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The William Allan Memorial Award Address: On Phosphate Transport and Genetic Screening. "Understanding Backward—Living Forward" in Human Genetics

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The joy I feel in receiving the William Allan Award from the Society is great, but still less than the happiness I have in learning and serving our discipline—human genetics. It is also less than the enduring pleasure I experience in working with colleagues, friends, students, and teachers who, in fact, by their own efforts have made me a recipient of the Award. The generous citation by Barton Childs names some of those persons; others are known to you as coauthors of papers or as co-participants in various endeavours over the years. But there are other influences to be recognized. I thank my family, university, and hospital for understanding that knowledge and progress, however small, ultimately come about through work, and for that to happen, time and opportunity are required. They have supplied me with a generous endowment of both; several research grants have also helped. The continuing gift of a nurturing environment is a great reward; the rest is up to—nature.

Life can only be understood backward but it must be lived forward. (Kierkegaard)

Human genetics is Janus-like: deeply concerned with past events, it cares also for the future well-being of humans. One can use the philosopher's aphorism in this essay, because, among other things, the occasion encourages perspective. Therefore, a backward look at phosphate transport (while reflecting a particular interest of my own) is an outlook on a facet of evolution and a fascinating experiment of nature that molds the size and shape of bacteria, mice, and men. A forward look at genetic screening again recognizes my own interests, but it also perceives a union between population genetics and medical genetics, as well as the possibility of predictive health care for all. Thus, looking backward and living forward is not an occasional personal excercise; it is in the fabric of human genetics. Therefore, I assume my choice of themes will be of some interest to most of us.

UNDERSTANDING BACKWARD: PHOSPHATE TRANSPORT

Our cells respire and our skeleton endures against gravity, only because we have access to the chemical element—phosphorus. How organisms capture phosphorus is

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an interesting story. What happens when phosphorus transport fails is the stuff of which an "inborn error of metabolism" is made.

Origins of Phosphate Metabolism in Evolution

Organic molecular evolution began some 3.5 billion years ago [1–5]. About 2 billion years of Precambrian time elapsed before eukaryotes established themselves and evolved into multicellular plants and animals [6]. Not until 400 million years ago, in the Phanerozoic era,* did vertebrates appear [5]. These simple benchmarks of events that occurred in the lithosphere, hydrosphere and atmosphere of Earth, as well as in the evolution of species, are relevant to an understanding of phosphate metabolism as we know it.

Phosphate was sequestered in igneous rocks when the lithosphere solidified during the first billion years of Earth history [7]. Since the content of the molecular oxygen in the lithosphere was undoubtedly considerable, the phosphorus, most certainly, was present as phosphates. However, phosphate became available for biological evolution only when it had been leached from the rocks into the hydrosphere. The leaching process required a very long time. About 3 billion years elapsed from the time Earth was formed before phosphate accumulated in primitive oceans; its rate of turnover then increased, and as the seas pulsated, sedimentary deposits of condensed phosphates formed and were available to support life. Abundant phosphate became available for cellular metabolism, as opposed to random chemical polymerizations in solutions, only about 1.5 billion years ago [7]. Concordance between the availability of phosphate and the evolution of aerobic prokaryotes and eukaryotes implies that the anion was a key element in the origins of life.

While the phosphate biosphere was evolving, an equally important change occurred in Earth's atmosphere: an oxygen-rich environment appeared [3, 6]. The major source of oxygen was prokaryotic life itself [6]. Prokaryotes (bacteria and cyanobacteria or blue-green algae) are characterized by highly variable oxygen tolerances. We can deduce from this variation that prokaryotes were adapted to environments of various oxygen contents during evolution, prior to the appearance of eukaryotes. From the available facts, it is deduced that photosynthetic bacteria initiated oxygen filling of our atmosphere, and cyanobacteria and stromatolites completed it about 1.5 billion years ago [6].

A study of cellular metabolism in prokaryotes and eukaryotes reveals several interesting facts [6]: (1) energy is captured in the form of high-energy phosphate bonds; (2) the more efficient systems for extracting energy from cellular fuels, such as the citric acid cycle, are oxygen dependent; (3) the latter system is appended to a less efficient glycolytic pathway for energy metabolism; and (4) biosynthetic pathways often begin with anaerobic steps and end with aerobic reactions. These findings indicate that the capability of the organism to sustain aerobic metabolism apparently followed obligatory anaerobiosis in the scenario of cellular evolution. However, aerobic metabolism could prosper only if organisms could also collect phosphate

* Geologic time, after the Cambrian Period is known as the *Phanerozoic* era, because *fossil life is manifest* in all of its eleven geologic Periods.

efficiently. Accordingly, *phosphate transport mechanisms* and a capacity for aerobiosis are likely to have evolved concordantly in prokaryotes and emerging eukaryotes. Present estimates would place the evolution of cellular phosphate transport at least 1.5 billion years ago.

Cellular Organization: Membranes

Living cells have membranes composed largely of lipids and proteins. Thus, when cells collect phosphate, this water-soluble anion must permeate a hydrophobic domain to pass from one aqueous environment to another. The control exerted on chemical traffic across lipid bilayer membranes is one of the important achievements of evolution. When prokaryotic cells gained the ability to accumulate phosphate, organization of the transport process began; its refinement was a wondrous task for multicellular organisms.

In view of the importance of biological membranes in the lives of cells, it is rather surprising that treatises on Precambrian molecular evolution give so little mention to membrane evolution itself [2–4, 8]. The topic is important because membranes establish compartments whose chemical composition can be different, one from another, depending on the control exerted by the membrane on the flow of physical and chemical information across itself. One of the features of a living system is its specific chemical composition, which differs not only from its external environment, but also among its components. Accordingly, the appearance of functional membranes was a giant step in the evolution of living cells.

The first protocells were proteinoid (colloid) microspheres; they did not have lipid bilayer membranes [3]. However, in the late Precambrian time apparently, lipids were synthesized. We presume this because prokaryotes have lipid bilayer membranes. When lipids come into contact with an aqueous environment, an organizing force is exerted on them. That force is the hydrophobic effect, and it determines the assembly and organization of lipid bilayers in all biological systems [9]. Lipids were repulsed from the primitive hydrosphere, as they are today from any aqueous solvent. Assembly of lipids was under thermodynamic control then, as it is now, and they would have aggregated in micelles, bilayers or vesicles according to their chemical nature. Thus, the origins of cells with lipid membranes can be imagined. Moreover, in the absence of strong attractive forces between its constituent molecules, the lipid membrane is fluid and deformable. This key property of biological membranes sustains the plastic nature of cells which possess mobility, pack themselves into architectural arrangements, and reseal themselves after injury. These are vital attributes for successful cellular evolution.

Biological membranes play many functional roles, for example: receptor or binding activity, maintenance of chemical gradients, and uptake of fuels and building blocks. Such biological functions have extraordinary specificity, that is, they can select unique sets of reactants from larger populations of molecules. Only proteins can confer this property to biological functions. Thus, it is not surprising that membranes contain proteins. This was true of membranes even in the earliest living cells, and if phosphate transport was important to the evolution of cells, we can expect that proteins were involved in the process.

Cellular Organization: Phosphate Transport

Phosphate penetration of intact living cells deviates from that expected of its oil-water partition coefficient. Passive diffusion across the lipid bilayer membrane does not account for phosphate collection by cells, and mediation of the process must be proposed. Facilitated or exchange diffusion is one possibility; active transport is another. Both involve specificity of the process, in which the transport protein can apparently differentiate the functional groups on the phosphate molecule, strip it of its water of hydration, and position the solute properly for a vectorial translocation across $\gtrsim 75$ Å of membrane. Exchange systems for phosphate in prokaryotic membranes share the property of specificity with active transport systems [10]. Accordingly, mutation could impair either function. We know very little about the integration of phosphate transport proteins into membranes. They undoubtedly span the membrane [9, 11], and for some systems they may have an additional binding protein located on the external surface of the plasma membrane near to the transmembrane transporter [12].

Phosphate transport takes place across a single plasma membrane in prokaryotes. At least two membranes—plasma and mitochondrial—are involved in eukaryotes. The situation is even more complicated in metazoa, such as man, because transport also occurs across epithelia (fig. 1). Because of tight junctions between its cells, an epithelium is a permeability barrier between large compartments. Furthermore its cells are characterized by structural and functional asymmetry; their apical poles face one environment, their basilar pole, another. Thus, net vectorial transport occurs across two sets of membranes in epithelia, and these processes, perforce, are organized differently at opposite poles of the cells [13]. The kidney is a critical arbiter of phosphate homeostasis in man, and its epithelium regulates the content of anions in

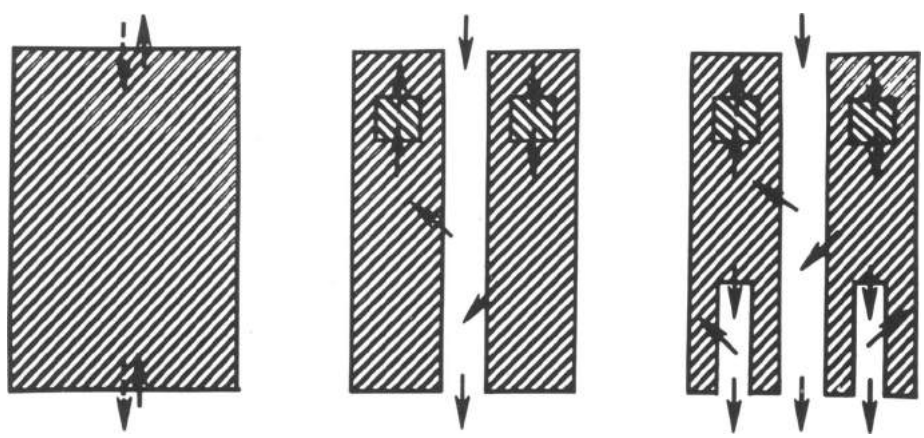

FIG. 1.—*Left panel:* The plasma membrane controls fluxes of solutes to achieve a difference in the composition of intracellular and extracellular pools for unicellular organisms. *Middle panel:* Symbolic eukaryotic multicellular organism with feeding channel (digestive tract); cells lining tract form an epithelium. Individual cells also have subcompartments bounded by membranes. Transport of nutrients and structural materials is now transcellular from "outside" to "inside" of organism; and transmembrane in cells and their compartments. *Right panel:* Further metazoan differentiation; development of an excretory (renal) system with further elaboration of "inside" and "outside" topology. Flux imbalance of solutes, to maintain total homeostasis, may be regulated by hormones and other factors.

urine and blood. As expected, phosphate transporters at luminal and basal-lateral membranes of renal epithelium are functionally different [14, 15].

Since net conservation of phosphate in man is ultimately determined by brush-border membrane activity [13], there would be a selective advantage in diversity of function in this membrane, under the control of several genes. It is not surprising then to find that more than one type of phosphate transport mediates both net renal reabsorption in vivo [16, 17], and net uptake by renal brush-border membrane vesicles in vitro [15]. Genetic evidence (to follow) suggests that this diversity of phosphate transport is under the control of autosomal and X-linked loci. On the other hand, because epithelial brush-border membranes and plasma membranes of internal cells serve different environments, phosphate transport properties in the erythrocyte, and kidney for example, should be different—and they are [18].

The Skeleton and Phosphate Homeostasis in Vertebrates

Vertebrates acquired rigid internal skeletons about 400 million years ago. The skeleton of later evolution is bone, a tissue that contains phosphate in great amounts. Phosphate transport serves the metabolic needs of bone, and it is vested with hormonal regulation at several levels in the organism.

Metabolic homeostasis of bone involves the turnover of hydroxyapatite, a complex crystalline phase composed of calcium and phosphate [19]. Accretion of skeletal mineral requires a constant intake of calcium and phosphorus from the environment. Vitamin D hormone ($1,25\text{-}(OH)_2D$) controls absorption of phosphate and calcium by the intestine [20]. Overmineralization of the skeleton is prevented by constant resorption, a process that also maintains extracellular calcium homeostasis [20]; mobilization of skeletal mineral is regulated both by vitamin D and by parathyroid hormone. Parathyroid hormone controls moment-to-moment homeostasis of the extra-skeletal pool of mineral, while vitamin D hormone is involved in its day-to-day regulation.

Cellular transport is a critical component of total phosphate homeostasis in vertebrates [21]. Active (concentrative) transepithelial transport not only gives the organism optimal access to a widely fluctuating extrinsic source of phosphate in the intestine, but also reclaims phosphate at the renal level. Because the phosphate environment is tightly regulated inside the body, access to the internal cells, such as the erythrocyte, can be served simply by a facilitated diffusional process [18]. Under the influence of parathyroid hormone, excessive removal of phosphate and calcium to the extracellular pool from bone could provoke soft tissue mineralization. Land-dwelling mammals compensate for this hazard by clearing phosphate from blood into the urine at an increased rate in the presence of the hormone, which achieves its effect by inhibiting net resorption of phosphate from filtrate in the pars recta segment of the nephron [16]. The relationships between vitamin D, parathyroid hormone, and the various phosphate pools are shown in figure 2.

Rickets, Phosphate and Mutation

Rickets is a complex, yet common, disease of man; and phosphopenia, secondary to impaired phosphate homeostasis, is the critical component in the pathogenesis of this

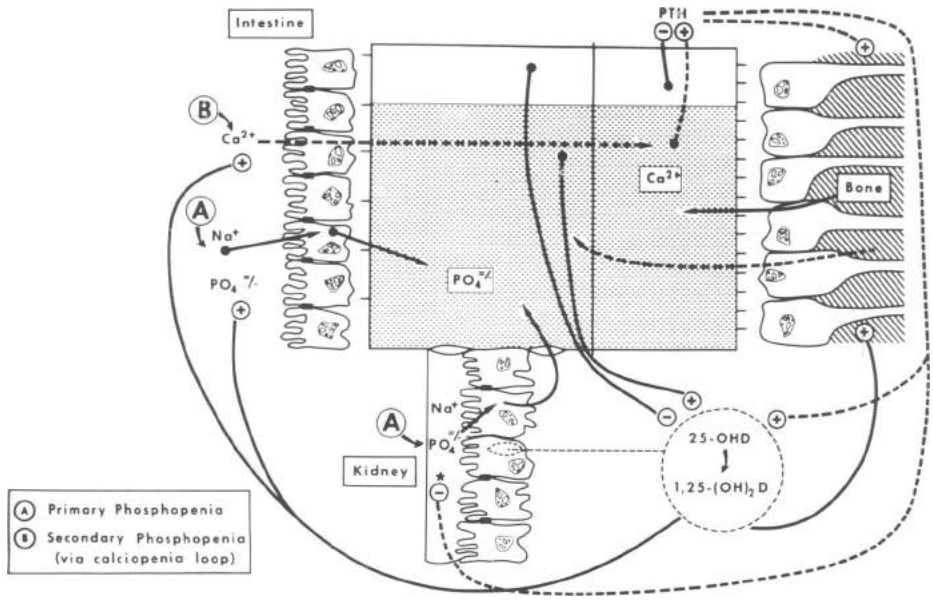

FIG. 2.—Interrelations of phosphate transport with mineral and bone mineral homeostasis in vertebrates such as man. Phosphate homeostasis in extracellular pools reflects the influence of a calcium-parathyroid hormone (PTH) limb (-----) and of a primary phosphate limb (—) independent of PTH. Vitamin D hormone ($1,25\text{-}(OH)_2D$) regulates calcium and phosphate transports as independent membrane (and epithelial) activities [19–21]. Phosphate homeostasis can be perturbed by calciopenic events (*A*), and by phosphopenic events (*B*); mutations impairing phosphate transport are *B* events. Other factors (e.g., calcitonin) that may influence homeostasis are omitted. Interactions terminating in (+), stimulate event indicated; those terminating in (−), inhibit.

condition. Virchow observed rickets in Neanderthal [22], and it has been continuously reported, for example, in the modern European since the 17th century [23, 24]. Rickets has been predominantly of environmental origin during human evolution, and impaired availability of vitamin D was, apparently, its major cause. Rickets in man today is usually of hereditary origin and phosphate homeostasis is always involved.

Loomis [25] proposed that the adaptation of early man to more polar latitudes followed a reduction of melanin pigmentation in the epidermis. This hypothesis is of interest because photolysis of 7-dehydrocholesterol by 300 nm wavelength ultraviolet irradiation of human epidermis is an important source of vitamin D. In the absence of an endogenous supply from the epidermis, man is dependent on his diet for the vitamin, the major sources being fish liver, fish-liver oils and egg yolk [20]. When he does not consume the appropriate diet, or when the intensity of ultraviolet irradiation to his skin is reduced, man is eligible for the consequences of vitamin D deficiency.

Vitamin D was unknown as such until recent times. Rickets related to deficiency of the substance was an endemic disease during and following the Industrial Revolution in most major cities of the temperate latitudes. The disease reflected a lifestyle among children consuming urban diets deficient in the vitamin and living under the pall of atmospheric pollution from the new industries. Biological research in the early 20th

century led to the discovery of the antirachitic vitamin [26, 27]. When it became available for prophylaxis and therapy, a dramatic decline in the incidence of rickets in populations at risk followed its use [28]. But rickets did not disappear; instead, its heritability increased dramatically! The inherited forms [29, 30], previously submerged, merely became more prominent as the flood of rickets of environmental origin abated.*

The key metabolic event in the pathogenesis of any form of true rickets is the appearance of hypophosphatemia [24, 31–33]. Mechanisms that may lead to phosphopenia (fig. 2) are [32, 33]: those that involve hypersecretion of parathyroid hormone causing increased renal clearance of phosphate (as in vitamin D deficiency and related "calciopenic" conditions); and those that modify the phosphate transport systems themselves to cause primary phosphopenia. The former are often "responsive" to vitamin D, while the latter are usually "refractory."

When hypophosphatemic rickets, refractory to physiological doses of the vitamin, began to be reported in populations that had experienced eradication of vitamin D deficiency, it was soon recognized that such cases were often familial. Two hypotheses followed these observations: one favored an inherited disturbance in the normal action of vitamin D in such patients [34]; the other favored a primary and hereditary abnormality of phosphate transport [35, 36]. A brief précis of three Mendelian forms of hypophosphatemia, each of particular interest to me, will illustrate how mutation comes to influence phosphate transport in man; and why careful interpretation of phenotype is both informative about cellular processes and beneficial to the affected proband.

X-Linked Hypophosphatemia (XLH)

This condition, the most commonly recognized form of "familial vitamin D resistant rickets," was not known to be an X-linked dominant [37] until long after its original formal description [34]. The possibility that XLH is a primary abnormality of phosphate transport (that is, a phosphopenic form of rickets [32, 33], rather than a primary disorder of vitamin D metabolism) was raised long ago [35, 36]. Yet it has taken more than 30 years to accumulate a compelling body of evidence for the transport hypothesis. This hypothesis is still not universally acclaimed, and the debate goes on and on [29]. . . . But, one new line of evidence, with a strong biochemical genetic flavor, offers considerable support for it. The witness is a rachitic mouse with vitamin D-resistant hypophosphatemia.

The *Hyp* allele is X-linked in the mouse, and the associated phenotype is hypophosphatemic rickets [38]. According to the doctrine of Ohno [39], the X-linked mutant gene products in man and mouse should be homologous. Therefore, any evidence for an intrinsic abnormality of phosphate transport in the murine mutant is likely to be informative about the true nature of the human disease. An intrinsic abnormality of phosphate transport is present in the renal brush-border membrane of

* This transition in heritability of rickets has taken place during the lifetime of many of us, and it offers an important lesson to human geneticists. It illustrates how futile it is to estimate the "heritability" of a condition or characteristic, when the environmental contribution is not clearly defined or is changing.

the *Hyp* mouse [15, 40]. An intrinsic defect in transepithelial transport of phosphate is also present in the small intestine of this animal [41, 42]. Thus, the X-linked disease can be classified as an inborn error of phosphate transport.

The mouse data reveal more information. The defect in *Hyp* renal membranes appears to be only partial [15, 40], because there is significant residual Na^+-dependent co-transport activity in the mutant hemizygote. This finding is not explained by postulating a K_m mutant (Tenenhouse and Scriver, unpublished data). Moreover, the evidence in the mouse is analogous to that for residual renal transport of phosphate in the affected male human patient [17]. Together, these findings strongly suggest endowment of the mammalian renal brush-border membrane with genetically heterogeneous phosphate transport systems, only one of which is affected by the X-linked mutation. Additional evidence in support of this hypothesis is available in an autosomal disorder of phosphate transport described below.

The transport hypothesis, for interpretation of the X-linked phenotype, offers a rational basis for the particular efficacy of dietary phosphate therapy in the murine and human diseases [38, 43]. Phosphate replacement by dietary means is euphenic because it offsets the primary phenotype; and it is as effective as it is, because an alternative form of membrane transport is available to facilitate phosphate replacement.

Autosomal Dominant Hypophosphatemic Bone Disease (HBD)

Elucidation of the XLH phenotype allowed another form of hereditary hypophosphatemia to be identified in man. The disorder is characterized by hypophosphatemia equivalent to that observed in XLH, but the bone disease is much milder and specifically non-rachitic [44]; for that reason it has been called hypophosphatemic "bone disease." A comparison of renal handling of phosphate in HBD and XLH indicates that the former is a disorder involving a mode of phosphate transport in renal epithelium different from that affected in XLH. Since HBD is not X-linked—male to male transmission having been identified in one critical pedigree—one perceives that the phosphate transporter involved in HBD is coded by an autosomal locus. The genetic evidence for diversity of phosphate transport gene products is thus compatible with the evidence of the physiologists [16] for two different types of phosphate transport in renal epithelium.

Another point of interest is raised by the HBD mutation. Since its bone disease is less severe, at phosphate levels in extracellular fluid equivalent to those associated with severe bone disease in XLH, there is clearly another factor that is also different in the two conditions. That factor is still unknown, but it might be an X-linked gene product involved in phosphate transport in bone [44]; or a difference in the amount of vitamin D hormone available to regulate bone mineral [45].

Autosomal Recessive Vitamin D Dependency (ARVDD)

Although associated with hypophosphatemia and rickets, ARVDD, or pseudovitamin D deficiency [32, 33], is not a primary disorder of phosphate transport. The hypophosphatemia is secondary to hyperparathyroidism, and the latter is a response to hypocalcemia associated with hereditary impairment of vitamin D hormone bioavailability. ARVDD exhibits genetic heterogeneity: one form of the condition appears to be

an abnormality of hormone biosynthesis [46]; another is thought to be a disorder of hormone binding to target sites [47]. Thus, ARVDD belongs to the group of Mendelian disorders known as the "vitamin-responsive inborn errors of metabolism," or "hereditary vitamin dependencies" [48, 49].

The ARVDD phenotype deserves mention here for only one reason: it is a realization of the condition Albright et al. [34] believed they were describing in their original report of "vitamin D-resistant rickets." The stature of Albright was Olympian, as any student of medicine knows, and because of that, his hypothesis about the nature of "vitamin D-resistant rickets" influenced the course of research on the X-linked disease. Alternate hypotheses received rather short shrift. But now that the case for a defect in phosphate transport has had its hearing, and the evidence for a striking benefit from phosphate therapy in the X-linked disease is also at hand, we can see the autosomal recessive condition for what it is: the genetic equivalent of vitamin D deficiency of environmental origin. In that discovery, the transition from environmental to genetic origins of rickets in modern man is further secured.

What is understood? This backward look has glimpsed only the peaks of a mountainous topic, but it reveals that cellular transport of phosphate is as important for human well-being as it was for aerobes throughout evolution. Human evolution has endured not only aberrations in the environment but also mutations that upset phosphate homeostasis and cause rickets. At times, the disadvantages incurred by these perturbations were endemic. Moreover, they undoubtedly had some deleterious influence on genetic fitness: either an effect on maternal survival and childbearing related to pelvic dystocia; or through increased infant mortality related to the convulsive disorder of vitamin D hormone deficiency [31]. However, homological evolution—that is, the ability to direct and relax selection acting on the human genome—has largely overcome such hazards in recent times. Their resolution came about with the advent of new knowledge and understanding. Living forward in fitness and in health will also benefit from an improved perception of the origins of disease in modern society and the use of new knowledge for the prediction and prevention of these diseases.

LIVING FORWARD: GENETIC SCREENING

Sir Thomas Browne (1605–1682), while considering his own palm prints, mused that "*even in things alike there is diversity*." The essence of human genetics lies in his statement, made in an age when science was evolving from a philosophical exercise to become a discipline that, in the words of Bacon [50], put nature "to the question." Out of that tradition came the science of Darwin, Mendel, and Garrod, and the modern appreciation of biochemical individuality. We believe that knowledge of human biological variation, and the understanding it brings, ought to benefit both the individual and society. But the knowledge is imperfect and slow in arriving, and its application is attenuated by our prevailing view of disease and how to improve human health.

While clinical medicine is often given the credit for improved standards of health and the increased longevity of citizens living in developed societies, more often than

not it is knowledge gained from research in other areas that has achieved such changes, as we have seen in the case of rickets and its medical management. The distinction is of some practical importance. The conquest of various forms of epidemic or endemic disease, when coupled to technological advances and perceived as a series of advances in clinical medicine, encourages a contemporary investment in bigger and better clinical medicine. Another result is a continuing war on disease.

Causes of morbidity and mortality, particularly those of nutritional or infectious origin, are, by tradition, extrinsic enemies. We use the language of World War I to describe our battles with them [51]. *Campaigns* subdued the endemic nutritional deficiencies such as scurvy, and infections such as smallpox; *breakthroughs* in chemotherapy and vaccines seemed to *conquer* infections such as tuberculosis and polio: cancer is now *bombed* with cobalt, and *killer* diseases such as Tay-Sachs need *eradication*. We make war on cancer, heart disease, diabetes, birth defects, etc., while our medical generals announce the strategies and tactics that will vanquish them.

When imprudent living in modern society seems to expose us to agents of disease, and to place us at increased risk for degenerative illness [52, 53], a change in lifestyle for each and everyone is urged by the generals, hoping that the barrage of illness will be lifted. A moral note is introduced, because disease of the collective is perceived as the outcome of profligate living. Disease that persists in the face of the earlier victories has a taint of sin. We are persuaded to hear the words of Juvenal as he describes a man succumbing to premature coronary heart disease because of an immoral lifestyle:

> "What a grossly ravening maw
> That man must have who dines off whole roast boar - a beast
> Ordained for convivial feasting! But you'll pay the price
> All too too soon my friend, when you undress and waddle
> Into the bath; your belly full of undigested
> Peacock-meat—a lightning heart-attack, with no time
> To make your final will."
>
> (Juvenal, *The Sixteen Satires*, translation by Peter Green, Penguin, 1967)

An old theme is being revisited while modern campaigns against diseases of lifestyle are mounted. Presumed causes of disease lurking in our diets, the air, the chemicals we encounter, and in our own behavior, must be overcome! But any argument that makes natural disease immoral, is unnerving; and, because it is so popular, it is extremely worrying, because it may be wrong; at least, in part.

Prudent living will avoid harm from excessive exposure to extrinsic and potential "causes" of diseases; no one will deny that. But attainment of a complete victory by that route will be limited by two factors: our own behavior and our personal inherited risks. Our behavior will make full compliance with lifestyle campaigns unlikely, both collectively and individually. Look, for example, at our handling of hypertension [54]! Our health behavior will also be uninformed if we, as individuals, are ignorant of whether compliance has any real personal relevance. The relevance comes from knowing that some of the risk of incurring a disease is sometimes related to inherited personal susceptibility.

A Genetic Paradigm of Health and Disease

Health and disease must be viewed in the paradigm of equilibrium between nature and nurture [55, 56]. Health is a state of equilibrium; disease is disequilibrium (fig. 3). Whereas citizens of developing nations have diseases primarily of extrinsic origin, those living in developed countries generally experience more subtle illness in which intrinsic factors, more often than not, play an important role. The origins of disease form a spectrum, embracing three typologic views: genetic, environmental and multifactorial (fig. 4). The origin of some diseases lies extrinsically, in the environment; acting upon the universal human genotype, it brings about an "environmentally caused" disease. In others, the origin is mainly intrinsic, in a particular genotype, which when expressed in the universal environment leads to the expression of "genetic" disease. When intrinsic and extrinsic factors are both important, as is the case in the majority of disease, they interact to cause "multifactorial" illness. Human genetics benefits the individual and society best when the specific contribution of intrinsic factors to disease expression is recognized in the practice of medicine. The "causes" of rickets, described earlier, illustrate the paradigm and reveal how transition from an "environmental" origin to a "genetic" origin of rickets was the direct result of advances in clinical medicine.

We are learning about the profound biological variation that exists between human beings. It is revealed in three quite different ways. First, there is the evidence in twins: only identical twin pairs share identical genes and few of us are identical twins. Second, there is the evidence from patients with inborn errors of metabolism; genetic

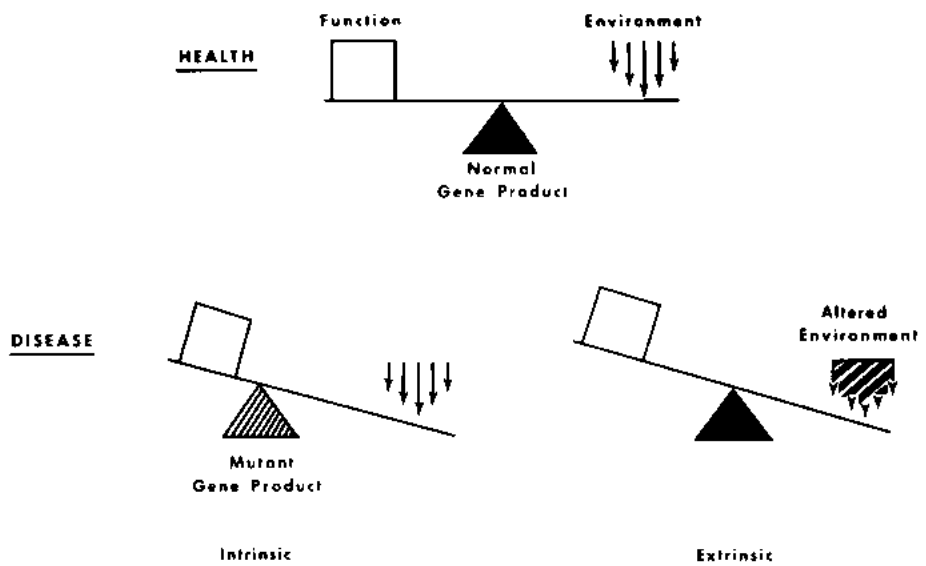

FIG. 3.—The Nature–Nurture paradigm. *Health* is seen as a state of equilibrium between organism and environment, and *disease* as disequilibrium. The fulcrum for interaction between environmental events and a particular biological function is a gene product (e.g., enzyme). Change in environmental "forces" may alter equilibrium; or mutation may modify the fulcrum and thus the equilibrium, other things being equal.

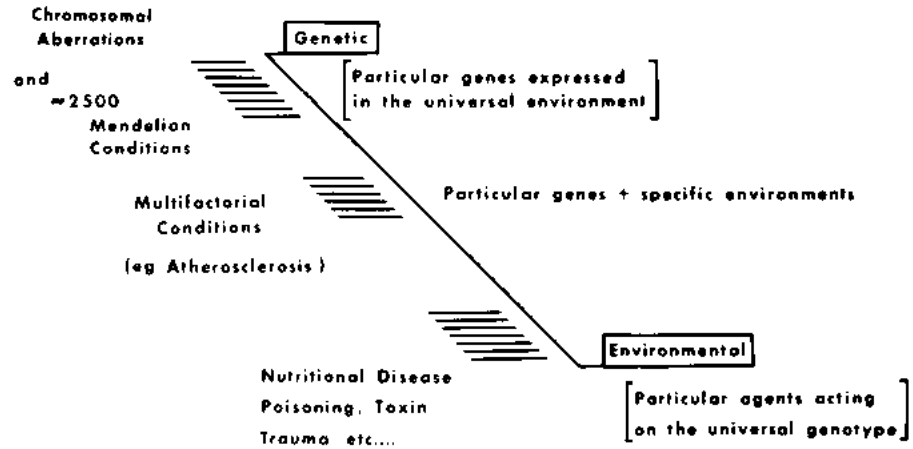

FIG. 4.—Origins of diseases seen as a spectrum covering those in which *genetic* (intrinsic) factors are more important, to those in which *environmental* (extrinsic) factors are more important. When intrinsic and extrinsic factors are both significant (mid-spectrum) the disease is said to be *multifactorial* in origin.

heterogeneity involving so-called "rare alleles" has been revealed at one locus after another. Third, there are the important studies of healthy human beings in whom prevalent heterozygosity involving common alleles, at virtually all loci, has been revealed. The issue of real importance for health maintenance is the biological significance of such extensive allelic variation in man. Whereas adaptability of the species appears to lie in its genetic diversity, we perceive that each human being is adjusted to the universal environment in a particular way because of genotype. *That is to say, each of us has a different and relative state of health*. Realization of this fact will have a far-reaching effect on the principles and practice of medicine, and in particular on the use of genetic screening to attain what one might call the goal of "predictive medicine." Population genetics, which is the study of inherited biological variation among the members of a population, and medical genetics, which is the study of genetic variation associated with disease in the individual, until now have been considered as separate disciplines. Henceforth, they will be seen, quite simply, to be two facets of a mutual problem [57]; and genetic screening is the catalyst that will fuse them.

Genetic Screening

Screening for non-genetic disease is a long-standing and well-established medical procedure [58]; genetic screening is more recent, and it is an activity with a particular goal. It can be defined as a search for persons within a population, who may be at risk for disease in themselves, or their descendants, in universal or particular environments, because of their genetic constitution [59].

There are three principal reasons for genetic screening. The first is screening for medical management; following presumptive case-finding through screening, specific diagnosis and treatment can prevent incipient disease from becoming established in an individual. Newborn screening for the hyperphenylalaninemias and thyroid hormone

deficiency observe this rationale [60]; and because of newborn screening, phenylketonuria, as an inherited cause of mental retardation, and congenital hypothyroidism, as a birth defect also causing mental retardation, are both disappearing in modern societies.

The second rationale is the counseling of persons about the consequences of their sexual reproduction. Individuals, who are themselves healthy, can have offspring with serious disease of genetic origin. Recognition of an intrinsic risk to offspring can lead to options that may modify this hazard. For example, selective screening for Tay-Sachs carriers and intrapartum screening for Down syndrome are reducing the frequency of these two "genetic" diseases in screened populations.

The third rationale has less immediate clinical application, yet it will serve disease prevention. Enumeration of mutant alleles and investigation of their distribution define loads of specific mutations in populations. Knowledge of such variation and its extent might then play a predictive role in the prevention of certain diseases among individuals.

Some Priorities

The future course of genetic screening is dependent on technology, but its general direction can be anticipated from priorities in health care that are announced from time to time. For example, perinatology, nutrition, industrial health, and premature aging (degenerative diseases) have been proclaimed by the minister responsible for health care and medical research in Quebec as priority areas for new initiatives. It is instructive to examine how the genetic paradigm in general, and genetic screening activities in particular, might serve these declared priorities.

Perinatology. Pregnant women in North America are likely to notice a poster with this message: "Be good to your baby before it is born." The intent of the message is to prevent birth defects through unnecessary exposure of the fetus to teratogenic insults. Indeed, 1.6% of liveborn and stillborn infants, of gestational age exceeding 20 weeks, have a serious malformation—defined as one with cosmetic significance or requiring surgery [61]; obviously, a reduction of this burden is desirable. Some of these infants have the fetal alcohol syndrome as a result of maternal alcohol intake; others were harmed by maternal cigarette smoking. The message on the poster might reach such mothers and deter lifestyles that can be harmful to the fetus. But the larger burden of birth defects has a different origin. Multifactorial inheritance accounts for 44% of serious malformations; Mendelian inheritance, 25%; and chromosomal aberration, 13% [61]. These findings underscore the importance of genetic factors in the origin of severe birth defects. They also question the intent of the poster campaign, when its target is, in fact, birth defects with high heritability, since guilt rather than understanding is likely to be produced after the fact. A more positive approach would be to find ways to screen for mothers with genetic risks, so that a predictive activity could be adopted; of course, that tactic is more difficult than a poster campaign.

Another problem in perinatology, of particular interest to biochemical geneticists, is maternal hereditary metabolic disease. Half of the patients with phenylketonuria and like diseases are girls. Should they become mothers, their metabolic condition must be treated throughout pregnancy to prevent profound brain damage and extensive

malformation in their offspring. A registry of mothers at risk, and research on intrapartum treatment so that they may have healthy children, is a realistic challenge, as yet unmet.

Nutrition. Genetic factors influence nutritional requirements of individuals [62], and national dietary standards, in general, accommodate for this [63]. Nonetheless, some diseases reflect bizarre nutritional requirements imposed on individuals by specific genetic variation. For example, some patients with schizophrenia have an inborn error of folate metabolism [64]. Screening of schizophrenic patients for homocystinuria associated with the specific folate disorder, and treatment with pharmacologic doses of folate, ameliorates this form of schizophrenia.

Thiamine supplementation of alcoholic beverages has been proposed as a novel and cost-effective approach to the prevention of the Wernicke-Korsakoff syndrome in alcoholics [65]. However, the proposal is more traditional than novel, because it is cast in the familiar mold of public health, and it ignores the possibility of an intrinsic predisposition for the syndrome. Not all alcoholics develop neuropsychiatric symptoms, and of those who do, most are of European extraction [66]. An abnormality of the thiamine-dependent enzyme, transketolase, was recently identified in European patients with the syndrome [67]; in such persons there is a specific increase in the thiamine requirement to maintain an appropriate cellular activity of the enzyme. The finding provides an alternative to the strategy that would impose thiamine-fortified spirits, wines, and beers upon the population at large. The genetic approach would screen diagnosed alcoholics for transketolase activity and determine the kinetics of thiamine binding early in their disease, so that subjects with the binding defect could receive specific thiamine therapy to prevent the neuropsychiatric complications of their alcoholism.

Precedent for the genetic approach to thiamine nutrition in alcoholics already exists in the prevention of rickets. For example, there is a rational nutritional standard for vitamin D in the normal population [48, 63]; and in some geographic regions dairy milk and other foods are fortified with vitamin D_2 to prevent deficiency rickets. Nonetheless, autosomal recessive vitamin D dependency is still found in the "protected" population, for the reasons discussed earlier. Does that mean the nutritional standard for vitamin D should be raised 100-fold to protect the whole population? Of course not! Instead, the genetic approach is used to protect patients with ARVDD. Affected patients are diagnosed early in the course of their disease and treated with a pharmacological dose of the vitamin (about 100 times the Recommended Daily Allowance); or they are given the particular form of vitamin D that they require (the hormone, for example). The guardians of milk appear to have a message for zealous arbiters of the nutrient quality of alcoholic beverages.

Occupational Medicine. Most developed nations have environmental standards for the major primary and secondary industries. When properly applied, these standards protect the worker from particular exposures that would otherwise be toxic. Nonetheless, some workers still develop trade illnesses. Imperfect standards or improper compliance may explain the persistence of occupational illness under these standards. But why only some workers are affected, and not others beside them in the same environment, also begs the question of personal susceptibility in the former. Genetics

could play a role in occupational medicine by identifying an intrinsic link between a particular environment and its occupational disease. When the link is found, it might then be possible to screen persons who apply to work at an occupation with a known health risk, and to advise them of their particular risk for the illness in accordance with their genotype at the relevant gene locus, or loci. Whether such preemployment screening should be compulsory and what rights the employee and employer should have are new facets of the problem already under consideration [68].

Premature Degenerative Disease. Intrinsic factors contribute to the process of premature aging. The importance of a genetic component is apparent in the disorders of cholesterol metabolism, controlled by low-density β-apolipoprotein, that are associated with premature coronary heart disease [69]. Hyperlipidemic premature myocardial infarction can be classified into non-genetic, multifactorial and Mendelian forms, and the latter are further subdivided into three types [70, 71]. Low-density-lipoprotein hypercholesterolemia is one of the Mendelian disorders. While homozygotes for this particular mutant gene constitute only about one-in-a-million live births, the corresponding heterozygote frequency is 0.2%. Heterozygotes are more susceptible than homozygous normal subjects to develop precipitous coronary heart disease, and they become 10% of those with hyperlipidemic premature myocardial infarction. These persons are not immoral beings, pursuing profligate lives. Their heart disease is dominantly inherited, and they have myocardial infarctions because they have a deviant process of absorptive endocytosis in the low-density-lipoprotein cholesterol pathway [69]. Their endogenous cholesterol metabolism is disregulated, and hypercholesterolemia is established early in life. Such persons cannot adjust their cholesterol metabolism to normal levels simply by prudent living. Their hope for healthy longevity lies ultimately in two activities: one is the development of a pharmacological agent that can bypass the mutant membrane transport step and yet still control intracellular cholesterol biosynthesis; the other is a screening test to identify those who need the drug.

Of course, familial hypercholesterolemia is not a universal model for the genetic approach to prediction and prevention of premature coronary heart disease. Age, sex, hypertension, cigarette smoking, diabetes, obesity, lack of physical activity, and personality each contribute to the risk status of an individual [72]. Nor is the risk status in the Mendelian disease unchangeable; elevated plasma levels of high-density α-apolipoprotein reduce the risk of premature coronary heart disease [72]. However, in context, familial hypercholesterolemia is seen as a harbinger of the significant predictive opportunity available in genetic typing, and of the role screening will play in the improvement of health care.

Predictive Medicine: A Goal for Genetic Screening and Education

Genetic screening will become a major predictive activity for identifying quantitative risks to personal health only when our prevalent heterozygosity is perceived as a potential origin of disease. Most physicians are unfamiliar with this concept, and they are not yearning to know the niceties of their patients' genotypes. It follows that a reform in the medical curriculum, with a new emphasis on the genetic paradigm of disease, is required if their patients are to benefit from an instauration of predictive

medicine. The problem of genetics in medical education was elegantly dissected in an earlier Allan Award address [73].

The education of citizens is another facet of the problem. Current experience with genetic screening indicates that self-knowledge of heterozygosity is not necessarily an uplifting experience. Afro-American blacks perceived little benefit in sickle trait screening programs, as they were practiced in recent times, and knew little or nothing of the selective advantages in their own heterozygosity (R. Murray, personal communication, 1977). Many Jews experienced anxiety at some point during revelation of their own Tay-Sachs heterozygosity, even though they could perceive medical advantages in the new self-knowledge [74–76]. A change in the education of citizens about their "biological rights" may be required to help them to understand the significance of heterozygosity, and the predictive value it may have for their personal health maintenance.

Two recent studies of human genetics education in the high schools of North America [77, 78] reveal that students of biology may be aware of human heterozygosity; but they scarcely perceive that they are themselves touched by it. The level of awareness is likely to be no better among students who take no biology whatsoever during their educational career; and that may be the majority of students in some school systems, unfortunately [77]. The nature of the educational system in America has encouraged these deficits in our awareness of human nature. The problem goes back to the Scopes Trial [78] and continues in the present conflict between Creationism and Evolutionism in the school curriculum [80]. Whereas student preferences strongly favor more emphasis in the curriculum on evolution, human biology, and genetics [77], the current text books, as good as they are, determine curriculum content and classroom teaching, and they contain relatively little human biology and less genetics; furthermore, the genetic material that they do present has a low human profile. Small wonder, then, that students lack a simple basic knowledge of human genetics, can be confused about the difference between a gene and a chromosome, and are uncertain how Mendel's laws apply to their aspirations to be parents of healthy offspring [77, 78].

While these findings describe contemporary students, the situation is likely to be far worse for their parents who were not exposed while in school to post-Sputnik texts, even with their limitations. That may explain why citizens are anxious about their genetic screening test results when their personal heterozygosity is revealed to them.

The challenge ahead for the educators of citizens and health professionals is clear. There is, in all likelihood, a wide illiteracy in human genetics; consumer, health professional, legislator—none appears to be exempt [59]. It has been said that the importance of learning "lies not in what meaning it makes of the world, but what it makes of the learner" [cited in ref. 81]. The goal in genetics education is to have all of its students become "more knowledgeable, independent, and mature in their approaches to the problems of the modern world" [81]. To that end, a beginning is under way to improve human genetics education and the text books that sustain it. If health education in the schools can be seen as a valuable resource for preventive medicine in other parts of the world [82], surely it is possible to improve human genetics education in the schools of nations when genetic heterozygosity is emerging as an important factor in the pathogenesis of disease.

Socio-Economic Implications

Predictive medicine will have socio-economic consequences. Because advances in knowledge and their application to public health policies will continue, health care will be further improved and life expectancy will increase as it has during the past century. Among the various possible outcomes will be a restructuring of social security and old-age pensions [83]. Continued pursuit of health maintenance and improved longevity will also increase the cost of medical care further. During the past 30 years in America, the per capita expenditure on disease care has increased by more than 300%, yet longevity has increased by only 15%; moreover, the greatest burden of illness is in the older population today. Accordingly, the goal of *healthier* longevity continues to elude us; and there will be little change while the emphasis in health maintenance remains on the control of extrinsic (environmental) causes of disease. We need to reorient the prevailing wisdom about "origins" of disease. A shift from exclusively medical and public health views of disease to include more of the genetic view could do that. It might then be possible both to contain the costs of disease care and to attain healthier longevity.

Predictions

Some predictions about the future of genetic screening can be attempted. I suggest the following: (1) The current programs of newborn screening for hereditary metabolic diseases provide a useful model for alternate forms of genetic screening still to come. (2) Heterozygote screening will play an important role in predictive medicine, and medical genetics will be the predictive discipline. (3) Genetic screening for a research rationale will play a greater role in the development of policies for health maintenance and disease prevention. (4) The practical reality of biochemical individuality will restore and reorient the importance of clinical medicine, as opposed to collective health care that perceives persons as if they were all at similar risk. An individual has his own specific risk for a given disease and possesses his own personal and relative state of health. (5) The physician will practice predictive medicine optimally, and the patient will benefit from it maximally, only when there is improved human genetics education for both parties.

EPILOGUE

At the beginning of this essay, I used a quotation from Kierkegaard. It has allowed me to weave together rather different themes from my work. To close, I quote a poem by Siegfried Sassoon, composed when he was nearly 50 years old on the occasion of his son's birth. Sassoon, who survived the Great War and saw many friends die in that campaign to cure the world of evil, knew that understanding is backward and living is necessarily forward. That awareness was the wellspring of the poem.

Meeting and Parting

Myself reborn, I look into your eyes;
While you unknowing, look your first time on me.
Thus will you stand when life within me dies
And you, full knowing, my parting presence see.

Alone I stand before my new-born son;
Alone he lies before me, doomed to live.
Beloved, when I am dying and all is done
Look on my face, and say that you forgive.

Siegfried Sassoon

Human geneticists are privileged both to understand the atavistic and the prophetic tones of this poem and to translate them into personal action on behalf of their fellow man.

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