Genetics and the Lipidoses[1]

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Abstract

Formal genetic analyses of family data in cases of errors of lipid metabolism are able to distinguish monogenic vs. multigenic and nongenetic disorders. These data, together with population data, provide criteria for the homogeneity of cases which can be useful in the interpretation of biochemical findings. The peculiarly elevated incidence of three main genotypes of sphingolipidoses among Ashkenasic Jews has no reasonable explanation except for heterozygote selection. This inference has strong implications for the biochemist in that it suggests a) the existence of some biochemical abnormality is common among the heterozygotes for these conditions and b) the utility of such a heterozygote abnormality in the defense of the body against some adverse environmental stress, such as some specific infectious disease.

The detection of heterozygous carriers by biochemical means could be important not only for this reason but also as a means for anticipating marriages which may produce a lethal lipidosis. Finally, as long as preventive measures are either imperfect or not practiced, major attention is directed toward therapy, which in turn requires not only more knowledge of the basic defect but awareness of the fact that such knowledge may not be helpful without the development of more advanced devices than those employed in, for example, phenylketonuria or galactosemia. The deep-seated nature of the presumed lesions in the lipidoses seems to require a different type of corrective measure, such as gene replacement or transformation.

Introduction

ALTHOUGH THE UNRAVELING of the mysteries of the lipidoses is primarily a task for the biochemist, something hopefully heuristic may be offered by the geneticist. From the genetic point of view the lipidoses can be examined with respect to family, population, and biochemical data. Each of these has implications for further biochemical studies.

The criterion for classification of a disorder as a lipidosis is the abnormal intracellular accumulation of lipid; the identification is essentially chemical. The establishment of such a disorder as a distinct entity depends upon a synthesis of clinical, pathological, and chemical findings. A further separation into hereditary forms is essential because a single basic biochemical defect is implied.

One of the important lessons of biochemical genetics is that a defect in a single gene is associated with a defect in a single enzyme or, more broadly, a single protein, which is a lesson of considerable importance for therapy. Hand-Schüller-Christian disease, which was formerly classified with the lipidoses because cholesterol-containing foam cells are found in many tissues, is a nonhereditary condition and cannot be expected to be associated with a single basic enzyme defect. It follows then that the demonstration of the hereditary nature of a disorder should be a matter of practical significance to the biochemist.

The incidence of any hereditary disease in a population is strongly affected by the action of natural selection upon the gene which causes the disease. Man has the capacity to perform artificial selection when an individual harboring a disease-producing gene is identifiable. The biochemist plays a major role in providing the information necessary to render technically possible the eugenic modification of disease incidence. In the process he may also discover clues to the mechanisms of natural selection.

The rational path to therapy of these serious disorders must lead through the biochemistry laboratory. However the identification of the basic defect may per se be unhelpful to the physician until new therapeutic approaches are available. A consideration of the contributions of the biochemical geneticist suggests some possible untried approaches.

Lipidoses as Genetic Entities

On the basis of these criteria, five main categories (Table I) of sphingolipidoses have been recognized by many investigators (1). Considerable heterogeneity of cases is encountered under these headings. The least clinical variability is shown within the category of Fabry's disease. The principal clinical features of this disorder are pains in the extremities, vascular skin lesions on the lower trunk and thighs, and urinary abnormalities. Onset is usually in childhood, and death most commonly occurs in the fifth decade from cardiovascular-renal disease. The primary pathological stigma is a foamy cytoplasm of vascular smooth muscle and endothelial cells, and of cells in the kidney and nervous system. Chemical analysis has revealed an accumulation of ceramide di- and tri-hexosides (2); the latter (ceramide-glucose-galactose-galactose) probably is the principal lipid (1). No data on an enzymatic block are available, but a strong presumption can be made for a deficiency in an enzyme in the degradative pathway of a visceral globoside, ceramide-glucose-galactose-galactose-acetylgalactosamine.

All severely affected cases are male, and family analyses disclose that Fabry's disease, like hemophilia, is inherited as an X-linked recessive disorder (3). The finding of single-factor inheritance encourages one to suspect a single basic metabolic defect, in accord with the one gene-one enzyme hypothesis of biochemical genetics. All males, having but one X-chromosome, carry only an abnormal or only a normal gene with respect to Fabry's disease. Females, having

TABLE I

The Sphingolipidoses

Clinical entity	Principal storage lipid
Gaucher's disease	cerebroside
Niemann-Pick disease	sphingomyelin
Tay-Sachs disease	ganglioside
Metachromatic leucodystrophy	sulfatide
Fabry's disease	glycolipid

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two X-chromosomes, may have two normal genes (homozygous normal), two abnormal genes (homozygous abnormal, affected like males but very rare), or one normal and one abnormal gene (heterozygous carriers). In contrast to the other lipidoses, carrier females, heterozygous for Fabry's disease, are partially affected. This observation, noted also in X-linked muscular dystrophy, is undoubtedly attributable to the Lyon effect (4). The Lyon phenomenon is one in which the somatic genes of only one X-chromosome are active in individual somatic cells; the other X-chromosome is inactivated with respect to these genes and physically condensed so that it stains more deeply in early mitosis (Figure 1-A) and maintains this state in nondividing cells, forming the sex chromatin body (Figure 1-B). If a particular gene on one of the X-chromosomes is defective, a tissue may show 50% activity for that gene's function, not because each cell is half-active but because in 50% of the cells the X with the normal gene is active and in 50% the X with the defective gene is active. Such a tissue is a mosaic with respect to the gene in question. Variation in the relative numbers of active normal and active abnormal cells can account for the variation in phenotypic expression in females. If some tissues were not mosaic with respect to the storage of lipid, the female cases would further inform the investigator that the disorder is not a generalized cellular abnormality but rather one involving primarily some tissues (the mosaic ones) and secondarily other tissues (the uniformly affected ones).

For Tay-Sachs disease or infantile amaurotic family idiocy, clinical and chemical heterogeneity is obvious. In classical Tay-Sachs disease the cerebral deterioration and characteristic changes found in the ocular fundus are unaccompanied by obvious visceral manifestations, and death commonly supervenes in the third or fourth year of life. The stored lipid, which causes great ballooning of neurones, is a monosialoganglioside, ceramide-glucose-galactose-acetylgalactosamine-neuraminic acid, which normally comprises a minor fraction of brain gangliosides (5). In the rare generalized form of the disease, known variously as Tay-Sachs disease with visceral involvement (6), familial neurovisceral lipidosis (7), late infantile systemic lipidosis (8), and generalized gangliosidosis (9), death usually occurs in the first or second year of life. The lipid stored in the brain is the major cerebral monosialoganglioside, ceramide-glucose-

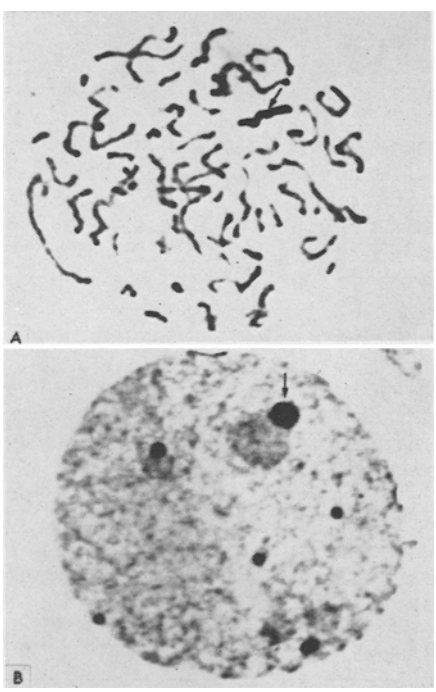

FIG. 1. Somatic interphase and prophase nuclei of a female cat (*Felis domestica*) from a direct squash of fetal liver, stained by Feulgen's procedure. A, in a prophase nucleus the sex chromatin of preceding interphase is visualized as a single chromosome condensed along its entire length (arrow). B, an interphase nucleus with the sex chromatin (arrow) attached to a nucleolus.

acetylneuraminic acid
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galactose-acetylgalactosamine-galactose (8,9,10). In passing, it should be noted that the so-called congenital, juvenile, and adult forms of amaurotic family idiocy have never been clearly shown to involve an accumulation of lipid although storage of the main disialoganglioside of normal brain has been reported in the late infantile form (11).

Genetic heterogeneity is also demonstrated between the classical and generalized forms of Tay-Sachs disease. A familial incidence compatible with recessive inheritance is displayed by both, but no one sibship contains a case of each disease. It may safely be concluded therefore that the two conditions are caused by two different mutations. Further evidence in support of this conclusion is supplied by the finding that most (more than 80%) of the cases of classical Tay-Sachs disease are children of East European Jewish (Ashkenasic) extraction whereas a majority of the generalized cases are not Jewish. One of the two mutations occurs with exaggerated frequency in one subgroup of the European and American Caucasion population, again indicating that the mutations cannot be identical with variable phenotypic effects.

No families containing, by accident, both mutations have been described so it cannot be decided on genetic grounds alone whether the two are changes in the same gene or in different genes. The chemical data favor the latter alternative in view of the one gene-one enzyme hypothesis since it is unlikely that one defect in an enzyme could result in the accumulation of one ganglioside while another defect in the same enzyme could lead to the storage of a different ganglioside. Discovery of different enzymatic defects for the two conditions may therefore be anticipated.

Two clinical forms of Gaucher's disease are also known. A so-called chronic cerebral form of Gaucher's disease is rare, and not enough biochemical information is available to establish its affinity to the two classical forms. Visceral involvement, notably in the spleen, liver, and bone marrow, is typical in both, but in one form cerebral degeneration and death in infancy occur whereas in the other the brain escapes and survival into adulthood is usual. The lipid storage cells of the two are identical, cerebral storage is not observed in either, and, in fact, the cause of the cerebral disorder is not known. In both forms the stored lipid is a cerebroside, ceramide-glucose, to be differentiated from cerebral cerebroside, ceramide-galactose. Glucocerebroside is not normally found in tissues. It may represent an intermediate in the pathway of degradation of a tissue lipid, such as the globoside referred to in the discussion of Fabry's disease, to which it is probably metabolically related (1).

Definite evidence of genetic heterogeneity is also

demonstrated by Gaucher's disease. The chronic noncerebral and infantile cerebral forms, both inherited recessively, are never found in the same sibship, indicating that different mutations are involved. Here again, for one form (noncerebral) many of the cases are Ashkenasic Jews whereas for the other (cerebral) there is no predilection for this group. It is not clear whether the two mutations for the main types occur at the same genetic locus or at different loci. Since the biochemical finding is the same for each, the mutations may be at the same gene (alleles), differing simply in the completeness of the defects they produce or in the times of phenotypic expression. In the experience of the author, for example, typical Gaucher cells were not present in a five-month-old infant but were at eight months whereas they are present at birth in the infantile cases (12). The recent report (13) of a considerably decreased activity of a glucocerebroside-splitting enzyme in the spleen of patients with the chronic noncerebral form is interesting in this connection. If it can be demonstrated that, in the infantile form, this enzyme has no activity, a case can be made for allelic mutations; one destroys all enzymatic activity, causes manifestations earlier in life, and interferes with brain development whereas the other reduces activity, produces delayed visceral effects, but has no cerebral effects.

Metachromatic leucodystrophy is a clinico-pathological entity which is homogeneous in the sense that it is a neurological disorder with delayed onset of motor abnormalities and blindness; a metachromatic sulfatide, cerebroside sulfate (ceramide-galactose-sulfate) accumulates in the brain. However the time of onset is variable; the ages of onset cluster around different modes in late infancy, childhood, and adulthood; the first is by far the most common form. Family data suggest autosomal recessive inheritance for all forms, and when more than one child in a sibship is affected, the ages of onset are similar. No ethnic differences have been noted so there is no compelling genetic evidence for heterogeneity. Whether different mutations are involved cannot be decided at present. The fundamental defect in all seems to be the deficiency of a sulfatase (14); possibly quantitative or ontogenetic differences account for the various clinical forms which are seen.

For Niemann-Pick disease the situation is the most confusing. This is a condition beginning in early infancy with an enlargement of liver and spleen and a failure to grow and develop normally. Blindness ensues, and death usually occurs in the second year. In many tissues, notably liver, spleen, bone marrow, and brain there is an enormous accumulation of lipid foam cells, which store large quantities of sphingomyelin. Many cases of the disease are delayed in onset and more prolonged in course (15). These display smaller accumulations of the above lipids in the affected tissues. The typical cases are often of Ashkenasic Jewish extraction whereas the delayed cases are not, again suggesting different mutations but not necessarily involving different genes. A third form with a prolonged course has been reported in a population isolate in Nova Scotia and probably represents still another mutation (15). Finally, a fourth type, without cerebral involvement, has been reported in a few cases. Now that a deficiency of a sphingomyelin-splitting enzyme has been found in a study of six patients with the classical infantile form (16), a biochemical comparison of the various forms may be undertaken.

Population Genetics of Lipidoses

All the conditions discussed are serious, and most are fatal even before adulthood is reached. All are hereditary so natural selection is effective in reducing their frequencies to low levels. The observed frequencies reflect a balance of forces, that of recurrent mutation of normal genes to abnormal genes serving to replace alleles lost by selection. A mutation rate of 10^{-5} per locus per generation can maintain an infantile lethal recessive disorder, such as Tay-Sachs disease, at an incidence of 10 per million per generation. Mutation rates for genes generally in man are estimated in the range $10^{-4} - 10^{-6}$. By and large then, the incidences of these diseases reflect the interaction of mutations which produce heterozygous carriers from normals, and selection which results from the deaths of homozygous affected persons. A population which holds a disease at a constant rate by this mechanism is said to be in mutational equilibrium for the gene.

How then can the peculiar frequencies of three classical forms of sphingolipidoses among Ashkenasic Jews be explained? One possibility is that the frequency of carriers is no greater than for other subpopulations, but cousin marriages produce a greater homozygote frequency for the same carrier frequency. This possibility has been tested and is not correct. In a large series from New York City there were only two cousin marriages among 124 parent-couples of Jewish children with Tay-Sachs disease (17) whereas a first-cousin marriage rate of 50% or more would have been necessary to give the disease incidence which was observed among Jews with the carrier rate found among non Jews. The difference is, in fact, attributable to a higher carrier rate among Ashkenasic Jews. This is accentuated by the observation in Israel that nearly all cases are Ashkenasic while the non-Ashkenasic Jewish population, which comprises about 40% of the total population of Israel, has a higher cousin-marriage rate.

Another possibility is that the elevated carrier rates for these three disorders are attributable to the chance variations observed in small populations, a phenomenon known as genetic drift. The population of Ashkenasic Jews is too large to permit this kind of random fluctuation to have been causative in recent times. Even in earlier periods it is doubtful that their number was small enough, but, if it were, one could hardly anticipate that three physiologically related entities should all show such marked genetic drift.

Finally, one must examine the assumptions about mutational equilibrium. For example, mutation rates for these conditions might be higher among Ashkenasic Jews although there is no scientific basis for such an argument. On the other hand, selection, which does have a physiological basis, could explain the discrepancy. Consider the coincidence that in parts of Greece there are populations with simultaneously high frequencies of the genes for sickle cell anemia, thalassemia, and glucose-6-phosphate dehydrogenase deficiency, all disorders of erythrocyte physiology. This coincidence is not inherent in Greeks but is correlated with the prevalence of falciparum malaria.

Heterozygous carriers of these genes in all populations are thought to be more resistant to an initial attack of this form of malaria than are noncarriers, who thereby have a lower survival rate than do carriers. Natural selection in such regions not only acts against abnormal homozygotes, who die of sickle cell

anemia, but also against normal homozygotes, who die of malaria, and in favor of carriers, who develop neither. The frequency of carriers is limited by the fact that, as they increase, marriage among them becomes more probable, more abnormal homozygotes are born, and more abnormal genes are lost from the population. An equilibrium can be reached under such conditions; the carrier frequency reflects the forces of selection on the two homozygotes. A balanced polymorphism is established, and the affected population is said to be in segregational equilibrium rather than in mutational equilibrium.

Coming back to the Ashkenasic Jews, one can now inquire whether they have sustained some selective forces to a greater extent than have adjacent subpopulations and whether these forces have been favorable to carriers of the genes which determine in homozygotes three classical sphingolipidoses (18). It is conceivable, for example, that this group, which was at one time localized primarily in Poland, Lithuania, and Russia suffered greater losses from certain plagues or famines than did other populations and that the carriers in question were peculiarly resistant. Even if these conditions no longer obtain, not enough generations have passed to permit a return to the original gene frequencies.

This suggestion is of particular interest with respect to possible biochemical mechanisms in disease resistance. Carriers of these genes may be anticipated to have lowered activity, approximately 50%, of the enzymes in question. Perhaps because of this deficiency or because of the presence of the abnormal protein which does not have normal enzymatic activity, there are changes in cell surface or cell reactivity which modify the invasion of some infectious agent. Careful investigation of biochemical deviations encountered in heterozygous carriers should be undertaken with the idea in mind that it may afford significant clues to mechanisms in resistance to infection.

Of course, man has the ability to manipulate these genetic equilibria and so effect a change in the frequencies of the various diseases. First of all, removal of the advantage enjoyed by carriers of genes deleterious in the homozygous state will disrupt any existing segregational equilibrium and convert it slowly to a mutational equilibrium, as has been happening for Negroes brought from the malarious slave coast of Africa to the United States, where falciparum malaria is not found. The discovery of a way to reduce mutation rates could reduce the frequency of any disorder held in mutational equilibrium although it must be admitted that, with the current contaminations of the environment, the prospect is for increasing mutation rates and increased incidences of diseases held in mutational equilibrium.

Selection can be put to man's advantage however if it is employed judiciously. Obviously there can be virtually no homozygotes if no children are produced by matings of two heterozygous carriers of the deleterious gene; a selective disadvantage would be placed on carriers as a group by a failure of some of their members to produce children. Even now many couples would prefer artificial insemination or adoption to having children in the face of one-to-four odds that Tay-Sachs disease or a similar disorder will result. The major limitation is the ability to recognize such marriages before they have resulted in disaster. Hopeful is the report (19) that heterozygous carriers of the Tay-Sachs mutation have a reduced blood level of fructose-1-phosphate aldolase. The main immediate application of the discovery of the basic enzymatic lesion in any of these diseases is its adaptation as a test for carriers.

Biochemical Genetics and Lipidoses

Owing to the inability to prevent these disorders at the present time, great attention is being paid to the development of an understanding of them which would have therapeutic consequences. In mind are the models afforded by some success in the management of such metabolic defects as phenylketonuria, galactosemia, and the adrenogenital syndrome. In the first two conditions harm is caused by the accumulation of substrate proximal to the enzymatic lesion. Since the substrate is dietary (in phenylketonuria) or immediately derived from a dietary constituent (in galactosemia), the accumulation can be circumvented by dietary limitation. In the third condition a metabolic clock in the synthesis of cortisone causes not only a deficiency in that hormone but an accumulation of androgenic steroids, which produce virilization. Administration of cortisone corrects the deficiency and, by inhibition of ACTH secretion, reduces the accumulation. These therapeutic maneuvers indicate that the genotype is not synonymous with the phenotype and that circumvention is feasible in at least some instances.

Circumvention in the case of lipidoses has been attempted by means of low fat diets but to no avail. This is not surprising as one contemplates the pathways of sphingolipid synthesis. Consider for example, the synthesis of the normal galactocerebroside of the brain (Figure 2); it is impossible to imagine a dietary omission that could impair its synthesis. One might visualize a means for blocking one of the synthetic reactions, as with an anti-metabolite or with competitive substrates. So far this has not been a fruitful approach, but perhaps adequate consideration has not been given to it.

A different kind of maneuver, one which has not been attempted, would take into account the nature of the defect in metabolic errors. From biochemical genetics it is learned that many mutations involve structural changes in proteins; well-known examples are the amino acid substitutions of various abnormal hemoglobins in man and of various defective tryptophan synthetases in *E. coli* and *Neurospora*. Many, perhaps even most, enzymopathies are associated not with the absence of enzyme protein but rather with the presence in normal quantities of structurally altered enzyme protein. Is it possible to use this knowledge to therapeutic advantage?

It is well known that a substantial "safety factor" exists for enzymes generally. Heterozygotes with 50% activity do not show signs of metabolic block. Individuals with one form of glucose-6-phosphate dehydrogenase deficiency have about 10% of normal activity yet have symptoms only under certain conditions, such as primaquine administration. By con-

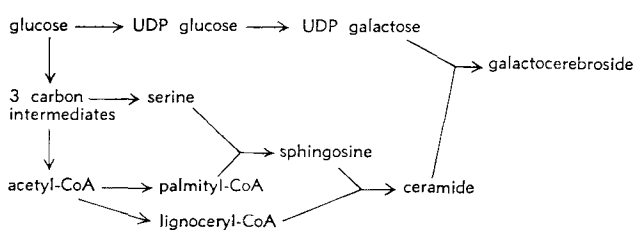

FIG. 2. The Relationship of Galactocerebroside Synthesis to Glucose Metabolism.

trast another form, with less than 1% activity, is associated with congenital hemolytic anemia in the absence of extraneous factors. Suppose then that a defective enzyme, with 1% of normal activity, were produced in 10 times the usual amount. Taking as a model one of the bacterial systems of induction of increased enzyme production, an assay should be made of potential inducers in animals or in cultivated cells. So too should the possibility of qualitative improvement in the defective protein be sought. Mutations in some cases probably result in weak binding of the cofactor to the apoenzyme, in which case a synthetic cofactor with greater affinity might be corrective.

Lacking the ability to circumvent a defect or to develop activity with a defective protein, the possibility of enzyme replacement must be investigated. Of course, the most direct method would be administration of normal enzyme isolated from another source, much as antihemophilic globulin is administered to hemophiliacs. Certainly this should be attempted in some model cases, but not much hope can be held that such an enzyme could be incorporated adequately into the normal metabolic sequence.

Other methods have to do with correcting the genotype itself (20, for example). One approach that is being tested in another context is that of tissue transplantation. Examples of this are afforded by the transfusion of blood in various hereditary defects of the erythrocyte. At present, the pressing need is for a method for overcoming host recognition of foreign genotypes. One method under active investigation in several laboratories involves attempts to induce "somatic hybridization." The object would be to accomplish a fusion of host and graft cells, perhaps even in tissue culture, with subsequent genetic recombination so that cells could be selected whose recognition sites are of host origin and whose allele for the gene in question is of donor origin.

Another way to overcome the problem of host rejection would be the selective conversion or replacement of the defective gene in question. About directed gene conversion actually nothing is known. However there are agents capable of converting one nucleotide base to another. The chief difficulty is that of specificity, i.e., of delivering the mutagen to the appropriate site. A method now available for achieving specificity of gene contact generates a hybrid molecule from gene DNA and its messenger RNA. It has been suggested that delivery of a mutagen to the proper DNA site might be accomplished with the appropriate RNA molecule.

Mechanisms for gene replacement are known from bacterial genetics. The study of the first, transformation, provided the knowledge that genetic material was DNA. Transformation has since been accomplished for many markers and in many organisms. It has even been reported in mammalian cells in vitro (21) although this work is unconfirmed. This hybridization with specific messenger RNA could conceivably be employed for the isolation of gene-specific DNA. Research along these lines will undoubtedly develop considerably in the next few years as the in-vitro somatic cell studies progress.

Another mechanism for gene replacement is that of transduction, a process mediated by latent virus infection. Some viruses can literally transport a gene from one cell to another, as in the transduction of the galactose gene by the lambda bacteriophage in *E. coli*. Models for animal cells are still lacking, but a number of latent viruses, some oncogenic, are available for study.

In summary, the lipidoses are a group of related hereditary disorders of lipid metabolism of serious clinical consequence and complex biochemical nature. Genetic analysis of family data can aid in their classification. Population studies suggest mechanisms for the persistence of the genes causing these defects and further suggest that biochemical analysis may yield information relevant to mechanisms of resistance to acquired disease. A sensible eugenic approach to the prevention of these disorders depends on the development of methods for carrier detection. Meanwhile therapeutic efforts seem seriously limited by the nature of the metabolic errors, thereby inviting a consideration of possible future means for dealing with genetic disorders generally.

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