SPECIAL ARTICLE

Patterns of Disease, Controlled Populations, and Experimental Design

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The records of the Philadelphia Zoological Garden from 1901 through 1928 once were taken to suggest that the "constitutional" characters of mammals determined their major patterns of disease. For example, elongate, slender-bodied animals, such as monkeys and small carnivores, usually had died of "gastroenteritis," whereas renal disease had been a much more frequent cause of death of the heavier, more compact herbivores. Further, "gastroenteritis" in monkeys (Cebidae) of the American tropics often had been complicated by skeletal deformities, while in raccoons and wild dogs (Procyonidae and Canidae) it usually had been combined with thyroid hyperplasia that sometimes terminated in malignancy.1-3

Twelve years later (1940) these and many other earlier examples of disease had virtually disappeared. This was one result of completely redesigned feeding practices (1933-1935) and demonstrated that malnutrition, not "constitution," had determined to a very large degree the earlier patterns of disease.4-6

These and subsequent experiences suggest that studies of spontaneous disease, as it may develop in any group or population, must consider all environmental factors. Otherwise, these studies may be completely unreliable in suggesting tentative explanations of etiology and pathogenesis. Obviously too, studies that ignore the probable relations of environmental factors to disease may be equally valueless as guides to the design of experiments that test these hypotheses.

Indeed, we suggest that much of the continuing confusion in the design and interpretation of experimental studies of etiology and pathogenesis will disappear when the influences of environment have been more fully understood. For example, the interactions of host and parasite that characterize infectious disease probably depend far more upon nutrition and upon behavioral responses to other features of environment than upon any inherent qualities of either parasite or host.7-10

Similarly arteriosclerosis (and atherosclerosis), of man or other animals, usually does not depend upon either drastic dietary manipulations, or debilitating physiologic disturbances.11-14 In fact its development in adequately nourished animals in the Philadelphia Zoological Garden has been independent of the diets.6,15 Instead, its occurrence and its rate of development apparently have been closely related to behavioral responses to other environmental factors.16-19

Experience with the adequately nourished collection of mammals and birds in the Philadelphia Zoological Garden has allowed us to identify tentatively features of environment other than nutrition that contribute to the genesis of disease, especially arteriosclerosis. This experience has been supplemented in recent years by a detailed study of a large "free-ranging" population of woodchucks, ground-hogs, or eastern marmots (Marmota monax). The pattern of disease in these wood-
chucks has been so similar to the pattern in zoo animals that environment in both situations has been examined closely for corresponding features.\textsuperscript{20, 21} A review of these observations as they apparently relate to etiology and pathogenesis may provide more reliable guides to experimental design than have been hitherto available. This review is presented in the three generalizations or postulates that follow. Each of these is accompanied by supporting evidence.

1. Adequately Nourished Mammals and Birds Develop a Highly Uniform Pattern of Disease

For the present this statement neither excludes nor includes neoplasms. Our experience with this type of lesion has been relatively limited (less than 2 per cent of all autopsies), but the neoplasms that have been encountered do not suggest differences in susceptibility except under peculiar circumstances.\textsuperscript{22} We will also exclude vascular disease of the central nervous system, since our material does not include a sufficient number of examinations of the brain to justify its discussion at this time.

Otherwise the characteristic expression of disease in the mammals and birds of this zoo and in the woodchucks has been arteriosclerosis, primarily arteriosclerosis of the cardiac coronary arteries, and renal glomerular sclerosis.\textsuperscript{6, 15, 16, 23} For the purposes of this discussion, these lesions are assumed to represent one process of disease, differing in morphology with the segment of the vascular system that may be involved.

The morphologic features of arteriosclerosis generally in the animals of the Philadelphia Zoological Garden, and of coronary arteriosclerosis in particular, are in keeping with this assumption.\textsuperscript{6, 15, 16} Coronary arteriosclerosis in the woodchucks corresponds completely in appearance to this lesion in the animals of this zoological garden.\textsuperscript{21} Similarly, glomerular sclerosis in the birds and mammals of this garden and in the woodchucks suggests an identical process.

Arteriosclerosis of the coronary arteries and of the aorta may develop simultaneously with glomerular sclerosis and apparently both progress at about equal rates. However, one or another phase of this disease complex usually is dominant. Its development in one segment of the vascular system seems to be independent of its development and progress in another. The distribution of the lesions has not been related to size, sex, body type, taxonomic group, climatic adaptations, food habits or diets in this garden. However, the series at this time includes relatively few of the smaller mammals of the size of mice, voles, and rats, although in experimental populations mice develop renal glomerular sclerosis much more readily than coronary arteriosclerosis.

2. Arteriosclerosis and Renal Glomerular Sclerosis of Adequately Nourished Animals Reflect Responses to Adverse Environmental Factors

Environment has been defined as the sum of the interactions of external and internal forces that encompass the individual, the colony, or the species.\textsuperscript{24} Survival demands that organisms of all species maintain internal factors in a satisfactory state of equilibrium in the presence of highly variable external factors. The concept presented here is that arteriosclerosis and renal glomerular disease develop in response to failure to maintain internal forces in equilibrium.

The more obvious of the external forces are climate (shelter, cover), food, and intraspecific relationships. Climate and food have not been recognized as obviously adverse features of the environment for the mammals and birds of the Philadelphia Zoological Garden since 1935. Similarly, observations on the woodchuck population have indicated that neither climate nor food was an adverse environmental factor.

However, intraspecific relationships or social interaction, i.e., the social environment, may become a major source of injury for the individual, the colony, and the species. Changes in the intensity of social interaction may be reflected in the capacity of the individual to develop and maintain resistance to infectious agents.\textsuperscript{9, 10} Moreover, birth rates and viability of offspring, as well as the sex ratio and growth potential of offspring are
readily influenced by the intensity of social interaction.\textsuperscript{25-28} And recent evidence strongly suggests that this factor also may be highly significant in the pathogenesis of coronary arteriosclerosis and renal glomerular sclerosis.\textsuperscript{15, 19, 20, 22} We suggest therefore that an understanding of the more obvious features of social interaction, or the social environment, may provide a key to problems of experimental design.

The social environment of a "free-ranging" population of woodchucks, for example, apparently is limited to interactions between the animals of this one species. Associated animals, even predators and closely related species, seem to contribute little, if anything, additional. Competition within the species must always be more intense because interspecific contacts are necessary to the survival of the species.

Interactions between individuals of a "free-ranging" species will depend upon behavior, which is conditioned by sex, age, physiologic state (mating, pregnancy, lactation, etc.), population density, and individual aggressiveness. Thus, in part, the intensity of interaction is density-dependent but obviously the effects of density reflect behavior, not numbers per unit of space.\textsuperscript{27, 29}

The social environment of animals in this zoological garden apparently is more complex, for these animals often respond to other species including man (attendants and visitors). Obvious behavioral responses are induced by smells, sights, and especially sounds from adjacent cages, from the visitor space, and from air, rail, and highway traffic. These stimuli may be assumed to be additive, and to increase the intensity of interactions among adequately nourished animals. The environment that is usually provided for experimental animals also includes this class of stimuli.

The recent increases (about 10-fold) in the frequency and severity of coronary arteriosclerosis in the mammals of this zoological garden have been related to increases in the intensity of social interaction. Food, shelter, and climate have been eliminated as contributing factors. Under these conditions, therefore, social interaction has been the most adverse feature of environment. Thus to summarize, we suggest that the rate at which coronary arteriosclerosis or renal glomerular sclerosis develops, and the age after sexual maturity at which deaths from these diseases occur will reflect chiefly the following two factors: (1) the capacity of a species to respond to its environment, (2) the nature of that environment.\textsuperscript{15, 16, 18}

3. Adverse Social Environment Operates Through Physiologic Adaptive Mechanisms to Induce Disease

Hypertrophy of the adrenal cortex and medulla is a characteristic response of mammals and birds to confinement in groups. This response may be especially evident when the animals are grouped before sexual maturity and followed through this period and for some time beyond. It usually is most pronounced in subordinate males and least so in dominant males.\textsuperscript{25, 26, 30, 31} "Free-ranging" mammals also respond by adrenal hypertrophy to increased population density, particularly when combined with the more intense interactions that must accompany reproduction, lactation, and dispersal of young animals within the habitat.\textsuperscript{32} Observations on man and other primates, also in highly competitive or highly stimulating experimental situations, have indicated that increased size of the adrenal gland reflects increased functional activity both of cortex and medulla.\textsuperscript{18, 33}

Adrenal hypertrophy under these circumstances usually has been accompanied by reduced function of the gonads of adults and delayed sexual maturation in the young. This has been reflected in reduced fertility of both male and female, and in failure to maintain pregnancy and lactation. It has been associated also with a reduced growth potential and delayed maturation of the offspring.\textsuperscript{25-29, 34} These effects upon productivity may be assumed to depend upon decreases in pituitary gonadotropins as adrenocorticotropins are increased. A reduced growth potential of offspring is not so readily explained.

The effects of grouping animals into the exhibition areas of the Philadelphia Zoo have not always produced such clear-cut changes.
in the size and apparent functions of the adrenals and gonads. On occasion however adrenal hypertrophy and suppressed gonadal activity have been clearly evident in these groups, and in at least one instance these changes were accompanied by pronounced thyroid hyperplasia with histologic appearances suggesting thyrotoxicosis.\(^{28}\)

However, the more common response of mammals to the social environment of this zoological garden has been moderate grades of adrenal hyperplasia, often with the medulla apparently having undergone the greater increase. At the same time productivity usually has been impaired. This has been evident especially with groups of mammals that contained two or more mature females with one mature male.

At this time only endocrine adaptive mechanisms have been studied in any detail, although it seems probable that social factors also operate through other pathways. We suggest therefore that observations on the effects of social interaction must continue to explore all evident physiologic adaptive mechanisms for indications of pathogenesis. Current evidence, at best, merely suggests relationships between the social environment and the origins of vascular disease. Nevertheless it is difficult to ignore these suggested relationships when an obvious increase in the intensity of social interaction has been followed closely by an exponential increase in the frequency of occlusive coronary arteriosclerosis.\(^{16}\)

A major need at this time is objective criteria for quantitative measurement of social interaction. This, we suggest, may provide another key to experimental design.

**Discussion**

To a very large degree these generalizations are based upon observations of wild animals in highly artificial habitats: the pens and cages of a zoological garden where "natural" foods have been replaced by mixed rations.\(^{4, 6, 10}\) Thus the first response may be to dismiss them as unreliable guides to etiology and pathogenesis in "free-ranging" populations, including urban man.

But, the records of the Philadelphia Zoo are unique. They are continuous since 1901. Accordingly, they also represent the period during which the development of disease-control has made possible mankind's greatest experiment in urbanization.\(^{11}\)

Further, these records have been accumulated by competent observers, with descriptions of disease in over 18,000 animals; approximately 5,000 mammals, 11,000 birds, and 2,000 reptiles and amphibians. About one half of these mammals and birds have come under observation, since feeding practices were redesigned in 1935. And since 1935, other environmental factors have been more completely documented. Thus, an increasing percentage of all mammals and birds that have become available for postmortem study since 1935 have been under observation in well-defined surroundings throughout their lives in this zoological garden. At this time (1962) this collection contains few animals that entered the collection prior to 1935. And a great majority of all animals either enter the collection as immature specimens or are born here.

This, then, has been a study of disease in well-controlled populations of mammals and birds for over 25 years. Thus, with the control of nutritional disturbances, which aided immensely the control of infectious agents, expressions of disease have become increasingly uniform.

Recognition of the probable significance of the social environment as a major factor in etiology and pathogenesis is more recent. It followed naturally from the obvious parallels between the behavior of animals in exhibition groups and in experimental populations which, in turn, had provided evidence to explain some of the reactions of "free-ranging" populations.\(^{16, 19, 23-29}\) Moreover, this highly uniform pattern of disease in animals of this zoological garden corresponds closely to that of the "free-ranging" woodchuck population.\(^{16, 20, 21, 23}\) These woodchucks are one of the very few populations of "free-ranging" animals to have been studied adequately (for at least two generations) and in sufficient de-
tail to evaluate its physiologic status.\textsuperscript{26, 27, 29, 32} Parenthetically, this investigation also demonstrates the complete inadequacy of casual collection of organs and tissues from other free-ranging populations of wild animals.

The evidence presented here demonstrates as well that further studies of "free-ranging" animals as guides to etiology and pathogenesis are unnecessary. Observation on wild animals in adequately managed zoological gardens will be less costly and at least equally valuable. Adequate management demands, of course, controlled diets, an awareness of other environmental factors, and continuity in the study.

And last, an effective use of these observations in experimental studies of disease demands experimental subjects that react to environmental factors quite as fully as animals in a zoological garden, or in a native habitat. This requirement eliminates the ordinary laboratory rat and mouse. These creatures represent purposeful selection for a lack of response, especially to the social environment, which, of course, increases productivity of a breeding stock and allows greater numbers to be caged as units in the colony.

References

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PATTERNS OF DISEASE IN THE ZOO


The Creative Environment

Every definite substance, whether inorganic, organic or organized, is autonomous; that is to say, it has characteristic properties and exhibits independent action. Nevertheless, each one of these bodies is inert, that is, it is incapable of putting itself into action; to do this, it must always enter into relation with another body, from which it receives a stimulus. Thus every mineral body in the cosmic environment is stable; it changes its state only when the circumstances in which it is placed are rather seriously changed, either naturally or through experimental interference. In any organic environment, the substances created by animals and vegetables are much more changeable and less stable, but still they are inert and exhibit their properties only as they are influenced by agents outside themselves. Finally, anatomical units themselves, which are the most changeable and unstable of substances, are still inert, that is, they never break into vital activity unless some foreign influence invites them. A muscle-fibre, for instance, has the vital property peculiar to itself of contracting, but this living fibre is inert in the sense that if nothing changes in its environmental or its inner conditions, it cannot bring its functions into play, and it will not contract. For the muscular fibre to contract, a change must necessarily be produced in it, by its coming into relation with a stimulation from without, which may come either from the blood or from a nerve.—Claude Bernard, M.D. An Introduction to the Study of Experimental Medicine. New York, The Macmillan Company, 1927, p. 78.
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