microbiological assay (Dent *et al.*, 1954a; Doolan *et al.*, 1957), and quantitative column chromatography (Arrow and Westall, 1958; Brigham *et al.*, 1960).

Frimpter (1961) has shown that a fifth amino-acid, the mixed disulphide of cysteine and homocysteine, is also excreted in excess in cystinuria. This amino-acid presumably occurs from oxidation by the cytochrome enzyme system (Keilin, 1930) of mixtures of cysteine and homocysteine, or by disulphide exchange (Kolthoff *et al.*, 1955; Ryle and Sanger, 1955; Pihl *et al.*, 1958) in mixtures of cystine and homocysteine. Although the average excretion by cystinurics of the mixed disulphide is about one-tenth that of cystine (Frimpter, 1961), cystine calculi contain less than 1% of the mixed disulphide, indicating that its solubility is much greater than that of cystine. This property of mixed disulphides is discussed later in relation to penicillamine therapy. Other than the specific transport defect of group 2 amino-acids there is no other abnormality of renal function except that which may be secondarily imposed by the formation of cystine calculi. These secondary defects, which are common to all types of nephrolithiasis, are caused by mechanical obstruction or bacterial infection.

Hartnup Disease

This disease, first described by Baron *et al.* (1956), is a recessive hereditary condition analogous to cystinuria except that in this case there is a similar transport defect of group 1 amino-acids. All the amino-acids of this group are in greatly increased concentration in the urine whereas their plasma levels are normal or reduced, the amino-acid clearances being correspondingly abnormally high (Cusworth and Dent, 1960). A total of 15 cases have been described (Hickish, 1955; Baron *et al.*, 1956; Jonxis, 1957; Henderson, 1958; Weyers and Bickel, 1958; Hersov and Rodnight, 1960; Albers and Wadman, 1961; Hooft *et al.*, 1962). None of the amino-acids concerned are particularly insoluble, and, provided that the protein intake is satisfactory, the amount lost in the urine is of negligible nutritional importance. If the abnormality was confined to the kidneys the disease would cause no symptoms, and, indeed, would have been unlikely to be recognized. Accidental routine chromatography of urine from an individual with such a rare anomaly would have been extremely unlikely. In Hartnup disease the associated intestinal transport defect is the important factor in causing the two cardinal signs of the condition—a pellagrous rash and intermittent short-lived attacks of cerebellar ataxia. In this respect Hartnup disease and cystinuria differ, as in the former it is the intestinal defect which is clinically important, and in the latter the renal defect.

The Intestinal Transport Defect in Cystinuria and Hartnup Disease

Recognition of a renal transport defect *in vivo* is simpler than that of a corresponding intestinal defect. By modern clearance techniques it is relatively simple to show an increase of amino-acid clearance despite a normal or reduced plasma concentration. This suffices to prove that there is defective transport of the amino-acid concerned from the glomerular filtrate to the peritubular capillary blood. An intestinal defect can be inferred by at least five methods.

1. After ingestion of the amino-acid to be tested there should be a lower plasma concentration than occurs in normal control subjects. Proof should also be given that after intravenous injection of the amino-acid the rate of decline of plasma concentration is identical in the patient and controls. Analyses of urine are also useful to prove that there is not a significant excess loss of amino-acid by this channel. This technique has been used to prove a transport defect of arginine in cystinuria (Asatoor *et al.*, 1962), and suggestive results have been obtained for tryptophan in Hartnup disease (Milne *et al.*, 1960).
 2. After ingestion of the amino-acid to be tested more should appear unchanged in the faeces than in normal controls. This method has proved positive for arginine, lysine, and ornithine in cystinuria (Milne *et al.*, 1961; Asatoor *et al.*, 1962), and for tryptophan in Hartnup disease (Milne *et al.*, 1960).
 3. After ingestion of the amino-acid to be tested a greater amount of products of bacterial degradation of the amino-acid should appear in the faeces than in normal controls. Positive results have been found for indole and tryptamine derived from tryptophan in Hartnup disease (Asatoor *et al.*, 1963), and for citrulline, ornithine, and putrescine derived from arginine in cystinuria (Asatoor *et al.*, 1962).
 4. After ingestion of the amino-acid to be tested a greater amount of urinary products of bacterial degradation of the amino-acid should be excreted than in control subjects. These may be either identical with the product absorbed from the colon or further changed by metabolic processes within body cells. Positive results are listed in Table III. Previous papers (Milne *et al.*, 1961; Asatoor *et al.*, 1962) have quoted a relatively unimportant site for formation of urinary piperidine and pyrrolidine by cystinurics (Fig. 1). It was stated that these heterocyclic amines were formed from absorbed cadaverine and putrescine by tissue diamine oxidase. This may possibly account for a small proportion of their urinary output, but the major part is formed directly within the colon by action of bacterial diamine oxidase and is excreted unchanged after absorption from the gut (Asatoor and Milne, 1963). The particular bacterial species involved has not been determined with certainty although it is known that some varieties of *Pseudomonas pyocyanea* have enzyme systems which could convert putrescine to pyrrolidine (Jakoby and Fredericks, 1959).
 5. The malabsorption of certain amino-acids from the jejunum allows a greater amount to reach the colon, and thus the bacteria are chronically exposed to an environment containing an abnormally high concentration of certain amino-acids. This may induce preferential selection of unusual bacterial strains which are stable on subculture. In cystinurics the *Escherichia coli* cultured from the stools have been shown to be unusually active in the decarboxylation of lysine and ornithine to the corresponding diamine (Asatoor *et al.*, 1962), but no corresponding unusual bacterial strain was cultured from stools of cases of Hartnup disease (Asatoor *et al.*, 1963).
- These investigations have conclusively shown that there is an intestinal absorption defect for tryptophan and probably for the other group 1 amino-acids in Hartnup disease, and for group 2 amino-acids in cystinuria, similar to the transport defect in the proximal renal tubules. There is no evidence, however, that the defect is present in other body tissues or cells.

TABLE III.—Metabolic Products Derived from Breakdown of Unabsorbed Amino-acids by Colonic Bacteria in Cystinuria and Hartnup Disease

Amino-acid Ingested in Excess	Derivatives in Faeces	Derivatives Excreted in Urine
Cystinuria:		
Lysine	Cadaverine Piperidine	Cadaverine very occasionally Piperidine
Ornithine	Putrescine Pyrrolidine	Putrescine very occasionally Pyrrolidine
Arginine	Citrulline Ornithine Putrescine Pyrrolidine	Citrulline Putrescine very occasionally Pyrrolidine
Hartnup disease:		
Tryptophan	Indole Tryptamine Indolyl-3-acetic acid Indolyl-3-propionic acid	Indican (indoxyl sulphate) bis-Indolyl-indoxyl Indolyl-3-acetic acid Indolylacetylglutamine Indolyl-3-acrylic acid Indolylacrylylglycine
Phenylalanine	β -Phenylethylamine Benzoic acid	Phenylacetylglutamine Hippuric acid
Tyrosine	Tyramine	p-Hydroxyphenylacetic acid and other phenolic acids

Unsolved Problems in Cystinuria and Hartnup Disease

These diseases are recessive hereditary conditions causing defective transport of groups of amino-acids in the proximal renal tubules and the jejunum. By modern views this should mean that an abnormal gene has resulted in the absence or defective synthesis of a protein which is presumably an enzyme involved in amino-acid transport. The dual location of the defect in each disease implies either that amino-acid transport is mediated by identical mechanisms in the jejunum and renal tubules, or that at least an essential portion of the process is identical in each site. Amino-acids are almost certainly absorbed by a combination of active transport and simple diffusion. In the case of sugars it is known that active transport is 10 to 20 times as important as is diffusion, but such accurate data are not available for amino-acids (Wilson, 1962). In his monograph on intestinal absorption Wilson (1962) states that "it is quite probable that without the active transport systems severe nutritional deficiency would result." No such severe deficiency occurs in cystinuria or Hartnup disease, although there is a slight but statistically significant reduction of the mean stature in these conditions (Colliss *et al.*, 1963). It follows, therefore, that either Wilson's statement is incorrect or the intestinal transport defect in the two diseases is partial rather than absolute.

FIG. 1.—Chromatograms of DNP-derivatives of urinary amines. Effect of ingestion of 10 g. L-ornithine hydrochloride by a cystinuric patient (right) and a normal control subject (left). In the former there is a gross increase of urinary pyrrolidine and a slight rise of urinary piperidine, but there is no change in the normal control. AMM=DNP-ammonia. M=DNP-methylamine. DM=DNP-dimethylamine. E=DNP-ethylamine. PYR=DNP-pyrrolidine. PIP=DNP-piperidine. PRE-ORN=control urine. POST-ORN 1=10-12 hours after ornithine. POST-ORN 2=12-24 hours after ornithine.

There is some experimental evidence that the latter alternative is correct. Ingestion of lysine in cystinuric patients reduces absorption of dietary arginine by further saturation of the existing transport system (Milne *et al.*, 1961), and, conversely, ingestion of arginine depresses absorption of dietary lysine (Asatoor *et al.*, 1962). There is no correlation between the percentage of separate amino-acids reabsorbed from the glomerular filtrate in Hartnup disease and the apparent molecular volume of the amino-acid concerned (Fig. 2). A significant inverse correlation would be expected if reabsorption from the tubular lumen was due entirely to passive diffusion rather than to residual active transport. The available evidence,

therefore, suggests a deficiency of unknown enzymes in each condition which are not absolutely essential for transport but which facilitate the process and increase its rate and maximum capacity.

FIG. 2.—There is no correlation between the percentage reabsorption of various amino-acids from the glomerular filtrate, in a case of Hartnup disease, and the apparent molecular volumes of the absorbed amino-acids. This suggests that reabsorption of amino-acids in Hartnup disease is chiefly by active transport rather than by simple diffusion. Clearance data taken from Cusworth and Dent (1960).

Probably these two diseases would be of greater nutritional significance in primitive communities dependent on hunting rather than on agriculture. In such races there are almost invariably chronic periods of protein insufficiency punctuated by successful "kills" which are followed by the ingestion of enormous quantities of animal protein in as short a time as possible. Experimentally, it is in these circumstances, when large quantities of amino-acids must be absorbed in a very short period, that there is the greatest functional inadequacy in both cystinuria and Hartnup disease. The more efficient transport system of the normal individual may well have been evolved to meet the needs of a more primitive existence where protein is ingested irregularly and in infrequent but large amounts. The regular and adequate ingestion of protein by agricultural societies has existed only for the last 12,000 years, which is a very small fraction of evolutionary time.

Unlike that in cystinuria, the intestinal defect in Hartnup disease is of greater clinical significance than the renal defect. The pellagra is easily explained by tryptophan deficiency, considerable quantities of the amino-acid being lost in the faeces and urine, or degraded to products such as indole, indican, and indolyl-acetic acid, which are useless nutritionally. Less tryptophan is therefore available for conversion to nicotinamide via kynurenine. Pellagra is more severe in the child, where demands for an essential amino-acid for protein synthesis are greater than in the adult. It responds satisfactorily to nicotinamide therapy. The toxæmia causing the attacks of cerebellar ataxia has not been adequately investigated, as it has not been a prominent feature of more recently described cases of the disease. Probably it is due to intoxication from bacterial degradation products of amino-acids present in excess concentration in the gut. Possible toxic agents are indole, tryptamine, β -phenylethylamine, tyramine, isoamylamine, and other amines derived from the decarboxylation of amino-acids. Theoretically, the ataxia should respond to measures used in the treatment of hepatic coma or pre-coma (Sherlock, 1958), but this must remain speculative until opportunities occur for treatment of cases of Hartnup disease in relapse.

Further research on these two diseases is hampered by their rarity and by the difficulties imposed by observations in human patients. Advances in the future may well be made by studies of canine cystinuria. In the past, investigation of this disease in the dog has tended to follow discoveries made in the human disease; in the future it may well lead the way.

Canine Cystinuria

Canine cystinuria is remarkably similar to the human disease. A much greater proportion of urinary calculi is due to cystinuria in the dog than in man. There are two important clinical differences. In the dog the disease is due to a sex-linked recessive factor and therefore it is almost confined to male animals, whereas in man it is an autosomal recessive disease and occurs equally in the two sexes. In man, most cystine calculi are within the renal pelvis, whereas in the dog they are chiefly within the bladder and cause retention of urine by impaction in the posterior urethra. There are also minor biochemical differences. There is a pure renal amino-aciduria involving cystine, lysine, arginine, and ornithine, as in the human disease (Treacher, 1962). The mixed disulphide of cysteine and homocysteine (Frimpter, 1961) has not been described in the urine of the cystinuric dog to date, but may well be present in small concentration. There is often, however, an abnormally high urinary output of the amino-acids citrulline and threonine (Treacher, 1962). Citrulline can be detected in human cystinuric urine after ingestion of large amounts of arginine, and is probably secondary to bacterial conversion of arginine to citrulline in the colon (Asatoor *et al.*, 1962). Possibly, therefore, the more prominent and constant output of citrulline by the dog is merely an index of a greater amount of arginine within the colonic contents, due to the higher protein intake of a carnivorous animal. Excess output of threonine has not been satisfactorily explained to date. It is probably not due to a renal leak mechanism, as it is a neutral amino-acid unlikely to be involved in a transport defect confined to group 2 amino-acids.

Treacher (1963) has shown that there is an associated intestinal transport defect as in the human disease. He found that 17 hours after giving two doses of 6 g. of lysine monohydrochloride to two cystinuric dogs there was a marked increase of urinary piperidine and a small rise of urinary pyrrolidine, whereas there was no change in the excretion of these heterocyclic amines after ingestion of lysine by two normal control dogs. This is identical to the results in human cystinuria (Milne *et al.*, 1961), and indicates defective intestinal transport of lysine in cases of the disease in both species.

Techniques are now available for the *in vitro* study of amino-acid transport in isolated intestinal loops (Agar *et al.*, 1954; Wilson and Wiseman, 1954a; Crane and Wilson, 1958; Wiseman, 1961), and these could obviously be easily applied to the cystinuric dog. Comparison of transport of group 2 amino-acids in intestinal preparations from cystinuric and normal dogs might well define the mysterious enzymatic defect of the disease and be of major importance in the elucidation of the mechanisms of amino-acid transport. The close similarity of the canine to the human disease makes it highly probable that the results would be applicable to cystinuria in man. In addition, it would be relatively simple to ascertain whether the defect of amino-acid transport was partial or complete, and to investigate the nutritional effects of the disease when protein intake was inadequate and irregularly spaced throughout the day.

IV. Renal Amino-aciduria Associated with Generalized Proximal Tubular Damage

The fundamental differences between this variety of amino-aciduria and the more specific type in cystinuria and Hartnup disease are given in Table IV. There is generalized proximal tubular damage due to a known or unknown toxic agent, and in many cases there is still more widespread renal involvement with disorders of distal tubular and of glomerular functions. Many poisons are known which will cause this type of tubular damage in the experimental animal—for example, uranium (Rothstein and Berke, 1949; Clarkson and Kench, 1956), cadmium (Clarkson and Kench, 1956; Kazantzis *et al.*, 1963),

lead (Wilson *et al.*, 1953; Marsden and Wilson, 1955; Chisolm *et al.*, 1955), oxalic acid (Emslie-Smith *et al.*, 1956), lysol (Spencer and Franglen, 1952), phosphorus (Crane *et al.*, 1959), and maleic (Harrison and Harrison, 1954), and trans-aconitic (Fellers and Vawter, 1961) acids. In view of the close connexion between renal tubular and intestinal transport systems it is not surprising that many poisons will damage both sites provided that a sufficient concentration is reached

TABLE IV.—Comparison of Pure Renal Amino-aciduria (Cystinuria and Hartnup Disease) and Renal Amino-aciduria Due to Generalized Proximal Tubular Damage (Fanconi Syndrome, Wilson's Disease, etc.)

	Pure Renal Amino-aciduria	Generalized Proximal Tubular Damage
Proteinuria	No	Yes. Unusually high proportion of globulin
Other proximal tubular abnormalities. (Renal glycosuria. Changes in C ₁ urate and C ₂ p and Tm _{PAH}) ..	,	Yes
Distal tubular abnormalities. Alterations in acidification and concentration of urine ..	"	Frequent
Variability of pattern of amino-acid loss between patients and at different times in the same patient ..	"	Yes
More than one amino-acid transport group involved ..	"	"
Associated jejunal transport defect ..	Yes	No, unless the jejunum has been damaged as well
Tendency to progression of the proximal tubular defect	No	Yes

in the cells concerned. Neomycin is a poorly absorbed antibiotic which temporarily impairs many intestinal absorptive mechanisms (Jacobson *et al.*, 1960a, 1960b; Faloon and Jacobson, 1961; Jacobson and Faloon, 1961; Hvidt and Kjeldsen, 1963) when given orally at ordinary therapeutic doses. If administered parenterally it is well known to be nephrotoxic. Emmerson and Pryse-Davies (1963) have shown that the earliest and most severe damage in the rat kidney is in the proximal renal tubule, and if the injections are continued there are permanent lesions with secondary calcification localized entirely to the proximal nephron (Fig. 3). There is impairment of amino-acid transport in both the intestine and the kidney, the defect involving all four amino-acid groups but with least effect on transport of group 2 amino-acids (Fig. 4).

Metabolic diseases may cause secondary damage of proximal tubular function by the production of abnormal metabolites or unusually high concentrations of normal metabolites. In

FIG. 3.—Transverse section of rat kidney four weeks after parenteral neomycin. There is massive calcification confined to the outer cortex. (Van Kossa stain. ×16.)

Wilson's disease the toxic agent is almost certainly copper deposited in renal tubular cells. Histologically there is necrosis of proximal tubular epithelium followed by desquamation into the tubular lumen (Wolff, 1962). The proximal tubular damage is often permanent even if copper deposits are removed by dimercaprol, penicillamine, or other effective chelating agent. Untreated infantile galactosaemia is associated with proximal tubular damage probably due to accumulation of galactose-1-phosphate within the affected cells (Cusworth *et al.*, 1955; Isselbacher, 1960). Similar tubular damage may also be caused by multiple myelomatosis possibly from reabsorption of Bence Jones protein (Sirota and Hamerman, 1954; Dragsted and

FIG. 4.—Effect of ingestion of neomycin sulphate 4 g. daily on faecal amino-acids. One-way chromatography of deproteinized extracts. 1-4=Control days. 5-8=Days on neomycin. 9-14=Days after neomycin. The antibiotic causes a great increase of the faecal amino-acid content.

Hjorth, 1956; Engle and Wallis, 1957; Wrong and Davies, 1959), and also in severe cases of the nephrotic syndrome, especially in childhood (Tegelaers and Tiddens, 1955; Stanbury and Macaulay, 1957). Vitamin deficiency may also damage proximal tubular transport as in dietary rickets (Jonxis *et al.*, 1952) and infantile scurvy (Jonxis and Huisman, 1954).

In the de Toni-Fanconi syndrome the cause of the proximal tubular damage is often uncertain. Only a minority (Dent and Harris, 1951; Ben-Ishay *et al.*, 1961) of cases of the adult type are due to a recessive hereditary gene, and even in these cases the cause of the lesion is completely unknown. Lowe's syndrome, or oculo-cerebro-renal syndrome (Lowe *et al.*, 1952), is a separate entity inherited as a sex-linked recessive disorder, and with a characteristic association of ocular, cerebral, and renal disease. Again, the actual cause of the widespread tissue damage is unknown. The type of generalized proximal tubular defect associated with cystinosis, and referred to as the Lignac-Fanconi syndrome, is of especial interest and worthy of separate description.

Lignac-Fanconi Disease with Cystinosis

Characteristically, this is a recessive hereditary disorder of infants and children in which there is a combination of cystine deposits in many tissues, including the cornea, lymph nodes, bone-marrow, spleen, Kupffer's cells of the liver, and the kidneys, and generalized proximal and sometimes distal renal tubular hypofunction (Abderhalden, 1903; Lignac, 1924a, 1924b; Fanconi, 1931, 1936; McCune *et al.*, 1943; Bickel *et al.*, 1952; Worthen and Good, 1958). Cases of cystinosis

without obvious renal damage (Hottinger, 1947; Drablos, 1951) are easily explained if the view is held that the renal lesion is not a necessary and primary manifestation of the disease, and would correspond to cases of Wilson's disease without kidney involvement or of galactosaemia in which adequate treatment has prevented renal damage. Bickel (1955) has described a case in which renal damage was delayed for some time after cystinosis was clearly present. Cases of generalized proximal tubular damage in infancy and childhood without cystinosis (Royer and Prader, 1957; Dubois *et al.*, 1958; Stanbury, 1962) should not be regarded as cases of Lignac-Fanconi disease. Most of these cases have not been associated with similar disease in relatives. There is obviously no reason why the infantile kidney should not be damaged by unknown causes any more than the adult organ. Stanbury (1962) has described a case in which hydro-nephrosis in infancy produced generalized tubular functional defects indistinguishable from those of the Lignac-Fanconi disease. In summary, this disease should not be diagnosed unless cystinosis is demonstrated either in life or at necropsy, or typical renal tubular lesions are found in a relative of a known case of cystinosis.

Experimental evidence in animals strongly suggests that excessive cystine or, more particularly, methionine feeding causes proximal tubular damage (Stave and Schlaak, 1956; Schwarz-Tiene *et al.*, 1957). Methionine is converted to cystine in the body, but owing to its greater solubility is more adequately absorbed from the gut. After about two weeks of excess intake of these sulphur-containing amino-acids there are functional lesions in the kidneys of the rat and rabbit very similar to those of cystinosis. In particular, there is a generalized amino-aciduria of the renal type in addition to the overflow amino-aciduria of the sulphur-containing amino-acids fed to the animal. The available evidence, therefore, suggests that the renal functional defects are secondary effects of the cystine deposits in the kidneys and that cystinosis is the primary lesion.

Cystine deposits in tissues are primarily intracellular in location. They never produce any secondary inflammatory reaction, whereas injections of cystine crystals into the extracellular space cause an intense polymorphonuclear cellular reaction with secondary fibrosis. Analysis of plasma in cases of cystinosis shows a normal cystine content but an excess of cystine (average normal value 0.95 mg./100 ml.; values in cystinosis 1.21–1.51 mg./100 ml.), the cystine/cysteine ratio being increased from an average value of 3 to over 4 (Brigham *et al.*, 1960). Since there is a deposition of cystine crystals intracellularly, the intracellular fluid must contain cystine in saturated solution—that is, over 200 mg./100 ml.—and therefore the normal intracellular-fluid/extracellular-fluid ratio of cystine is greatly increased. Chromatographic results (Baar and Bickel, 1952) suggest that the intracellular concentration of other amino-acids, including leucine, valine, alanine, taurine, lysine, serine, glycine, and glutamic and aspartic acid, is also abnormally high.

Attempts to find a metabolic block in the metabolism of cystine have been unsuccessful, or unconfirmed by later work. Worthen and Good (1961) concluded that cystine reductase, which tends to reduce the cystine/cysteine equilibrium ratio in body fluids, was deficient in samples of blood from two cases. Seegmiller and Howell (1961) reported a low activity of a system catalysing the transfer of hydrogen from reduced glutathione to cystine (glutathione-cysteine transhydrogenase). Patrick (1962) has carried out a detailed examination of enzyme systems concerned in cystine metabolism in tissue obtained at necropsy of three cases of cystinosis, and has failed to confirm these postulated enzymatic deficiencies. Cystine reductase was normal in liver and kidney, and glutathione-cysteine transhydrogenase was in normal amount in cystinotic liver. The glutathione reductase activity and amount of reduced glutathione in the livers were also normal. Cysteine, cystine, and cysteinesulphinic acid were completely oxidized by cystinotic liver,

and the patients in life had excreted a normal amount of inorganic sulphate in their urine. Conversion of cysteine and cystine to hydrogen sulphide by cystinotic liver, and the trans-amination of cysteine, cysteic acid, and cysteinesulphinic acid with α -oxoglutaric acid by liver enzymes were also normal. Similarly, this tissue was capable of decarboxylation of cysteine and cysteinesulphinic acids at normal rates. Degradation of cystine, therefore, appears to be normal in this disease, and the chromatographic results of Baar and Bickel (1952) suggest that cystinosis may also be a disease of amino-acid transport, but, in this case, of transport between the intracellular and extracellular phases of the affected cells. The peculiar location of cystine deposits in cells of the reticuloendothelial system, the cornea, and the kidney makes bulk biopsy of affected tissue for research purposes a difficult and often unethical procedure. The recent demonstration (Korn, 1960) that leucocytes in the peripheral blood are also involved in the metabolic abnormality and may actually contain cystine crystals may be of great importance in future investigation.

Penicillamine in Treatment of Cystinosis and Cystinuria

D-Penicillamine was first used in practical therapeutics as a chelating agent, active by mouth, for copper in Wilson's disease (Walshe, 1956), and for lead in cases of poisoning (Goldberg *et al.*, 1963). Chemically, it is $\beta\beta$ -dimethyl-D-cysteine and is oxidized by the cytochrome system in the body to the corresponding disulphide:

where Pe represents the penicillamine radical $\text{COOH.CH}(\text{NH}_2).\text{C}(\text{CH}_3)_2$. The same enzyme also oxidizes cysteine to cystine (Keilin, 1930). In the normal subject D-penicillamine is chiefly excreted as the disulphide (Pe.S.S.Pe), but there are also small quantities of free penicillamine (Pe.SH), and the mixed disulphide of penicillamine and cysteine (Pe.S.S.Cy) produced by disulphide exchange.

where Cy is the cysteine radical $\text{COOH.CH}(\text{NH}_2).\text{CH}_2$. The reaction is slow and reversible, and almost certainly continues in secreted urine within the renal pelvis and urinary bladder. In the cystinuric, again, very little free penicillamine is excreted, but there is usually more mixed disulphide in the urine than penicillamine disulphide. Formation of the mixed disulphide is accompanied by a corresponding reduction in the amount of urinary cystine (Fig. 5). Unfortunately, no data are as yet available of the partition of excreted penicillamine and its derivatives in cystinosis, as comparison with that in cystinuria would give some information regarding the amount of disulphide exchange which occurs before the urine reaches the renal pelvis.

The rationale for the use of penicillamine in cystinuria and probably also in cystinosis depends on the peculiar solubility relationships of cystine, cysteine, penicillamine, and their derivatives. The thiol compounds penicillamine and cysteine are extremely soluble in water, while the symmetrical disulphides cystine, homocystine, and penicillamine disulphide are the least soluble. Of the three, cystine is the least soluble; homocystine is about twice as soluble as cystine, and, although precise information is not available, penicillamine disulphide crystallizes out from both watery and urinary solutions much less readily than cystine. There is no record of crystalluria even after extremely high dosage of penicillamine in the experimental animal. The mixed disulphides are intermediate in solubility. That of penicillamine and cysteine is about 20 times as soluble as cystine (Emmerson and Milne, 1963), while the disulphide of cysteine and homocystine excreted by cystinurics (Frimpter, 1961) is obviously more soluble than is cystine. The quantity of the disulphide excreted by cystinurics averages about 10%

of the amount of cystine, but the amount found in cystine calculi is less than 1% of the cystine content. The greater solubility of the mixed disulphides depends on the asymmetry of the molecule. The symmetrical molecule of cystine packs more easily into solid crystals, and therefore is less likely to enter the aqueous phase. Penicillamine treatment in cystinuria is beneficial because most of the insoluble cystine excreted is converted to the more soluble mixed disulphide. It is, however, more likely to prevent the formation of new calculi or the further growth of established calculi than to dissolve large cystine stones.

FIG. 5.—Effect of penicillamine ingestion on urinary amino-acids in a case of cystinuria. There is a marked decrease of cystine output with a corresponding rise of the mixed disulphide of penicillamine and cysteine. Left—before penicillamine. Right—after penicillamine.

Conversion of urinary cystine to the more soluble penicillamine-cysteine disulphide was first recorded by Crawhall *et al.* (1963). Evered (1963) has suggested that the drug might possibly reduce urinary cystine by competitive inhibition of cystine absorption from the gut. This view is unlikely, as urinary cystine in cystinuric patients is chiefly derived from methionine (Lewis *et al.*, 1936). Any substance inhibiting methionine absorption would inevitably cause serious nutritional disturbances, as this is an essential amino-acid. This has never been recorded, even after prolonged penicillamine administration in Wilson's disease. Further cogent arguments against this interpretation have been given by Scowen and Crawhall (1963) and by Hartley and Walshe (1963).

Carefully controlled long-term studies are necessary before the use of penicillamine in cystinuria can be regarded as established therapy. The drug is expensive, and causes toxic effects consisting in fever, lymphadenopathy, dermatitis, granulocytopenia, thrombocytopenia, proteinuria, and a reversible nephrotic syndrome in some patients (Sunderman *et al.*, 1963; Yonis and Karp, 1963). Penicillamine therapy in cystinuria causes an enhanced excretion of the sum of urinary inorganic plus ethereal sulphate as well as of neutral sulphur. In two patients the former fraction increased about 1.3 times as much as the neutral sulphur (Milne and de Rousse, 1963). This indicates that a considerable amount of ingested penicillamine is oxidized to inorganic sulphate and is therefore useless therapeutically,

as only the fraction excreted as neutral sulphur can participate in disulphide exchange. We have found that approximately 2 g. of D-penicillamine hydrochloride a day is necessary to produce an adequate fall in cystine output. The two results agree satisfactorily, as the average cystinuric excretes about 1 g. of cystine a day, and approximately equal amounts of cysteine and penicillamine combine to form the soluble mixed disulphide. Possibly its place will chiefly lie in the prevention of redevelopment of cystine calculi after nephrolithotomy in severe cases of cystinuria which have not responded to the high-fluid-intake regime of Dent and Senior (1955). On present evidence it should not be regarded as routine therapy.

The rationale of the use of penicillamine in Lignac-Fanconi disease with cystinosis is more controversial. Clayton and Patrick (1962), who have described its use in two cases of Lignac-Fanconi disease, consider that there is a deficiency of sulphhydryl enzymes in this disease. They demonstrated an almost total lack of succinic dehydrogenase activity in fresh necropsy specimens of cystinotic liver, but this was reactivated by preincubation with cysteine. In addition, it was found that blood pyruvate concentrations were abnormally high both fasting and after glucose ingestion. They considered that thiol compounds (both dimercaprol and D-penicillamine) were beneficial in that they reactivated or maintained thiol-dependent enzyme systems. The argument would obviously have been stronger if it had been demonstrated that these non-physiological thiols were as effective as cysteine in the reactivation of succinic dehydrogenase *in vitro*.

There was, however, little doubt that therapy with both thiols was beneficial, as the blood pyruvate was restored to normal within four days and this was followed by at least temporary clinical improvement and gain in weight. An alternative explanation would be that the thiols are not directly active in the reactivation of sulphhydryl enzymes, but that their action is indirect by removing cystine crystals from tissue by formation of soluble mixed disulphides. Administration of excess cystine and methionine in the experimental animal has been shown to reduce kidney alkaline phosphatase as well as producing severe proximal tubular functional defects (Stave and Willenbockel, 1956). It is not, however, known whether sulphhydryl enzymes are also affected. A similar doubt occurs in the use of penicillamine in Wilson's disease, another metabolic disease associated with an abnormally high blood pyruvate content (Walshe, 1961). In that article Walshe asks the important but unanswered question, "Do chelating agents such as B.A.L. and penicillamine act by removing copper from the tissues or by supplying -SH groups to replace those blocked or oxidized by the metal?" The question in relation to penicillamine therapy of cystinosis is of more than academic importance. If the main action lies in the solution of cystine deposits, a constant level of penicillamine must be maintained in body fluids, necessitating a dosage spaced at intervals of not more than six to eight hours. If the thiol is directly reactivating sulphhydryl enzyme systems, then more infrequent dosage would be adequate. Clayton and Patrick (1961) were of the latter view, as after an initial regime of 100 mg. of penicillamine t.d.s. for three days further maintenance doses were given only at intervals of 6 to 17 days.

Clayton and Patrick's therapeutic trial of thiols in cystinosis, a fatal disease of childhood, is a brilliant but preliminary report. One unavoidable disadvantage was that therapy was started late in the course of the disease, when the children had been known to suffer from cystinosis for over a year. Reports of therapy given earlier in the course of the disease, and with more prolonged and intensive dosage, will be awaited with great interest. Present evidence suggests that this is by far the most useful treatment in a serious and otherwise fatal hereditary disease. The small cystine crystals in cystinosis are obviously more likely to go into solution by formation of soluble mixed disulphides than are the large, hard masses of cystine in cases of cystinuric calculous disease.

Conclusion

Diseases of amino-acid transport, although rare conditions, are of disproportionate interest because of the clues they give to normal physiology and metabolic processes. Clinical management of these conditions is not yet standardized, and every case is a challenge to the critical powers of observation of the clinicians and biochemists in charge of the case. There are now promising signs of substantial improvements in therapy in the next few years. Especially promising avenues of future research will be on investigation of disordered amino-acid transport in the intestinal epithelium of the cystinuric dog, and further explanation of the disordered physiology and the therapy of human cystinosis.

Thanks are expressed to my colleagues, and especially to Dr. A. M. Asatoor, for assistance in the writing of this paper, and to the photographic department, Westminster Hospital Medical School, for the illustrations.

REFERENCES

- Aberdalden, F. (1903). *Hoppe-Seylers Z. physiol. Chem.*, **38**, 557.
- Agar, W. T., Hird, F. J. R., and Sidhu, G. S. (1954). *Biochem. biophys. Acta (Amst.)*, **14**, 80.
- Albers, F. H., and Wadman, S. K. (1961). *Maandschr. Kindergeneesk.*, **29**, 102.
- Allan, J. D., Cusworth, D. C., Dent, C. E., and Wilson, V. K. (1958). *Lancet*, **1**, 182.
- Armstrong, M. D., Yates, K., Kakimoto, Y., Taniguchi, K., and Kappe, T. (1963). *J. biol. Chem.*, **238**, 1447.
- Arrow, V. K., and Westall, R. G. (1958). *J. Physiol. (Lond.)*, **142**, 141.
- Asatoor, A. M., Craske, J., London, D. R., and Milne, M. D. (1963). *Lancet*, **1**, 126.
- Lacey, B. W., London, D. R., and Milne, M. D. (1962). *Clin. Sci.*, **23**, 285.
- and Milne, M. D. (1963). Unpublished observations.
- Auerbach, V. H., di George, A. M., Baldrige, R. C., Tourtellotte, C. D., and Brigham, M. P. (1962). *J. Pediatr.*, **60**, 487.
- Baar, M. S., and Bickel, H. (1952). *Acta paediat. (Uppsala)*, (Suppl. 90), **42**, 171.
- Baron, D. N., Dent, C. E., Harris, H., Hart, E. W., and Jepson, J. B. (1956). *Lancet*, **2**, 421.
- Ben-Ishay, D., Dreyfuss, F., and Ullmann, T. D. (1961). *Amer. J. Med.*, **31**, 793.
- Bickel, H. (1955). *Helv. paediat. Acta*, **10**, 259.
- Smallwood, W. C., Smellie, J. M., and Hickmans, E. M. (1952). *Acta paediat. (Uppsala)*, (Suppl. 90), **42**, 27.
- Booth, C. C. (1963). *Scientific Basis of Medicine: Annual Reviews*, p. 171.
- and Kanaghinis, T. (1963). *J. Physiol. (Lond.)*, **167**, 18P.
- Brigham, M. P., Stein, W. H., and Moore, S. (1960). *J. clin. Invest.*, **39**, 1633.
- Brown, J. L., Samiy, A. H., and Pitts, R. F. (1961). *Amer. J. Physiol.*, **200**, 370.
- Childs, B., Nyhan, W. L., Borden, M., Bard, L., and Cooke, R. E. (1961). *Pediatrics*, **27**, 522.
- Chisolm, J. J., Harrison, H. C., Eberlein, W. R., and Harrison, H. E. (1955). *Amer. J. Dis. Child.*, **89**, 159.
- Clarkson, T. W., and Kench, J. E. (1956). *Biochem. J.*, **62**, 361.
- Clayton, B. E., and Patrick, A. D. (1961). *Lancet*, **2**, 909.
- Collins, J. E., Levi, A. J., and Milne, M. D. (1963). *Brit. med. J.*, **1**, 590.
- Conden, R., Gordon, A. H., and Martin, A. J. P. (1944). *Biochem. J.*, **38**, 224.
- Crane, C. W., Ebeling, P., and Bentley, A. B. (1959). *Aust. Ann. Med.*, **8**, 66.
- Crane, R. K., and Wilson, T. H. (1958). *J. appl. Physiol.*, **12**, 145.
- Crawhall, J. C., Scowen, E. F., and Watts, R. W. E. (1963). *Brit. med. J.*, **1**, 588.
- Crumpler, H. R., Dent, C. E., Harris, H., and Westall, R. G. (1951). *Nature (Lond.)*, **167**, 307.
- Cusworth, D. C., and Dent, C. E. (1960). *Biochem. J.*, **74**, 550.
- and Flynn, F. V. (1955). *Arch. Dis. Childh.*, **30**, 150.
- Dancis, J., and Levitz, M. (1960). *The Metabolic Basis of Inherited Disease*, edited by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- Davies, H. E., and Robinson, M. J. (1963). *Arch. Dis. Childh.*, **38**, 80.
- de Koninck, L. (1835). *Ann. Chem. Pharm.*, **15**, 75.
- Dent, C. E. (1950). *Schweiz. med. Wschr.*, **80**, 752.
- and Harris, H. (1951). *Ann. Eugen. (Lond.)*, **16**, 60.
- Heathcote, J. G., and Joron, G. E. (1954a). *J. clin. Invest.*, **33**, 1210.
- and Rose, G. A. (1951). *Quart. J. Med.*, **20**, 205.
- and Senior, B. (1955). *Brit. J. Urol.*, **27**, 317.
- and Walshe, J. M. (1954b). *J. clin. Invest.*, **33**, 1216.
- Doolan, P. D., Harper, H. A., Hutchin, M. E., and Alpen, E. L. (1957). *Amer. J. Med.*, **23**, 416.
- Drablos, A. (1951). *Acta paediat. (Uppsala)*, **40**, 438.
- Dragsted, P. J., and Hiorth, N. (1956). *Dan. med. Bull.*, **3**, 177.
- Dubois, R., Vis, H., and Loeb, H. (1958). *Ann. paediat. (Basel)*, **191**, 1.
- Efron, M. L., Bixby, E. M., Palattao, L. G., and Pryles, C. V. (1962). *New Engl. J. Med.*, **267**, 1193.
- Elsden, S. R., Gibson, Q. H., and Wiseman, G. (1950). *J. Physiol. (Lond.)*, **111**, 56P.
- Emmerson, B. T., and Milne, M. D. (1963). Unpublished observations.
- and Pryse-Davies, J. (1963). Unpublished observations.
- Emslie-Smith, D., Johnstone, J. H., Thomson, M. B., and Lowe, K. G. (1956). *Clin. Sci.*, **15**, 171.
- Engle, R. L., and Wallis, L. A. (1957). *Amer. J. Med.*, **22**, 5.
- Evered, D. F. (1963). *Brit. med. J.*, **2**, 120.
- Faloon, W. W., and Jacobson, E. D. (1961). *Gastroenterology*, **40**, 447.
- Fanconi, G. (1931). *Jb. Kinderheilk.*, **133**, 257.
- (1936). *Dtsch. med. Wschr.*, **62**, 1169.
- Fellers, F. X., and Vawter, G. F. (1961). *Fed. Proc.*, **20**, 417.
- Fölling, A. (1934). *Hoppe-Seylers Z. physiol. Chem.*, **227**, 169.
- Fowler, D. I., Harris, H., and Warren, F. L. (1952). *Lancet*, **2**, 544.
- Frimpter, G. W. (1961). *J. biol. chem.*, **236**, 51PC.
- Haymovitz, A., and Horwith, M. (1963). *New Engl. J. Med.*, **268**, 333.
- Ghadimi, H., Partington, M. W., and Hunter, A. (1961). *Ibid.*, **265**, 221.
- Gibson, Q. H., and Wiseman, G. (1951). *Biochem. J.*, **48**, 426.
- Goldberg, A., Smith, J. A., and Lochhead, A. C. (1963). *Brit. med. J.*, **1**, 1270.
- Govaerts, P., and Lambert, P. P. (1949). *Acta clin. belg.*, **4**, 341.
- Hagihira, H., Lin, E. C. C., Samiy, A. H., and Wilson, T. H. (1961). *Biochem. biophys. Res. Commun.*, **4**, 478.
- Harris, H. (1957). *Biochemical Disorders in Human Disease*, edited by R. H. S. Thompson and E. J. King. Churchill, London.
- Penrose, L. S., and Thomas, D. H. H. (1959). *Ann. hum. Genet.*, **23**, 442.
- Harrison, H. E., and Harrison, H. C. (1954). *Science*, **120**, 606.
- Hartley, B. S., and Walshe, J. M. (1963). *Lancet*, **2**, 434.
- Henderson, W. (1958). *Arch. Dis. Childh.*, **33**, 114.
- Hersov, L. A., and Rodnight, R. (1960). *J. Neurol. Neurosurg. Psychiat.*, **23**, 40.
- Hickish, G. W. (1955). *Arch. Dis. Childh.*, **30**, 195.
- Hoof, C., de Lacy, P., Timmermans, J., and Snoeck, J. (1962). *Acta paediat. belg.*, **16**, 281.
- Hottinger, A. (1947). *Ann. paediat. (Basel)*, **169**, 272.
- Huisman, T. H. J. (1957). *Arch. franc. Pédiat.*, **14**, 1.
- Hvidt, S., and Kjeldsen, K. (1963). *Acta med. scand.*, **173**, 699.
- Isselbacher, K. J. (1960). *The Metabolic Basis of Inherited Disease*, edited by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- Jacobson, E. D., Chodos, R. B., and Faloon, W. W. (1960a). *Amer. J. Med.*, **28**, 524.
- and Faloon, W. W. (1961). *J. Amer. med. Ass.*, **175**, 187.
- Prior, J. T., and Faloon, W. W. (1960b). *J. Lab. clin. Med.*, **56**, 245.
- Jagenburg, O. R. (1959). *Scand. J. Lab. clin. Invest.*, **11**, Suppl. 43.
- Jakoby, W. B., and Fredericks, J. (1959). *J. biol. Chem.*, **234**, 2145.
- Jolliffe, N., Shannon, J. A., and Smith, H. W. (1932). *Amer. J. Physiol.*, **100**, 301.
- Jonxis, J. H. P. (1957). *Ned. T. Geneesk.*, **101**, 569.
- and Huisman, T. H. J. (1954). *Pediatrics*, **14**, 238.
- Smith, P. A., and Huisman, T. H. J. (1952). *Lancet*, **2**, 1015.
- Kamin, H., and Handler, P. (1951). *Amer. J. Physiol.*, **164**, 654.
- Kazantzis, G., Flynn, F. V., Spowage, J. S., and Trott, D. G. (1963). *Quart. J. Med.*, **32**, 165.
- Keilin, D. (1930). *Proc. roy. Soc. B*, **106**, 418.
- Knox, W. E. (1960). *The Metabolic Basis of Inherited Disease*, edited by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- Kolthoff, I. M., Stricks, W., and Kapoor, R. C. (1955). *J. Amer. chem. Soc.*, **77**, 4733.
- Korn, D. (1960). *New Engl. J. Med.*, **262**, 545.
- Kuroda, Y., and Gimbel, N. S. (1954). *J. appl. Physiol.*, **7**, 148.
- La Du, B. N., Howell, R. R., Jacoby, G. A., Seegmiller, J. E., Sober, E. K., Zannoni, V. G., Canby, J. P., and Ziegler, L. K. (1963). *Pediatrics*, **32**, 216.
- Levin, B., Mackay, H. M. M., and Oberholzer, V. G. (1961). *Arch. Dis. Childh.*, **36**, 622.
- Lewis, H. B., Brown, B. H., and White, F. R. (1936). *J. biol. Chem.*, **114**, 171.
- Lignac, G. O. E. (1924a). *Arch. klin. Med.*, **145**, 139.
- (1924b). *Ned. T. Geneesk.*, **68**, 2987.
- Lowe, C. U., Terrey, M., and MacLachlan, E. A. (1952). *Amer. J. Dis. Child.*, **83**, 164.
- McCune, D. J., Mason, H. H., and Clarke, H. T. (1943). *Ibid.*, **65**, 81.
- McMurray, W. C., and Mohyuddin, F. (1962). *Lancet*, **2**, 352.
- Rossiter, R. J., Rathbun, J. C., Valentine, G. H., Koegler, S. J., and Zarfas, D. E. (1962). *Ibid.*, **1**, 138.
- Rathbun, J. C., Mohyuddin, F., and Koegler, S. J. (1963). *Pediatrics*, **32**, 347.

- Marble, A. (1932). *Amer. J. med. Sci.*, **183**, 811.
- Marsden, H. B., and Wilson, V. K. (1955). *Brit. med. J.*, **1**, 324.
- Mayrs, E. B. (1923). *J. Physiol. (Lond.)*, **57**, 461.
- Menkes, J. H., Hurst, P. L., and Craig, J. M. (1954). *Pediatrics*, **14**, 462.
- Milne, M. D., Asatoor, A. M., Edwards, K. D. G., and Loughridge, L. W. (1961). *Gut*, **2**, 323.
- Crawford, M. A., Girão, C. B., and Loughridge, L. (1959). *Biochem. J.*, **72**, 30P.
- (1960). *Quart. J. Med.*, **29**, 407.
- and de Rousse, A. R. (1963). Unpublished observations.
- Patrick, A. D. (1962). *Biochem. J.*, **83**, 248.
- Pihl, A., Eldjarn, L., and Nakkem, K. F. (1958). *Act. chem. scand.*, **12**, 1357.
- Poulsen, L. T. (1930). *J. Physiol. (Lond.)*, **69**, 411.
- Reubi, F. C. (1951). *Helv. med. Acta*, **18**, 69.
- Robson, E. B., and Rose, G. A. (1957). *Clin. Sci.*, **16**, 75.
- Rothstein, A., and Berke, H. (1949). *J. Pharmacol. exp. Ther.*, **96**, 179.
- Royer, P., and Prader, A. (1957). *16e Congrès des Pédiatres de Langue Française*, June 1, 263. Expansion Scientifique Française, Paris.
- Ruszkowski, M., Baertl, J. M., and Gabuzda, G. J. (1960). *Pol. Tyg. lek.*, **15**, 1679.
- Ryle, A. P., and Sanger, F. (1955). *Biochem. J.*, **60**, 535.
- Schafer, I. A., Scriver, C. R., and Efron, M. L. (1962). *New Engl. J. Med.*, **267**, 51.
- Schwarz-Tiene, E., Careddu, P., and Labassa, N. (1957). *Minerva pediat.*, **9**, 231.
- Scowen, E. F., and Crawhall, J. C. (1963). *Brit. med. J.*, **2**, 250.
- Scriver, C. R., Schafer, I. A., and Efron, M. L. (1961). *Nature (Lond.)*, **192**, 672.
- Seegmiller, J. E., and Howell, R. R. (1961). *Clin. Res.*, **9**, 189.
- Shaw, K. N. F., Redlich, D., Wright, S. W., and Jepson, J. B. (1960). *Fed. Proc.*, **19**, 194.
- Sherlock, S. (1958). *Amer. J. Med.*, **24**, 805.
- Sirota, J. H., and Hamerman, D. J. (1954). *Ibid.*, **16**, 138.
- Spencer, A. G., and Franglen, G. T. (1952). *Lancet*, **1**, 190.
- Stanbury, S. W. (1958). *Advanc. intern. Med.*, **9**, 231.
- (1962). *Renal Disease*, edited by D. A. K. Black. Blackwell, Oxford.
- and Macaulay, D. (1957). *Quart. J. Med.*, **26**, 7.
- Stave, U., and Schlaak, E. (1956). *Z. Kinderheilk.*, **78**, 261.
- and Willenbockel, U. (1956). *Ibid.*, **78**, 563.
- Sunderman, F. W., jun., White, J. C., and Sunderman, F. W. (1963). *Amer. J. Med.*, **34**, 875.
- Tegelaers, W. H. H., and Tiddens, H. W. (1955). *Helv. paediat. Acta*, **10**, 269.
- Treacher, R. J. (1962). *Vet. Rec.*, **74**, 503.
- (1963). Personal communication.
- von Mering, I. (1888). *Z. klin. Med.*, **14**, 405.
- Walshe, J. M. (1953). *Quart. J. Med.*, **22**, 483.
- (1956). *Amer. J. Med.*, **21**, 487.
- (1961). *Clin. Sci.*, **20**, 197.
- Webber, W. A. (1963). *Canad. J. Biochem.*, **41**, 131.
- Brown, J. L., and Pitts, R. F. (1961). *Amer. J. Physiol.*, **200**, 380.
- Weyers, H., and Bickel, H. (1958). *Klin. Wschr.*, **36**, 893.
- Wilson, T. H. (1962). *Intestinal Absorption*. Saunders, Philadelphia.
- and Wiseman, G. (1954a). *J. Physiol. (Lond.)*, **123**, 116.
- Wilson, V. K., Thomson, M. L., and Dent, C. E. (1953). *Lancet*, **2**, 66.
- Wiseman, G. (1953). *J. Physiol. (Lond.)*, **120**, 63.
- (1955). *Ibid.*, **127**, 414.
- (1961). *Methods in Medical Research*, edited by J. H. Quastel, **9**, 287. Year Book Medical Publishers, Chicago.
- Wolff, S. M. (1962). *Clin. Res.*, **10**, 45.
- Wollaston, W. H. (1810). *Philos. Trans.*, **100**, 223.
- Worthen, H. G., and Good, R. A. (1958). *Amer. J. Dis. Child.*, **95**, 653.
- (1961). *Ibid.*, **102**, 494.
- Wrong, O., and Davies, H. E. F. (1959). *Quart. J. Med.*, **28**, 259.
- Yonis, I. Z., and Karp, M. (1963). *Lancet*, **2**, 689.

Cancer in an African Community, 1897—1956*

An Analysis of the Records of Mengo Hospital, Kampala, Uganda : Part 2

J. N. P. DAVIES,† § M.D. ; SALLY ELMES† ; M. S. R. HUTT,‡ M.D., M.R.C.P. ; L. A. R. MTIMAVALLYE‡
R. OWOR,‡ L.M.S. ; LORNA SHAPER‡

Brit. med. J., 1964, **1**, 336—341

Cancer at Special Sites

Oro-Facial Region

Lip cancer probably represents the only genuine change in the cancer pattern disclosed by these records. It seems to have declined steadily over the six decades until to-day it is an uncommon tumour. In the Mengo series there were 11 females and 5 males ; in the Registry series 4 females and 4 males. All 16 Mengo patients were elderly ; in 15 the lower lip or angle of the mouth was affected, and many were recorded as confirmed smokers using primitive pipes with thin wood or stem pipes. The decline in this tumour may be due to a change in smoking methods rather than in the habit.

The infrequency of carcinomas of the tongue, mouth, tonsils, pharynx, and larynx is notable and corresponds with recent experience ; this infrequency would seem to be of long standing in Uganda. The low figures of the Mengo series might be due to diagnosis as out-patients without admission of inoperable cases, but this does not seem likely, as even the few admitted were advanced cases. The incidence of syphilis in Uganda in

these decades has been high, but there does not seem to be any reflection of this in the frequency of tongue, mouth, and cheek or laryngeal cancers.

The infrequency of laryngeal cancers and of lung cancers may be connected with the infrequency of oral, tonsillar, and pharyngeal cancers. Tumours of these sites were diagnosed in Indians and Europeans.

Experience with salivary tumours at Mengo again concords with recent findings, notably in the distribution of mixed salivary tumours and the frequency with which they arise in sites other than the parotid gland. Of the Mengo series the parotid was involved in 16 cases, the submaxillary gland in 6, and the lip and palate in one case each. There were also two tumours of the lacrimal gland, one of which behaved in a malignant fashion in 1906. Lacrimal-gland tumours are to-day relatively much more common in Africans than in Europeans.

Cancer of Sex Organs

Mengo Hospital experience with cancers of the sex organs is in close conformity with recent findings, as is shown in Table VI, in which neoplasms are given as percentages of all tumours in the appropriate sex. There appears to have been virtually no change over the years as regards the frequency

* The beginning of this paper appeared last week.

† Department of Morbid Anatomy, Postgraduate Medical School, London.

‡ Makerere College Medical School, Kampala, Uganda.

§ Present address: Albany Medical College of Union University, Albany, New York.