MANAGEMENT OF WILDLIFE IN CAPTIVITY:
TAL ATHEROSCLEROSIS IN GROUP AND SOCIALLY
APANESE QUAIL (COTURNIX COTURNIX JAPONICA)

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ABSTRACT

MANAGEMENT OF WILDLIFE IN CAPTIVITY: EXPERIMENTAL ATHEROSCLEROSIS IN GROUP AND SOCIALLY ISOLATED JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

by Prem Shankar Prasad

Management of wildlife in captivity, from the standpoint of managing a zoological garden, is reviewed. Administrative organization of a zoological garden, with particular reference to biology of animals, is discussed. Problems of confinement, limited space, proper display of animals, feeding, breeding, exercise, training, handling and disease control are discussed in some detail. These are based upon the recent advancements made in the field in the 420 zoological gardens of the world. The many links between zoo biology and animal psychology are given prominence throughout the text.

Zoological gardens of today are not only a place for public entertainment, they are also biological institutions and should fit the community needs in size and service. Both the people managing a zoological garden, and those visiting a zoo, thus, have certain responsibilities. These are discussed throughout this work.

Recent advancements in the field of animal nutrition and animal sanitation, and their application in the

zoological gardens, have eliminated most of the infectious, mal-nutritional, and deficiency diseases. However, arteriosclerosis, among "well-fed" and well-kept varied wildlife of zoological gardens is posing a constant problem.

In an attempt to evaluate the effect of environmental factors on dietary-induced atherosclerosis, Japanese quail (Coturnix coturnix japonica) were held in groups and in complete social isolation caged singly (each provided with one square foot of space). The experimental birds were kept on an atheromatous diet, while the diet for controls was diluted with unsaturated vegetable oil to make the two rations comparable.

A significant increase in the weight of adrenal glands was found in all the birds kept either at higher density level, or caged singly in complete social isolation. This correlated well with the amount of social interaction in the birds. Social hierarchy in the males develops after they attain sexual maturity (at about 8 weeks).

Adrenals were heaviest in the birds kept singly in complete social isolation. Olfactory, acoustic, or visual stimulation seems to evoke adrenal responses in these birds.

A highly unnatural situation in the form of intense crowding, or complete social isolation, enhanced atherogenesis.

Isolation of various interacting factors, and the process or processes involved regarding their neural mechanism, however, is not yet clear.

A survey was made of the electrophoretic pattern of the serum protein of birds kept in the natural and experimental conditions. Each pattern from the experimental group was sufficiently unique to differentiate from the control birds.

Fundamental differences in the protein components of normal and experimental birds are described. These were generally in the form of increased beta-globulins and decrease in the components of $alpha_1$ and $alpha_2$ globulins. It has been suggested that electrophoretic pattern of serum protein may even be a reliable guide of the developmental stage of the disease.

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INTRODUCTION

A comprehensive literature exists on how to manage domestic animals, dealing at length with every conceivable detail. Feeding, breeding, transmission of hereditary characters, pedigree, distribution, pathology, training and so on have long been the subject of profound research, and each has become a specialized branch of an impressive science. On the other hand, the knowledge of how to keep wild animals in captivity can hardly boast of even the most general outline; all it has to show is a collection of more or less fragmentary pieces of advice and some facts.

Despite such limitations, the zoological gardens of today have progressed far beyond the scope and status of a mere menagerie. During the present century, great strides have been made toward the development of management techniques that can satisfy the physical as well as psychological needs of the animals and at the same time allow them to be shown so that at least some segments of natural habitats and life cycles are illustrated. Changing world conditions that endanger the wildlife of many areas, have brought the zoological gardens into new prominence in the field of propagation of threatened species. Also, zoos have far reaching values in the field of education and research.

The biology of zoological gardens, however, is such a complex field that it is difficult for a single individual to get a complete view. It ranges, for example, from zoology to psychology (including human psychology), and from ecology to pathology. The present study, therefore, endeavors to provide a review of some useful relationships between the most important of these aspects.

The first part of the text deals with some major problems of a zoo which provide the basis for the present study. The contents in this section are discussed under two separate headings:

a) Administrative problems of a zoo

b) Operational problems of a zoo.

These are further subdivided into smaller groups and discussed in some detail.

The second part presents an independent research dealing with an emerging problem of the pathological aspect of captive animals, the so-called "disease of civilization."

It presents an intricate relationship between the physiological and psychological state of the animal, a clear understanding of which is still far from accomplished.

PART I

MANAGEMENT OF WILDLIFE IN CAPTIVITY

ADMINISTRATIVE PROBLEMS OF A ZOOLOGICAL GARDEN

The major consideration under this heading is the administrative organization of a zoological garden. Through administrative planning, long and short range objectives are determined, policies are formulated, organizational structure is streamlined for efficiency, and the work of the organization is given coordination, direction, and control. An organization, thus, accomplishes its objectives through people.

Organization of a zoological garden

Inconsistency in the organization of zoological gardens is such that scarcely two are alike. Most zoological gardens of the world seem to have developed quite independently from one another--a fact which seems fundamentally wrong with such an important organization. A number of factors may have contributed in varying degrees to this independent growth. Poor communication, in some cases, undoubtedly resulted from the great distances separating some zoological gardens. A friendly spirit of competition, as it does to a certain extent today, may have existed, creating a delaying effect on the flow of new ideas.

Hediger (1950) in his book "Wild Animals in Captivity" stressed that

> Zoological gardens cannot have isolated existence today. They are necessarily linked up with the long history of development which the keeping of wild animals has undergone in the course of centuries, and this means they have responsibilities. Every zoological garden is a part of the whole system and must fit into the picture along with all other zoos. The important knowledge gained in past and present zoological gardens demands suitable recognition.

To better understand the organization of a zoological garden it seems imperative to mention a brief historical background of zoological gardens from the beginning up to the present day.

History of zoological gardens

No record is available to indicate who founded the first zoo. Man's enthusiasm for keeping pets probably was the original impetus; a desire to gain favor by presenting some strange beast to a ruling potentate was probably another. Certainly the custom of collecting wild animals is ancient.

The earliest zoological garden for which there is a definite record was established in China by the first Emperor of the Chou Dynasty, who reigned about 1000 B.C. (Cadwalader, 1949). This was known as the "Intelligence Park," and its name implies that it served an educational purpose.

The keeping of animals for pleasure and exhibiting them for show or entertainment was highly regarded by the Egyptians, the Greeks, and the Romans (Berridge, 1932).

The great animal collection of Ptolemy II (309-246 B.C.), when exhibited in a procession through the city of Alexandria, as a part of the festival of Dionysus, took all day. A remarkable assemblage of animals formed a part of this parade. Elephants and groups of other animals in harness drawing chariots, camels laden with spices, sheep, oxen, bears, leopards, lions, and birds in cages were included. 24,000 hounds also passed in review, and there were 150 men carrying "trees to which were attached wild animals and birds of all sorts" (Jennison, 1937).

Hediger (1950), speaking about the evolutionary history of keeping wild animals by man from paleolithic time to the beginning of the twentieth century, divided the subject into three main groups:

1. The age of cults: The incentive for keeping animals was based on religious beliefs, especially for use as sacrifices. In ancient Egypt, animals such as lions, baboons, crocodiles etc. were considered sacred and suffered from a special type of pampering. These "animal gods" were often kept in temples and surrounded with pomp fatal to them. They

were provided with food chosen on the basis of what would suit human tastes; were bathed, anointed and perfumed. The sacred crocodiles of Lake Moeri that came out at the call of their priest keepers were decked out with bangles and necklaces (Loisel, 1912). These unnatural treatments caused pathological conditions in many, as Lortet and Gaillard (cited by Loisel, _op_. _cit_.) have pointed out in their exhaustive researches on mummified animals.

2. The profane age: The chief consideration being
   a) usefulness;
   b) entertainment (animal fights and hunting).
3. The scientific age: The interests being mainly
   a) systematics and anatomical;
   b) ecological and psychological.

These divisions show how the emphasis in man's attitude to towards animals gradually shifted and reoriented.

As civilization spread westward and northward, the custom of keeping wild animals continued. During the middle ages they were held captive mostly for sports. Eventually, zoological gardens were established in many of the larger cities of central Europe, France, and in England. The collections in Paris under Louis XIII and Louis XIV were well known.

The zoological gardens now existing in the world are all derived in some manner from Jardin des Plantes of Paris which appeared at the end of January, 1793 (Loisel, 1907). "This was a place set aside for the study of nature, in the interests of science and the liberal arts, for the scientists and the artists (Loisel, _op_. _cit_.).

Of all the zoological gardens of the world, the Zoological Garden of London seems to be the oldest and the largest (both in total number of specimens and number of species exhibited; total specimens 5,654; number of species on exhibit 1,544). It was founded by the Zoological Society of London in 1826 for the purpose of "the advancement of zoology and for the introduction into England of new and curious animals" (Loisel, _op_. _cit_.). It was incorporated by Royal Charter in 1829. This garden belongs to the Zoological Society of London which serves the dual purposes of maintaining the garden as well as publishing Proceedings, Symposia, Transactions, Nomenclator Zoologicus, Zoological Records, International Zoo Yearbook, Zoo Magazine, Zoo Guide, Zoo Plan and various scientific and non-scientific leaflets.

On North American continent, the Zoological Garden of Philadelphia is the first and foremost zoo, equipped with a modern research laboratory. It is affiliated with the University of Pennsylvania. The garden, likewise, is managed by the Zoological Society of Philadelphia and is governed by a Board of Directors, none of whom receive remuneration.

Philadelphia Zoological Garden was founded by the Zoological Society of Philadelphia in 1859 and initially covered an area of about 33 acres. Currently the garden occupies over 42 acres of land and is exhibiting 1,595 specimens representing 555 different species of mammal, bird, reptile, and amphibian. It publishes a quarterly magazine and zoo guide besides several scientific papers coming out of its Comparative Pathological Laboratory.

Jarvis and Morris (1961), emphasizing the need of a zoological garden in the field of propagation of threatened species, have said, "The policy of zoos all over the world must surely be conservation; whether rare species of animals are preserved in a natural or captive environment is of secondary importance." India, however, where some of the noblest and most beautiful animals of the world are to be found, is most backward in this respect. In his forwording note for "The Wild Life of India" by E. P. Gee (1964), the late Prime Minister Jawaharlal Nehru has said, ". . . in no country is life valued in theory so much as in India, and many people would even hesitate to destroy the meanest of the most harmful animals. But in practice we ignore the animal world." Since the inception of the Indian Board for Wild Life in 1952, a list of 13 rare fauna of India was published (Gee, 1964). At least two of them, the pygmy hog and the Indian Cheetah, are now believed to be extinct. The Indian Lion (Panthera leo persica), whose existence in their natural habitat is most threatened, however, is doing well at the Calcutta Zoological Garden (census of rare mammals in captivity, International Zoo Yearbook, 1961).

Currently, in India, there are 10 zoological gardens of which the Victoria Zoological Garden, Bombay, is the oldest and the Alipore Zoological Garden, Calcutta, is the largest. The Alipore Zoological Garden was founded in 1875 by the Government of Bengal. It now occupies 50 acres of land and has a total exhibit of 1,965 animals. The financial support for the garden is met by the State Government of West Bengal and a part of it comes from the city corporation. The Zoo has an attached laboratory for pathological and zoological research and also publishes an administrative report which is distributed free of cost. Display and housing for most of the animals, however, are not very up-to-date.

Governing authority

Individual zoological gardens can operate under a variety of governing authorities. One means of control may be successful in one community but inadequate in another and local conditions generally determine what type of governing responsibility will be established.

The superintendent or the director of a zoological garden may have authority vested in him through the city manager or the city commissioner or the heads of such departments as public works or animal husbandry. Under any system, however, the cooperation of a good zoological society or an advisory board of influential and interested citizens can be of great value in promoting the growth of the program and maintenance of high garden standards.

A zoological society is a group of interested and often influential people who may direct or act as an advisory body in the development and operation of the garden. Members of the society are more likely to take greater interest in the garden.

The primary objective of the society's interest is to place the development and operation of the institution in the control of a non-profit organization, free from political influences and unaffected by frequent changes in the administrative affairs of the zoo (Mitchell, 1935). For the zoological garden it provides continuity of management, and to the officials and employees permanent employment during faithful and satisfactory service, both vital features in the building up of any organization. For other advantages of a zoological society to a zoological garden see Luehrmann (1931).

Administration of a zoological garden

The director or the superintendent is in charge of over-all operation of the garden. He directs and carries out the policy and is directly responsible to the Board of Directors. Divisional supervisors are directly responsible to him. It is the director's duty to plan, coordinate, and direct the work of the entire zoological garden. He is assisted by his secretarial section in matters incident to the shipment and receipt of specimens, including insurance and custom clearance, also mail receipt and transmission, public relations and interpretive programs, and maintenance of the central files.

In most modern zoos, a separate public relations division integrates publicity and other informational activities through all kinds of public communication media. It deals with membership fees and subscriptions, signs and posters, publications, public information, radio and TV programs, lecture series and reference library, and guided educational as well as public tour services.

The public service division operates restaurants and refreshment stands, vending operations, films and other accessories, and souvenirs of the garden. It also caters to special groups and the general public through picnic facilities.

In almost all zoological gardens there is a separate security division which may be further divided into building service section and a patrol section. The duties are to patrol and protect the garden properties, enforce security regulations governing exhibits and visitors' behavior, supervise public comfort, direct visitors, and render first aid service when and where needed.

Functional division of a zoological garden

Most of the big zoological gardens have their zoological department divided into four separate units that include:

1. The animal clinic and the laboratory: The work of this division is governed by a research director who supervises the veterinary work and other zoological research projects. The veterinarian and a research team work directly under him.

2. Ornithology division

3. Herpetology division which takes care of the aquarium and the reptile house.

4. Mammalogy division.

The last three divisions are often the responsibility of a separate curator who is further assisted by a team of workers including a head keeper, a junior keeper, and several animal attendants of various grades.

<u>Curator</u>

The curator of a division is subject to administrative direction from the superintendent or the managing director of the garden. He is responsible for the acquisition, health and maintenance of the animals under his division. The director and the curator of a division work together, planning and organizing long-range objectives of their respective units.

The curator supervises the work of the personnel employed in his division. He is responsible for feeding, breeding, receipt and shipment of animals. The animal's environment and its general health should be checked daily and any deviation from normal reported to the veterinarian and to the director. It is the responsibility of the curator to see that the advice of the veterinarian is strictly followed. As a zoological expert, the advice of the curator is important to the efficient care, handling, and exhibition of the animals.

Another aspect of biological nature directly concerned with administration is the complete inventory of all the animals at hand. This, however, varies widely from one zoo

to another. It should give continuous information about a number of similar species, as well as about many completely different ones. Also, this should not be confined to the individual, but should include the ancestors, the progeny, and special events in life such as growth, accident, illness, breeding cycle, as well as special behavior, change in social organization etc.

If possible, and if it does not involve too great expense, all animals must be weighed periodically and records kept. This provides a valuable reference about growth and development and also their general well-being. In almost all zoological gardens, a separate card is maintained of the periodic parasitic check of feces so that the parasitic state of the animal may be compared at a glance. This problem has efficiently been discussed by Peters (1933).

The capture of every animal means exclusion from its natural environment and man incurs full responsibility for providing adequate surroundings. The zoo, in this respect, should not play its role only as a "living museum" or a repository of the world's fauna. The surroundings should be designed and maintained in such a way that the wild animals housed in them not only live long in captivity but also live in conditions where they can reproduce themselves.

The loss due to capture, thus, can directly be compensated by releasing the offspring, in return for the individual loaned, as it were, by nature. This simple remedy, however, could be applied only in rare circumstances. One of the best examples of this is the restocking of the Swiss Alps with ibex from animals born in captivity. Hediger (op. cit.) is correct in saying that there is

> . . . only one criterion for suitable biological conditions and that is success in breeding. When breeding does not occur, something is wrong with the methods of keeping the animals; if breeding does occur, it is guarantee that the conditions are essentially right.

This important aspect should, therefore, be the greatest challenge of the curator or the director of a zoological garden, and every effort should be made to achieve this goal. There are still many species that have not been induced to breed in captivity, and our knowledge about their physiology and ecology is seriously lacking. In the interests of the maximum breeding potential of the stock of animals in all the zoological gardens of the world, close cooperation on an international scale is desirable, quite apart from its significance in many other fields.

Among zoo animals, a vast array of behavior of the mother-child relationship may often be observed. Since many kinds of teratological conditions arise due to possible physiological disturbances, in the same way the danger of qualitative or quantitative departures from the normal may occur in every single element of behavior in the mother-child relationship after birth. These deviations from the normal, as often credited, are not always due to under-nutrition. In some cases, close connection with hormone metabolism has been shown. Interesting cases of incredible nature have been described by Yerkes (1929) among his anthropoid apes. The mother may sometimes blow into the mouth of newborn which are unable to breathe. Once a mother chimpanzee, whose baby was not breathing, pulled its tongue out with her lips and blew until it started to breathe.

The hypertrophy of the maternal instinct, or the over-exaggerated care of the young, however, may sometimes prove fatal to the offspring, especially in the predatory animals. In order to hide their young in a more secure place, they drag them around by the scruff until they eventually succumb to exhaustion or even to serious injuries. Even a loud, disturbing noise nearby might bring evidence of that odd quirk of animal psychology which prompts carnivore mothers in captivity to kill their young at the first suggestion of danger (Brown, 1932).

Expectant mothers should, therefore, be given special care and individual attention by the curator. Breeding and successful rearing of rare fauna of the world is one of the zoo worker's greatest challenge, and its accomplishment is the greatest reward.

Animal keeper or the animal attendant

An animal keeper or the animal attendant is directly responsible to both the curator and the head keeper. The general condition of enclosures and animals under his care are reported daily to the head keeper. He must frequently watch the behavior and condition of animals under his charge for signs of illness, injury, or breeding condition. He is often called upon to assist in crating, moving, and shipment of animals.

Animals must be fed and watered strictly according to the schedule and the keeper incurs full responsibility for mishaps occurring due to any irregularity. In small zoos, it is also the duty of the keeper to prepare and mix the food of animals under his charge. Food containers and drinking bowls must be cleaned and sterilized daily.

It is the duty of the animal keeper to keep the animal quarters and their adjacent areas scrupulously clean, especially during the visiting hours. It is extremely important for the keeper to know the proper disinfectants, vermin repellants, and other cleansing agents for different situations since some may be dangerous to certain groups of animals. Most zoos, however, are not in favor of using disinfectants for cleaning the animal quarters (personal communication, Freeman Shelly, Director, Philadelphia Zoological Garden). Carnivorous animals of the cat family, for example, are highly susceptible to phenol poisoning.

It is generally known among keepers of zoological gardens that the same animal may react quite differently, even in opposite ways, to two or more men of apparently identical characters. Brown (1932) gives an account of a one-man elephant at the Philadelphia Zoo which always disapproved of the keepers except her own regular one. A fine specimen of an ibex, likewise, took dislike to a new assistant keeper at Basle Zoo (Hediger, 1950).

Hediger (op. cit.), analyzing the situation on the part of the keeper said that a man from the very start may either be sympathetic or antipathetic to the animal. According to him, in the best interests of both the man and the animal, and for a better relationship between the keeper and the charge, the pathetic attitude must be as positive as possible on the keeper's side. It makes for avoidance of accidents, and simplifies running of an establishment.

Also, in addition to this pathetic and antipathetic attitude between man and animal, there are certain people who feel themselves unusually happy with wild or ordinary animals, while others meet the situation with consistent difficulties. Hediger further feels this character to be innate and one that cannot always be acquired with the right sort of training.

Katz (1937) states that animal behavior of the above mentioned kind and their way of reacting is of a more primitive nature and does not seem to represent the individual emotional experiences which the animal transfers from one category of people to another. He goes so far as to say that animal behavior of this kind may even provide a new basis for the typology of man. From the biological point of view, therefore, the keeper should be selected from men having sympathetic attitude and an innate inclination for animals. Wild animals are unusually sensitive creatures, and their happiness depends to a great extent on the good qualities of the keeper.

Another important aspect of animal psychology having a direct bearing on the management of wild animals in captivity is the assimilation tendency. The animal sometimes considers a man as one of its kind, and treats him as a member of the same species. It is sometimes difficult to differentiate this assimilation tendency with that of Lorenz's "imprinting" which was originally ascribed only to birds (Lorenz, 1935). In contrast to imprinting, assimilation occurs slowly in mammals, especially with older animals (Hediger, 1950). This animalizing tendency of the animal consequently incorporates man as a fellow member into the social organization which has both practical advantages and disadvantages. Assuming man occupies the α-position (premier position), the advantages of being accepted into their social organization are of several kinds. The man enjoys the full privileges that the α-animal in the group possesses, namely complete freedom of movement, and all inferior animals must obey his orders without question. Kohler (1931) reports that illness makes a drop down on the social scale, and so an especially strong attachment to man or the keeper is often noticed in sick animals, which is very useful for giving treatment.

The chief disadvantage of this animalizing tendency is that the man thus accepted by the animal is often involved in disputes over social precedence and is, therefore, bound to assume the premier position unless he is willing to give up permanently close contacts with the animal. It is fortunate for the man, however, that quarrels about social rank among higher animals do not always end with physical superiority. Another most unpleasant situation commonly observed in zoos as a result of the animal's assimilation tendency is during breeding season. Man thus favored by the animal is also considered as his sexual rival and they either try to fight over him or to mate with him. Roebucks always regard man as a rival of the same sex and would never miss a chance to attack him during breeding season.

OPERATIONAL PROBLEMS OF A ZOOLOGICAL GARDEN

Maintenance of native and exotic wild fauna in captivity requires a wide knowledge of various aspects of their biology and involves certain responsibilities, too. The knowledge so far gained in this respect is limited and scattered and one, therefore, will always find some gaps and frequent omissions. It is only by the close cooperation of all the biological research establishments of various zoos throughout the world that this imperfect outline could be perfected and improved. The subject matter under this heading is divided into five different categories and each discussed separately in some detail.

Problem of confined space and its biological significance

Confinement of space for life in captivity is one of the immediate and most controversial concerns of zoo husbandry men. The popular conception of "golden freedom" is purely a product of human imagination and is certainly not a biological fact. Neither the fish in its shoal (Breder, 1959), nor the monkey in its troop (Chance and Mead, 1953), is free as an individual. Carpenter (1942) significantly remarks that the monkey or ape in its natural group in the

tropical forests has its freedom of movement strictly limited by the structure of its group.

Hediger (1950) speaking about this anthropomorphism has said, ". . . how wrong, from many points of view, are anthropomorphic methods of observation in biology. It is also necessary to get rid of a lot of the anthropomorphic tendencies still current in the practical management of wild animals, and in the biology of zoological gardens." Analyzing the problems of confined space (bars, cages, fencings, and other means of confining animals) from the biological point of view, he said that to the animals its main significance may be one of the following:

1. Prevention of flight reaction: The animal attempts to get away from man made barriers not to liberty, or home, but away from its chief enemy--man, and his surroundings which are perhaps biologically unsuitable. There are several examples where, once released or escaped from their confinements, animals have voluntarily returned to their cages. The animal does not cease to regard man as an enemy immediately after capture. This decisive change of significance occurs only in course of getting used to the new situation or of becoming tame (Hediger, 1935).

2. Prevention of encounters--with animals of its own kind and/or others.

3. Limitation of territory: This ideal situation quite often is achieved in most zoological gardens. The space given to the animal is defended against new arrivals in the same way as they would do in nature. For this reason, it is quite risky to put fresh arrivals with animals that have already settled down. It is much easier to get strange animals accustomed to each other by bringing them together in a new home instead of introducing one to another already settled in the quarters.

4. Protection against encounters: And finally, a cage may mean to the animal a protection against unwanted contacts with animals of the same or different species. Sheak (1924) describes a very delightful observation made on Sally, a tame chimpanzee. Sitting in a chair one morning when a leopard roared ". . . the chimpanzee jumped to the floor, ran to the cage, lifted up the door, hurried in, and closed the door behind her. She recognized the fact that the cage was as good a contrivance to keep enemies out as to keep her in."

For display purposes, wild trapped animals are always inferior to zoo bred specimens. Having known "freedom," it takes longer to get used to the new situation, sometimes only at the cost of serious losses. Each animal lives in its own specific world. The environment offers, as it were, a reservoir of stimuli from which the subject constructs its own specific world. By capturing it we utterly destroy the animal's previous world, and put it into a different situation. The animal must construct an entirely new world. This means an enormous task on the part of the animal, one which is not easy for every animal to tackle successfully. The transitional phase, from freedom to captivity, has been discussed in detail by Hediger (1950).

<u>Housing and display of animals</u>

Broadly speaking, there are two possibilities in which the animals in a zoological garden could be housed and displayed:

1. Adjustment of the animal's locomotor capacity to the given space;

2. Adjustment of the space according to the animal's locomotor capacity.

The first category consists of such mechanical devices as hobbles, nose-rings, collar chains, and ropes by which the animal's locomotor capacity can be accommodated to a confined space. Such devices, although usually applied solely to domestic animals, may be suitable for certain groups of zoo animals such as camels, elephants, and ponies.

Furthermore, in birds, the primaries of one side of the wing are clipped to cause asymmetry and thus restrict their flight movements. It causes the bird to fly in a circular course, or to drop back to earth, and they cannot escape. In the Calcutta Zoo, this was the usual practice for most birds of the family Anatidae kept in open enclosures. Since the clipped feathers grow again the following molt, the process must be repeated at least once a year for which the bird must be caught again, with all the accompanying disturbances and the risk of injury involved. To alleviate this probable danger, there is an operation called pinioning often performed on one of the wings of the birds.

The operation, as described by Young (1948), consists of exposing the metacarpal of the hand by making the incision at right angles across the wing just external to the outer wing joint. The metacarpal bones, excepting the thumb, well above the joint, are then severed together with the attached muscles and the wound is closed by appropriate sutures. This causes the wound to heal rapidly, giving a smooth appearance on the end, nicely covered with feathers, and leaves a presentable bird for exhibition. The birds, however, should be kept quiet after the operation or profuse haemorrhage, sometimes with fatal results, may

happen (personal communication, Dr. M. D. Pirnie, Mich. State Univ.). It is therefore advisable to do this operation when the birds are quite young.

There is no indication of post-operative complications, however, Lorenz (1940) reported a striking change in behavior in one grey goose. After the operation, the bird showed symptoms of severe depression with marked loss of appetite and went so much "off condition" that breeding activity was almost nil the following spring. This, however, may be an isolated case due to individual idiosyncrasy and may not justify a sweeping generalization.

The only disadvantage of this operation is that the birds are mutilated, even though slightly. To overcome this difficulty, several workers have suggested an alternative called radial-neurectomy or tenotomy (Young, op. cit.; Sperling and Faber, 1951). Under a suitable anaesthetic, a small piece of the radial nerve supplying this appendage is removed. The bird's skeleton remains completely intact, and so does its normal appearance. The operation simply prevents the bird from stretching out its wing to its full length causing asymmetry as a result of which the bird cannot fly. In powerful fliers (pheasants and gulls), however, this operation does not seem to help very much.

According to Hediger (1950), not all zoo birds are pinioned. Among the medium and larger kinds, it is usually the Anatidae (geese and ducks), the flamingos, the waders and the web-footed birds, and less often the Phasianidae are treated. Because of their marked sedentary nature, it is often simple to induce the Phasianidae and the peacocks to settle down in a particular place. This is also true with domestic pigeons because of their marked homing instinct.

Means of confinement under the second category (page 27) are aimed at adjusting the space according to individual's motor capability and not the other way around. These may further be grouped into:

a) Psychological or imaginary--true only for circus animals and not practical in zoos. The animal, by special training, learns to respect the ring or other such barricades which present a psychological barrier to the animal.

b) Natural--such as pits, moats, ditches and land-water barriers. These are called natural because similar obstacles are usually met with in nature.

c) Semi-natural--such as fencings, bars, wire nettings. These represent a close network of branches and dense creepers of the wild which the animal usually avoids because of possible injury.

d) Artificial--such as glass, light, and electricity. These are not encountered in nature as such.

The pit is the most primitive way of confining animals, both literally and historically. Palaeolithic man made use of this, at least temporarily, as a means of confining wild animals prior to killing for food. There is evidence of keeping bears, and even large mammoths, in hollows of the ground from where the animals could neither jump nor climb. Pits present a vertical barrier in contrast to ditches which are horizontal. There are still some older zoos in different parts of the world where such enclosures are used especially for such large carnivores as bears. The Zoological Garden of Lucknow, India, still has some large reptiles on display in straight-walled pits.

Moats differ from pits and ditches in the fact that animals do not live at the bottom. Moats surround modern, free enclosures on the level ground, the area of which depends upon the locomotor capacity of the animal. These moats may be dry or filled with water, when they serve as bathing pools.

The greatest difficulty encountered in this respect is that every species of animal has its specific type of locomotion and each has its own maximum capacity for

overcoming obstacles in both horizontal and vertical directions. In the construction of such "open cages," therefore, it is important to know the maximum locomotive capacity of the animal concerned. Unfortunately, accurate judgment or calculation of this critical maximum capacity for each species is not easy, and underestimation has led to a series of deplorable accidents (Mottershead, 1960). Fundamentally, the only sound method is to work out the capacity for overcoming the obstacles for as many individuals of a species as possible, using observations taken from the wild as an additional check. Currently, the London Zoological Garden is experimenting with over 20 different species of mammal to determine their maximum jumping distances (Morris, 1962).

Hediger (1950) has further warned that the capacity of an animal to clear an obstacle in a normal, balanced state of mind is far less than that which happens during extreme conditions of stress and excitement. This excitement factor, therefore, should always be considered while planning an open enclosure.

Bigalke (1961) recommended dry ditches or moats for confining wild animals because of the prohibitive cost of water filled ditches. Dimensions of moats required for various species of animals, including large carnivores, are illustrated in the second volume of International Zoo Yearbook (Anonymous, 1961).

Such water-land barriers, however, are not ideal for smaller cats simply because it would interpose too great a distance between the observer's eye and the relatively small size of the object; also, even a small cat can jump a considerable distance. Leyhansen (1962) cites an example where a serval (Felix serval), a long-legged African wild-cat, from a standing start, twice jumped a ditch 15 feet wide and landed 4.5 feet above the level on the opposite bank. For this reason, the improvements in the display of small cats were unmodified and they are still kept in much the same way as in the menageries of the Middle Ages. Leyhausen (op. cit.) has described a most modern ground plan of a small cat house at the Wuppertal Zoo, Germany, where the minimum area required for a pair of serval or caracels (Felis caracal) is 50 sq. yds. of outer enclosure and 12-15 sq. yds. of indoor cage. A pair of Indian leopard cats (Felis bengalensis), however, might do well with slightly less, but by no means as much less as would be proportionate to relative size. It is utterly wrong to think that smaller cats require less space than a tiger or a lion. If anything, they need more which also holds good

both for the indoor cages as well as the outside enclosures.

In designing and constructing animal enclosures, all practical measures must be aimed at making the animal feel safe and at home, and at the same time giving it enough opportunity for movement to keep them busy. In the fittings of indoor cages, therefore, a variety of tree trunks with at least some of their branches still intact, pieces of rock or equivalent, and some relatively broad boards at various heights on the walls for sitting and resting, should be liberally used. All these things must be arranged in such a way as to suggest to the occupant a great variety of pathways throughout the three dimensional space available. With a little skill and experience, these can be arranged giving a maximum sense of protection without unduly obstructing the visitor's view.

The semi-natural methods of confining wild animals include devices such as railings, bars, fencings, and wire nettings. With advancement in the display techniques, these devices are being rapidly replaced in many zoological gardens. In the case of big cats, bars have already been replaced by wide ditches and moats, or by much lighter, but sufficiently strong, interlocking wiring. This interlocking wire netting has another advantage of rebounding the animal unhurt when, under the impulse of great excitement and escape, they dash against it. Also, it obstructs the visitor's view less than a fense or a heavy iron bar cage.

Among the artificial means of confinement, glass and light seem to be the indespensable items of construction. Because of its transparency, glass is the ideal material for most small animals, especially fish, reptiles and amphibians. Also, behind glass panes, almost any type of climate desired can be produced. In addition, glass seems to be the only solution for undesirable feeding by the public and subsequent dangers of picking up infection.

The widest use of glass, however, is made for the fishes which, by the possession of their lateral line, are at a great advantage over amphibians, reptiles, birds, and mammals. This lateral line appraises them of the presence of the glass from a distance, thus enabling the fish to avoid direct collision. Other groups of animals, however, have no visual means of detecting glass panes. In spite of this drawback, more and more birds and mammals are now being kept and displayed behind glass. Birds learn by experience and discover the exact dimension of their flying space by bumping into it. Once they overcome this difficulty, they turn

smartly away close to the glass, but in conditions of stress and excitement such as a chase during breeding season, may disregard this space barrier and crash at full speed.

Despite these limitations, birds may get so accustomed to glass cages that they completely avoid this invisible barrier. This fact, biologically speaking, provides a direct solution of the problem of confined space. With birds fully accustomed to flying behind glass plates it is possible to remove the panes altogether, without the birds flying away. This psychological barrier is further reinforced by the action of light where the inside of the cage is kept bright while the space outside, for the public, is dark. This type of aviary--with no barrier between the exhibit and the spectator--has only recently been devised and is now successfully used in Frankfurt Zoo, Germany (Anonymous, 1962), Wassenaar Zoo (Gerrits and Diedruch, 1962), San Diego Zoo (Anonymous, 1961a), Zoological Garden, Philadelphia (Anonymous, 1949) and at Basle Zoo, Zurich (Hediger, 1950).

Methods of exhibiting and caring for great apes in captivity have also improved considerably during the past two decades. These methods are based on the knowledge that these animals, being extremely susceptible to cold and influenza viruses, and even to tuberculosis which leads to dangerous and often fatal illnesses, must, above all, be protected from infections carried by man. A glass screen erected in front of both indoor and outdoor cages effectively excludes these elements of danger. In the ape house of the Antwerp Zoo, this partition consists of "Polyglass." It is a sheet of glass 2 cm. thick to which a similar sheet 1 cm. thick is attached by gluing at the edges. The 1 cm. space in between the two sheets is filled with dry air. This device acts as an insulator against heat and noise and also prevents condensation. The strength of such a sheet of glass was tested at the Ghent University laboratory where it was established that it could withstand the weight of a 300 kilo gorilla charging from some distance above the ground, five feet away (Bergh, 1960). Presence of glass panes, however, causes light reflection which makes it difficult for the visitors to see animals behind their own reflected images. This could easily be prevented by fitting these panes at slightly tilted angles.

Another great stride toward exhibiting nocturnal animals has been made lately by the use of red light. It is based on the information that the retina of man contains two types of cells involved in visual perception. The first, which discriminate color and are excited only by relatively intense light, are called the cones. While the rods, the second type of cell, are extremely sensitive to even minute amounts of light, but do not respond to color as such. Rhodopsin, the photosensitive pigment of the rods, however, responds differently to lights of different wavelengths. Its maximum sensitivity lies in the range of green or blue-green, falling sharply toward the red end of the spectrum where sensitivity is almost nil (Walls, 1942). Red light, therefore, effectively shuts out the sensitivity of the rods, allowing the man to see clearly by means of his cones.

This duplicity of visual cells, however, is not shared by nocturnal animals, who have, exclusively or nearly so, only the thin and slender rods in their retina. Southern (1955), for the first time, utilized this knowledge in watching the behavior of nocturnal animals in his study to know the predator-prey relationships among birds and mammals. In the course of his study, he also found that badgers and foxes are also blind to red light and he thought this might be true with most of the carnivores who hunt during night.

In early 1961, the New York Zoological Park started a series of experiments designed to reverse the normal activity cycle of small nocturnal mammals and render them visible during the day in red light. The success of these experiments is evident from the fact that today 39 species of mammals, representing nine orders and 21 families, have become active by day under this red light activity reversal program (Davis, 1962). These animals are on display in the Small Mammal House of the New York Zoological Garden. The red light room operates on a 12/12 hour cycle, the "night" cycle beginning at 1100 hours and the "day" cycle at 2300 hours. The 11 o'clock onset of the "night" period allows for the keepers to clean the enclosures under white light of the "day." Under red light, a dirty floor is not clearly visible and makes the cleaning difficult. The principal advantage of such red light exhibits is that not only does the public watch the animals moving around, but keepers also are better able to watch the physical condition of their charges during normal working hours.

The only objection to this type of exhibition is that, in some instances, only a very dim colored light is used which gives rather a gloomy picture to the visitors. In order to avoid this trouble the Zoological Garden of London is currently experimenting with some small mammals trying to reverse their daily cycles under normal lighting conditions (Morris, 1962). The assumption is that the daily rhythm of small nocturnal mammals is based, not upon the absolute light strength, but on the relative strength of light present during the day and during the night. If this is true, it is possible to reverse their cycles by keeping them in slightly less light during the day, without changing the quality of light, and giving more intense and bright light during night. The results have thus far been encouraging, and it seems feasible, at least with certain species, to allow the public to view such animals in much stronger and more nearly normal lighting.

Problems of food and feeding

Feeding of wild animals in captivity is not a physiological matter alone, but is intimately tied up with their social and psychological conditions. The knowledge gained in this field during the past decade has been tremendous and the results include the increased life span, reduced mortality, increased resistance to various diseases, complete disappearance of almost all the deficiency and malnutritional diseases, and healthy, good looking animals in most zoological gardens of today. This knowledge has been derived directly from the scientific feeding of domestic animals where it is possible to correct most deficiency diseases by proper feeding. Despite a large variety of animals in zoological gardens, this is not impracticable, for the science of nutrition is based on the fact that all animals, from single cell amoeba to large mammals, need the same broad groups of nutrients, i.e., proteins, carbohydrates, and fats. Vitamins and minerals essentially play the same important roles in almost any species of animal as they do in humans and their domestic pets.

Wackernagel (1961), in his brochure "Modern Methods of Feeding Wild Animals in Zoological Gardens," cites several references where the importance of vitamins and minerals is shown in a variety of wild animals including birds, mammals, and reptiles. Scheunert (1933) believed that the "star gazing disease" of young tigers is caused by Vit. B_1 deficiency. Sievers (1957) studied in detail the importance of Vit. A, C, D, and the B-complex group in the periodical skin shedding of snakes.

Despite vast experience gained in the field of animal nutrition, the knowledge has not yet been fully exploited in the development of practical methods of disease control and maintaining healthy animals in zoological gardens. According to Hediger (1950), this problem has, so far, "been too one sided, too physiological and often anthropomorphic." Quite often, people fail to realize that the mechanics of providing high quality diets to zoo animals are much simpler than one would ever realize. For example, the ration for all the 1,595 animals of the Philadelphia Zoological Garden is prepared by just two men, who also pack and make allotments according to the exhibition area (personal communication, Freeman Shelly, director, Philadelphia Zoo). Also, we must get rid of the old belief that most wild animals are stenophagous or even monophagous. Leopold (1933) quotes several examples where tremendous varieties of food are eaten by most game species. Schaller (1963) listed a variety of food taken by the mountain gorilla in the wild. He, however, failed to find a single occasion of gorillas feeding on animal matter, which is quite contradictory to the belief of those who maintain zoological gardens. According to Freeman Shelly, meat is one of the indispensable items in the menus of the great apes. Chimpanzees, according to Goodall and Van Lawick (1963), have definitely carnivorous tendencies in the wild and, among others, they seem to prefer monkeys. Killing and tearing of the flesh is done by both the juveniles and the adults.

Quality and quantity of food

Considering the fact that no animal is strictly monophagous, careful attention should be given to the substitute diet. It must contain all the necessary nutrients as well as essential vitamins and minerals in proper proportions. A balanced diet promotes healthy growth and builds up a strong natural resistance against infectious and non-infectious diseases. This is evident from the fact that enteric diseases, bacillary dysentry, amoebiasis, balantidiasis, strongyloidiasis and esophagostomiasis in apes and monkeys of Philadelphia Zoo are successfully controlled and cured by just routine feeding (Ratcliffe, 1963). Also, according to this author, the lung mites of Asiatic monkeys disappear and the old lesions heal up within a couple years on the present diet.

For convenience, the animals of a zoological garden may be grouped as herbivores, carnivores, omnivores, and piscivores.

Feeding the herbivores

Herbivores, especially ungulates from various ecologic niches, constitute the major collections of most zoological gardens. These are represented by ruminants such as camel, llama, deer, giraffe, antelope, sheep and goats. Other members of the ungulates are the elephants, rhinoceros, tapir, all members of the horse family and the pygmy hippopotamus. The kangaroo, and a few rodents such as chinchilla, also are herbivorous. Hay of good quality should be given ad libidum to all of these animals, supplemented by a suitable concentrate. Hay, besides providing bulk, is a means of occupying the animal's attention and energy, and prevents restlessness. It also assists in preventing obesity. The concentrate mixture is of great importance, as the hay is usually not a complete diet, and the supply of fresh greens or pasture is usually limited or absent in a zoological garden. Below is the composition of a well balanced concentrate mixture designed for the herbivorous animals of the Basle Zoological Garden (Waekernagel, 1961):

Ground oats	15	Carob bean meal	4
Ground barley	27.2	Ground calcium carbonate	1
Ground grain sorghum.	8	Bone meal	2
Ground wheat	10	Salt	1
Soya bean oil meal. .	10	Magnesium sulphate . . .	0.2
Groundnut oil meal. .	8	Trace elements mixture .	0.1
Linseed meal	3	Vitamins mixture	0.5
Lucerne meal	10	Total	100.0

The composition of this mixture in terms of proteins, fats, and other nutrients is:

	Per Cent
Crude protein	18
Crude fat	2.5
Crude fibre	6.5
Calcium	1.2
Phosphorus	0.6

This mixture is made into pellets and is given to all the animals at the rate of 0.5 to 2 ounces per pound of body weight, depending upon the size and general condition. A roedeer thus receives about a pound, while four to five kilograms is correct for a wisent.

Feeding the carnivores

Fresh meat seems to be the indispensable item of food for carnivores and most birds of prey. O'Connor (1944), however, claims that lions and other carnivores may be maintained on the dry mix she recommended for omnivores. Muscle meat by itself is not completely balanced nutritionally. Supplementation of this meat, therefore, is justified not only from a scientific point of view but also from the observation in the wild where the animals consume the whole of their prey and not just the meat alone.

All meat fed to animals in the Basle Zoo is supplemented with a protein-mineral-vitamin concentrate in the proportion of 7 of this concentrate to every 93 parts of raw meat used. This supplement consists of 9 parts of dry yeast, 9 parts of skimmed milk powder, and 3.5 parts of mineral-vitamin mixture (Wackernagel, op. cit.). It is mixed either with the minced meat or sprinkled on the pieces of meat as they are fed to the large beasts of prey. Because of its palatability, it is liked very much by all the animals; also it adheres well to the chunks of meat. This feed is given to mammals and owls and to several other birds of prey.

The meat supplement for carnivores in the Philadelphia Zoological Garden consists of oystershell flour 50 parts, powdered skimmed milk 45 parts, and iodized salt 5 parts. This is mixed with raw meat in the quantity of 12 to 13 parts of the supplement to every 86 or 87 parts of the meat and 2 parts of Vit. A-D feeding oil. This is given at the rate of 0.5 to 2 ounces per pound of body weight (Ratcliffe, 1963).

For adult members of the Felidae, raw meat (preferably horse meat) with 1-4 ounces of the above supplement is given at the rate of 20 to 100 gm/kg of body weight. Leopards, cheetahs, and jaguars are fed a whole chicken (2 to 3 lbs.), with bones, once or twice each week. Smaller cats are given pigeons or ducklings once or twice a week. This change of diet is necessary in maintaining an animal's interest in food and also to improve its appetite. Raw liver acts as a laxative and stomachic and is relished by most animals. It is always given when animals are constipated and subsequently lose their appetite.

Feeding the piscivores

Fish-eating animals like sea lions, penguins, pelicans, and cormorants present a different situation. Fish, which are commercially obtained, may not be adequate for these animals, but according to Ratcliffe (*op. cit.*), there is no practical solution by which fish may be improved. Zoos, that are able to obtain oily fish, like herring and mackeral, however, report better results with fish-eating animals.

Penguins present a constant problem to most of the zoological gardens. They suffer from a special type of intestinal catarrah almost always accompanied by mycosis. Sladen's (1954) observation of penguins in the wild and in captivity indicates that these animals are remarkably free from disease in nature and it is only when they are brought into captivity that they become susceptible.

Wackernagel (*op. cit.*) believes that food-fish for the animals may, during transportation, lose some micronutrients due to decomposition and produce toxic substances causing intestinal catarrah and subsequent mycosis. He, therefore, suggests to fortify the fish diet and treat the fish with a bacteriostat. For this the fish on arrival is submerged into a solution of acromize (chlortetracycline) and stored on ice. His mineral-vitamin mixture consists of:

Standard mineral mixture	5 kg
Vitamin preparation	860 gm

The above vitamin preparation contains:

Vitamin A	3,000,000 IU/kg
Vitamin D_2	600,000 IU/kg
Vitamin E	60,000 IU/kg
Vitamin B_1	700 mg/kg
Vitamin B_2	2,300 mg/kg
Nicotinic acid	9,000 mg/kg
Calcium D-panthothenate	3,500 mg/kg
Vitamin B_6	1,200 mg/kg
Folic acid	300 mg/kg
Vitamin B_{12}	35 mg/kg

About 5 grams of the above mineral-vitamin mixture is put into a gelatin capsule and fed to penguins and sea lions at the rate of one to two capsules per animal. These capsules are put into the fish before being offered and are thus readily taken.

Feeding the omnivores

The diet developed at the Philadelphia Zoological Garden for omnivorous animals seems to be simple and quite adequate for a variety of animals. The basic diet consists of the following ingredients (Ratcliffe, op. cit.):

Ground yellow corn	15
Ground whole wheat	15
Ground whole barley	10
Ground rolled oats	10
Peanut meal	10
Soybean	10
Alfalfa meal	5
Brewer's yeast	10
Dried skim milk	10
Oystershell flour	2
Iodized salt	1
A-D feeding oil	2
	100

This mixture is prepared in bulk but never stored for more than two weeks. Nine parts of this are combined with one part of ground, boiled meat and sufficient meat broth or water to make a stiff mash. This is thoroughly mixed, pressed into shallow pans, refrigerated for 24 to 48 hours, and cut into pieces for feeding. Ordinarily, it is supplemented by oranges and carrots for apes, and carrots, kale or escarole for monkeys, baboons, and other animals. This ration supplies approximately four calories per gram and is given at the rate of 1 to 10 g/kg of body weight, depending upon the age, care and condition of the animal. This ration, with or without slight modification has been found to be completely adequate for the following mammals and birds at the Philadelphia Zoological Garden:

Family	Mammals
Pongidae	Gorilla, chimpanzee, orangutan
Cercopithecidae	Baboons, Asiatic monkeys including langurs, African monkeys including colobus
Lemuridae	Lemur
Cebidae	Cebus, spider, squirrel, and wooly monkeys, saki, uakari
Hylobatidae	Gibbon
Callithricidae	Marmoset[1]
Castoridae	Beaver
Muridae	Rats and mice
Cricetidae	Hamster
Chinchillidae	Chinchilla
Hystricidae	Old world porcupines
Erethizontidae	New world porcupines
Ursidae	Bear[2]
Procaviidae	Hyrax
Suidae	Swine
Tayassuidae	Peccary
Bradypodidae	Two-toed sloth

[1]For debilitated apes and monkeys, equal parts of this ration are mixed well with equal parts of carnivore ration (page 46) and finally ground in a meat grinder.

[2]Half of this ration is mixed with equal parts of carnivore ration (page 46), fish, suet, and carrots. For the polar and kodiak bears, 4 to 8 ounces of peanut oil is added to the carnivore diet.

The omnivorous diet discussed on page 49 has been found completely adequate for the following birds:

Family	Birds
Anhimidae	Screamers
Anatidae	Ducks, swans, and geese except those that eat only fish
Gruidae	Cranes and their allies
Rallidae	Coots, gallinules
Columbidae	Doves, pigeons
Tetraonidae	Grouse
Numididae	Guinea fowl
Meleagrididae	Turkeys
Struthionidae	Ostriches
Dromiceiidae	Emus
Rheidae	Rheas
Casuariidae	Cassowaries[1]
Phoenicopteridae	Flamingos
Psittacidae	Parrots, parakeets, lories, lorikeets, macaws, keas, conures, cockatoos and cockatiels[2]

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[1]Equal parts of omnivore and carnivore diet.

[2]Larger psittacines are fed on a mixture of 2/3 omnivore ration and 1/3 carnivore diet. For small lories, cockateels, and parakeets, half of the omnivore ration is mixed with 20 parts of ground raw carrots, 20 parts of ground cooked meat, 8 parts of ground hard-cooked eggs and shells, and 2 parts of A-D feeding oil. The final ration, thus prepared, is mixed with some seed, otherwise birds do not seem to recognize this ration as food which may reflect their innate behavior pattern.

For flamingos, 90 parts of the omnivore ration and 10 parts of ground boiled meat are mixed thoroughly and moistened with fresh pulverized carrots and carrot juice (1 gallon to 20 pounds of dry weight), refrigerated and used within 6 hours. 0.5 to 0.75 pounds of the ration divided into morning and evening meals is given to each bird per day.

Freshly ground carrot and carrot juice are essential for retaining plumage colors of scarlet ibis, flamingos, and other birds having carotenoids (red, yellow, and orange pigment) in their feathers. In the Basle Zoo, flamingos are provided with 1 per cent of fat-soluble extract of lucern to preserve their colors.

Social and psychological problems in feeding

Feeding wild animals in captivity, as said earlier, is not simply a physiological matter, but is closely bound up with the animal's social and psychological state. Flight reaction is the most significant characteristic of the wild animal's life in freedom, and it must be given prime concern in captivity, especially with newly captured ones. With untamed animals, flight comes before feeding.

Feeding freely and satisfactorily in the presence of man is one of the most important proofs that an animal has settled down. Many animals, from fish to mammal, have been known to go on hunger strikes in reaction to captivity. Brown (1932) cited such an example in an aard-wolf which did not eat or drink for several days when first brought to Philadelphia Zoological Garden. Hediger (1950) recommends that in such situations a restful atmosphere with lack of disturbance and darkness is quite helpful. Force should always be avoided as it only aggravates the animal's excitement.

It is a common experience that the amount of food eaten by an individual can be greatly increased if fed in the company of other members of the same species, or of other animals accepted in their social organization (animal's assimilation tendency). The influence of this companionship factor can be traced through all the vertebrate kingdom, from fish to mammal.

The effect of social factor on food intake has been the subject of intensive researches, especially on hens. Katz (1937) states that under the influence of the social factor the food intake in chickens can be increased over 60 per cent. Conversely, isolation of individual chimpanzees from a social group caused fasting for several days. In the best interests of the animal, therefore, it is essential not to keep them isolated. Lang (1960) said that all great apes are social animals, and it is important not to keep them isolated. Especially when young, they should be brought up in the company of their own kind, or of a closely related species. Slow orangs and gorillas, for example, are favorably influenced by the lively chimpanzees.

Another important aspect of the biology of zoological gardens is the feeding pattern in different animals. Hediger (1950) distinguished between two extreme types of feeding in zoo animals. These are the continuous and the occasional feeders which are found, again, throughout the animal kingdom, from fish to mammal. The continuous feeders are represented by plankton-eating fish, toads, tortoises, passerine birds, rodents, ungulates, elephant, brown bear and monkey. Examples of occasional feeders are predatory fish, snakes, birds of prey, and great cats.

Intake of food, like any other biological activity of the animal, is part of its space-time pattern. The free-living giant panda (Ailuropus melanoleucus), for example, spends from 10 to 12 hours to take sufficient food. It wanders along its habitual tracks in comparatively small territory of about one square mile, eating on bamboos, which are relatively poor in nutrition. Apparently, the chief feeding period is at night and thus the food intake, in this animal, is spread over time and space. Another extreme example perhaps is found among snakes which await at a particular spot until a victim appears. In a few moments this is captured, swallowed, and the animal retires until the victim is completely digested.

These occasional feeders react quite differently to the food stimuli than do the continuous feeders (bear, sea-lion, elephant). This power of food attraction, therefore, may be used for drawing the animal's attention and increasing the intimacy of the animal-man relationship. Continuous feeders, while training, must be rewarded for accomplishing every single performance with food such as sugar for bears, and fish for sea-lion. Training sea-lions without fish, and bears without sugar, says Hediger (op. cit.), is unthinkable. In occasional feeders like lions and tigers, however, food stimuli play no part at all.

Exercise and training

Considering the fact that wild animals are, in nature, constantly engaged with the impulses to avoid enemies and to seek food, and that both these highly significant elements suddenly disappear in captivity, it is obvious that the change must have some far reaching consequences. Since the captive animal's most important occupations are taken away from him, enormous amounts of energy are thus released, and these must somehow be utilized.

Apparently, the most immediate problem in the biology of zoological gardens arises from the want of occupation. Lack of engagment may have some harmful results, manifesting itself in various ways. Some of these secondary effects of captivity appear in the form of excessive anti-social behavior, hypertrophy of sexual activity, and stereotyped movements. The animal, in order to break through the boredom of life, resorts to several means of self amusement and to subsequent vices. These, in turn, may lead to such symptoms of captivity as reduced body size, muscular dystrophy, lighter skeleton etc. The captives, therefore, must be given a new interest in life, an adequate substitute for the chief occupation of freedom. A biologically suitable training, which also provides a sort of occupational therapy, seems to be the best solution.

Zuckerman (1932) believes that regular provision of food in zoological gardens releases energies in the animal which, in the absense of proper occupational therapy, are used up probably in more intense sexual behavior. This is much more so in those animals which breed continuously, or nearly so, the whole year around (e.g. giraffe and anthropoid apes). Since sexual behavior is closely connected with social behavior, this hypersexuality has far reaching effects in the biology of zoological gardens. Hypersexuality in captives makes possible an increase of socially conditioned fighting where the social rivals cannot get as far away from one another as in nature. Thus, after a fight, both the victor and the defeated partner still cannot lose sight of each other, and this state of affairs often leads to the conflict never being settled as long as two rivals live together. In freedom, the animals separate and can thus avoid a final solution of the conflict. But the only real solution in captivity seems to be the death of the rival. Zuckerman (*op. cit.*) gives several examples of serious losses from socially conditioned fights of this kind in a colony of captive hamadryad baboons (*Papio hamadryas*). The chief conclusion to be drawn from this fact is that it is often more suitable, and more correct biologically, to keep those species which fight under social stimuli in smaller, harmonious groups, and keep them busy by providing suitable training exercises. Naturally, the more highly organized is the captive animal, the more its lack of activity will be noticed. No wonder, then, that training exercises were first tried out on apes.

Training, however often it is used as a scientific auxiliary, was not a laboratory invention. It represents an age-old way of managing animals. It was used by primitive men, as it still is used intuitively by the trainers in the modern circus. Training as a scientific aid, as far as the fundamentals are concerned, is not even a century old, but these have certainly been borrowed from the circus or its forerunners. Hediger (1950; 1955) distinguished all the intermediate stages between freedom and domestication through which an animal has to pass in the course of being tamed or trained. Training, according to Hediger (1938), represents a transitional stage through which the animal passes to reach the fullest kind of relationship with man; the state of being completely trained.

In scientific training, the elimination of the animal-man relationship is at its maximum while in a circus this relationship is at its extreme intensification. This is quite natural considering the amount of money circuses spend on staff and the type of performance required from their animals. In circuses nearly every animal is almost continually in close contact with his trainer, the riding master, the groom, and the watchman. In the zoos, however, each keeper is assigned to a large area, covering several animals, and he has little or no time to spend on separate individuals. Zoo training, therefore, should be limited to simple exercise training in the best interest of the animal, as a part of occupational therapy; performance of high standards and sensational showmanship should be left to the circus.

The need of occupation evidently is found not only among apes but in many other species, for example sea-lions, many predatory animals, ungulates, birds, and even certain reptiles and fishes. Hornaday (1936) listed the following species, in order of their importance, whose zoological park training is a matter of necessity: elephants, bears, apes, hippopotami, rhinoceroses, giraffes, bison, musk-ox, wild sheep, goats and deer, African antelopes, wild swine and wild horses, asses and zebras. Of large birds the most conspicuous candidates for training are the ostriches, emus, cassowaries, cranes, pelicans, swans, egrets and herons, geese, ducks, pheasants, macaws and cockatoos, curassows, eagles and vultures. Among the reptiles, the best trained are the giant tortoises, the pythons, boas, alligators, crocodiles, iguanas and gopher snakes.

In most zoological gardens, sea-lions are seen taking hold of a piece of wood floating in their pond, tossing it up and catching it again. Many predators, too, appear to enjoy interruption of their monotonous life; and for this they are often provided with big wooden balls for play. This also causes regular wear and tear of their claw nails which otherwise overgrow and cause sore pads.

Many zoo animals that are kept in spacious enclosures can sometimes find their own specific games at will. "Mohan," the chimpanzee at the Calcutta Zoological Garden, for example, had a small table and a chair. Every day during lunch hour he would set his dining table and chair properly on the lawns of his island, sit straight on the chair, and would start his lunch from the bowl and the plate brought him by the keeper. After the meal, he would take the table and chair back to their position and would indulge in all sorts of acrobatics, including swing and horizontal bar exercises.

Through play and training, contact between man and animal is so intensified that often man is eventually accepted as a member of the species, and incorporated into the social structure of the group. This intimacy often has several practical advantages. The above mentioned chimpanzee of the Calcutta Zoo, for example, once suffered from a bad type of intestinal tuberculosis. The keeper used to bring the prescribed medicine to him in paper folds. The chimp would voluntarily accept the medicine, open it up, put it into his mouth, and chew up the whole thing without losing even a single grain. The result was a quick and complete cure without much trouble. This is quite unnatural as it has often been observed that no ape in captivity would eat even the best food offered to him once he has the slightest hint of some medicine mixed in it.

In addition, the material value of an animal, or of a group of animals, can be greatly increased through training because groups of trained animals have more show value than the untrained ones.

Morris (1961) has recently described an automatic feeder "Mark II Seal Rotor" for the gray seal of the London Zoo. These seals are housed in a large circular pool with rails installed around its rim, on which travels an electrically driven box with a capacity to travel up to 30 miles an hour. A fish-release button opens a series of flaps on the underside of the box as it moves around the pool, which could be pressed at any point in the run. This makes it impossible for the seals to learn exactly where the fish will appear, and forces them to follow the box competitively, in order to be the nearest one when a fish falls into the water.

In the improved variety of the "Rotor," the fish, instead of falling in the water, simply hang in a group from traveling arm. The seals, thus, have not only to chase after the box, but also have to leap repeatedly out of water to snatch the fish. This rotor, in addition to providing the seals with plenty of exercise, has become a major "feeding time" attraction for the public.

The London Zoo is currently experimenting to improve the design of this apparatus for a variety of species in which the animals will be required to perform various activities to obtain food.

Disease control and animal restraint

If wild animal reserves are to be built up, both to maintain the disappearing species and to provide a protein bank for the world's ever-growing population (Halloran, 1955), it is essential that scientists should learn more about the general welfare and disease problems of these animals. The popular conception that wilderness areas produced a healthy lot of animals is quite mistaken. As a matter of fact, there is not a single group of animals that is spared. Captivity, however, through numerous physical and mental derangements, often aggravates the situation. Unfamiliar, unnatural and unvaried food, change of climate and environment, physical and mental degeneration from disuse of muscle and brain, fear, ennui, nostalgia, lack of exhilaration of chasing and being chased, often unsatisfied sexual desire--all comprise a complex social and biological environment for the animal and react harmfully on the captive. To study the actions and interactions of all these elements in the vast array of the animal kingdom, a zoological garden provides the best laboratory.

During recent years, the interest taken by the veterinary profession in the diseases of non-domestic animals has been tremendous. Halloran (op. cit.) published a connotative bibliography of references to diseases in wild mammals and birds, both feral and captive, obtained from over 900 different publications throughout the world, covering a period of 120 years (1830-1950).

Graham-Jones (1962), in his concluding remarks on the 4th International Symposium on diseases in zoo animals, said that the reasons for these rapidly growing interests are: economic, ethical, a healthy competition among zoos to exhibit contented and disease free stock, and the study of various diseases mutually communicable between man and animals. This last impetus, thus, has led to establishment of various institutions of comparative medicine and comparative pathology throughout the world.

Since the time of Darwin, who systematized the world's knowledge of comparative biology, pathologists have been trying to explain the abnormalities in the light of Darwin's principles. Bland-Sutton's work, "Evolution and Disease," as early as 1890, was probably the first step in this direction.

On the occasion of the celebration of the 50th anniversary of Darwin's great work, "Origin of Species," ilya Metchinikoff, in his address at Cambridge, explained how the idea of evolution stimulated the discovery of phagocytosis and the development of the comparative pathology of inflammation. The study of evolution has proved that all living beings on earth are related to one another through origin from common ancestors. It presents an obvious basis for the suggestion, then, that certain pathological processes may appear in their most primitive forms in lower organisms, where their expression is particularly favorable for analysis.

Huxley (1958), in his book, "Biological Aspects of Cancer," strongly defended the idea that cancer is not merely a medical problem; it is a biological phenomenon, and its elucidation is closely connected with advances in a number of key branches of present-day biology. Despite these realizations, the application of evolutionary principles to the practical problems of pathology is still in its infancy.

Role of veterinary services in a zoological garden

The importance of veterinary services in the biology of zoological gardens can be justified for two reasons:

a) Maintenance of good, healthy looking animals free from disease or disease carrying agents. This includes the therapeutic as well as the preventive medicine.

b) Applying scientific methods of animal restraint in the day to day zoo-husbandry work. It includes both the mechanical and the chemical restraints such as sedative, anaesthetic, ataractic, and tranquilizing drugs.

Problems of disease and disease prevention

In the absence of any systematic guide to the diseases and treatment of wildlife, disease prevention is probably the prime concern of a veterinary program in a zoological garden. Diagnosis of disease in a wild animal, except of course those which are self-evident, is more often than not speculation and conjecture. It is not uncommon for animals

to come to autopsy presenting a galaxy of abnormalities, yet the antemortem observation may fail to reveal any unusual appearance. On the other hand, some specimens are opened which fail to reveal lesions even on close and careful examination. Careful observation, however, may sometimes lead to correct diagnoses.

Physical examination is limited to tractable beasts and to those which can be caught and handled without danger to personnel or unusual fright and damage to themselves. Anaesthetics and sedatives are not always practicable, especially when the animal is of bad temperament and/or seriously sick. Animals do not like to be molested; on the other hand they seem to enjoy attention. Whenever possible, handling should be avoided or done under tranquilizers. A discussion about the usage of tranquilizing drugs will appear in the forthcoming pages.

Effective cure of any illness depends on early detection and prompt care. It is important, therefore, that signs of illness or troubles be regularly watched by the keeper in charge. Some of the common symptoms to be looked for are lack of appetite, weight loss, lack of grooming, inactivity, dullness of eyes, unusual falling of hairs, looseness of bowels or constipation, convulsions, coughing, excessive

thirst, discharge from eyes or nose, and rapid or labored breathing. Appetite, as usually considered, however, is not always a reliable guide to the state of health. Many healthy animals refuse food through physical excitement and sick ones often take it until they collapse and die.

Another reason that poses far more difficult problem for the veterinary surgeon is the lack of acquaintance with the anatomical and physiological organization of wild animals. Knowledge of these disciplines is indispensable for any therapeutic measures since some animals show marked differences from closely related species. Often the knowledge of domestic species is applied to animals of similar physiology. Analogies, for example, can be made among canines, felids, and mustelids, yet perplexing differences are common.

Because of these and many other difficulties, treatment of wild animals *per se* is not at all encouraging. It is essential therefore to collect all the information available from the field and to make it readily available. A close cooperation on an international scale is desirable. A passive role, in the form of control and prevention of disease, therefore, should be the major objective of the veterinary services in a zoological garden.

The importance of a scientific, balanced diet for the control and prevention of disease can scarcely be overemphasized. Carefully designed diets certainly should be an essential feature of the environment of every zoo; first because the more obvious malnutritional diseases are thereby eliminated, and second, because adequately nourished animals develop and maintain high levels of resistance to many microorganisms that otherwise induce disease.

After the introduction of complete, balanced rations in the Philadelphia Zoo, one of the effects observed was a marked decline in the incidence of tuberculosis among birds (Ratcliffe, 1958). This has been a helpful factor in the development of resistance to tuberculosis, especially in the waterfowl collection (Ducks, geese, and swans). Similarly, enterocolitis of apes, monkeys, and baboons, which is a common affliction of newly imported animals, has been cured by appropriate diet. The carrier status usually persists for a time, but eventually the agents of disease are eliminated within a year or so without resort to specific therapy. The diet, likewise, has offered effective resistance among ruminants and non-ruminant herbivores against nematode parasites, including stomach worms (Haemonchus) which have been a common cause of mortality among these animals.

In addition, the introduction of a scientific, well planned feeding system makes obvious other limiting factors concerned with the successful keeping of animals. Thus, if, despite a complete ration, the condition of a species is unsatisfactory, then more attention must be paid particularly to their social factors, and also to the sanitary conditions of the pen and all of the animal's surroundings. Ratcliffe (1962), for example, listed 18 per cent of all deaths among mammals (representing primates, rodents, felids, bovids, and marsupials) as due to arteriosclerosis. This was evenly distributed among males and females, ranging in age from 2 to 245 months. Analyzing the situation in the light of other pertinent information, he came to the conclusion that environmental factors, especially the social environment (number, sex, age ratio), are a major factor in the development of this disease. In order to study the effects of social factors on the development of atherosclerosis a separate experiment was designed which is discussed in Part II of this thesis.

Control of infectious diseases in a zoological garden is highly important since there is reciprocal danger of picking up infection from man to animal and vice versa. Fox (1923), for example, mentioned that tuberculosis has not with certainty been diagnosed among animals living in a wild state, unless they have been in contact with human beings. Among zoo inmates, primates suffer most and the public is the source of infection. With very few exceptions, an airborn infection acquired through exposure to open cases of tuberculosis is the rule. However, with the improved management and display techniques, the disease has now practically been controlled. The glass partition erected in front of both the indoor and the outdoor cages effectively does away with the possibilities of infection passing from man to animal or from animal to man. Among other members of the primates, potentially dangerous to humans, are the gibbons and chimpanzees. Gibbons are frequently carriers of the shigella dysentry organism, and chimpanzees, of <u>Entamoeba histolytica</u>, <u>Balantidium</u>, and other parasites which live in the alimentary canal. Recently, chimpanzees have also been shown to be healthy carriers of the virus of infectious hepatitis, a very serious disease of adult humans (Fiennes, 1962).

Another indispensable measure in the control and eradication program of tuberculosis in apes and monkeys is the tuberculin test combined with proper quarantine. The method, by keeping a record of rectal temperature both

before and after the injection, has been fully described by Fox (1923). The procedure, although expensive in terms of time and personnel, is quite simple. However, temperature is not always a reliable guide as it may shoot up due to excitement.

The palpebral test, originally described by Kennard et al. (1939), is now commonly used in both large and small animal practice. The procedure for this test in apes and monkeys, after Ratcliffe (op. cit.) is as follows:

> Restrain the animal by hand or in a net and inject into the upper eyelid 10-15 mg. Old Tuberculin/kg. in 1 ml. or less of fluid. Redness and swelling sufficient to partially or completely close the eye usually develop within 24 hours and persist for 72 hours if the animal has tuberculosis. Non-tuberculous monkeys are not affected.

If some positive animals are found in a group of quarantined animals, the healthy ones are still kept isolated and retested after two months. These are passed for exhibition only when they prove negative on the second test. This test, coupled with a periodic X-ray examination of the keepers and attendants and an effective glass barrier between the visitors and animals, have been proved to be most effective in the control and eradication of tuberculosis.

Problems of animal restraint

Those responsible for day-to-day management of captive animals are fully aware of the problems associated with getting near an animal, without either provoking the animal's flight reaction and causing alarm and injury, or imposing upon themselves a greater danger than ordinarily is sensible. Knowledge of the techniques of controlled handling of wild animals is essential for their protection and welfare. Some of the aspects of zoo management which have been assisted by proper restraint of animals may be listed as:

a) Animal management: The problems of breeding and rearing are complicated sometimes by viciousness that can be controlled by modern drugs. Introduction of new mates, and acceptance of newborn by the mother, are frequently made easier (Gandal, 1962).

b) The shipping, crating, and transit of wild animals, previously a big task, is now greatly assisted by the use and development of modern tranquillizing and ataractic drugs.

c) The treatment of many diseases is assisted by the use of modern sedative drugs either indirectly to make the patient more amenable, or directly, if the illness is associated with a neurosis.

Mechanical devices like squeeze cages, crates, nets, and moving boxes, although indispensable items in the management of a zoological garden, are very limited in their range. These are resisted by force, and the animal may use its teeth, hands, and feet for defense. Furthermore, apes, monkeys and many more animals seem to possess a remarkable memory, and an imprint upon an animal of an unpleasant situation may make it more apprehensive of any repeat. Physical restraint, especially in primates, therefore is always undesirable.

Recent advancements in tranquillizing and ataractic drugs have opened up a fascinating field of wild animal restraint, both in the feral and in captivity. Successful immobilization of a variety of animals has been achieved in game reserves and in zoological gardens.

A standard solution of succinylcholine chloride (20 mg/cc) is extensively used to cause temporary paralysis lasting anywhere from 10 to 40 minutes. The drug is a neuromuscular blocking agent and blocks the transmission of the nerve impulse at the neuromuscular junction, the place where the nerve and muscle join. The action of the drug is accomplished chiefly by causing a persistent depolarization of the muscle end plates. Since the effect of the drug is only on voluntary muscle, and since the muscles of respiration fall into this category, respiration is seriously affected by doses sufficient to cause paralysis.

Heuschele (1961) used this drug on 44 different animals (lion, tiger, bear, sea-lion, deer, elk, and hyena) at the San Diego Zoological Garden using a projectile syringe in which seven animals were lost and five were not affected at all. He found a considerable species variation with respect to drug dose per pound body weight required to produce complete immobilization and with respect to the margin of safety of the drug. This means that careful experimentation with each species must be carried out to ascertain the safe, effective dosage limits for it. In handling highly nervous and fractious animals it is advisable to use tranquillizing drugs 1 to 2 hours prior to immobilization.

Another drug extensively used in the zoological gardens is chlorpromazine hydrochloride ("Largactil," May & Baker). This has been found more suitable than any other ataractic drug chiefly because it also has a soporific value which is of advantage in conditioning newly caught animals. Dosage range from 1 mg./lb. body weight for antelope to 0.125 for fully-grown giraffe. Harthoorn (1961) used this drug on 30 black rhinoceroses and on a number of giraffes and zebras for transportation without fatality. He further indicates that dosage rates for animals in zoological gardens seem to differ markedly from those advocated for free roaming animals. This may be due to the interactions of various factors working on animals in captivity which lower their resistance to such drugs. Dosage for most species is still unconfirmed and considerable related research is needed.

Interaction of public and zoo tenants

From the preceding discussions it is apparent that the public is the reason for the very existence of most zoological gardens. Experience, however, has shown that they also provide the greatest source of danger to zoo tenants. The duty of a zoo biologist, thus, lies not only in taking appropriate measures to protect the public from the animals, but also the other way around.

Visitors may become a source of danger to animals for three reasons. First, by carrying infective agents of diseases that are readily communicable from man to animals. This aspect has already been discussed in the section on disease control. A protection such as the glass partition provides the most effective and practical solution.

The second serious danger lies in offering unsuitable food to animals. Appelman (1961) indicated various problems involved in imposing a complete ban on such feeding by the public. He made a careful record of the various items and quantities of food given by the public to certain specific animals. Results of his observation indicate a clear behavioral difference among different species. The elephant, for example, virtually accepted and swallowed everything given to him without discrimination. The gibbon accepted everything, but ate certain foods and rejected the rest. The zebra, on the other hand, accepted only those items that seemed edible to him, but refused foods with a strong or strange smell. Below is the list of items offered to and eaten by an Indian elephant:

Items Offered as Food	Quantity Offered
Peanuts	1,706
Slices of bread	16 with ham 11 with cheese 28 with chocolate 14 with sugar
Sweets	1,330
Ice cream	2 whole 5 pieces
Sugared biscuits	811
Apples	17
Pieces of paper	198
Paper bags	3
Lady's white leather glove	1
Boot lace	1

From the list of items given, one can realize how disastrous offered food may be to the health of the animals, especially to those which accept and eat most everything offered to them.

Dobberstein (1936) reported instances of serious indigestion with fatal results, among animals of the Berlin Zoo after days when there had been large crowds. Quite often people fed tainted and spoilt food, perhaps not realizing that these could cause just as serious an illness in animals as in man. Hediger (1950) cited an example where a polar bear contracted severe haemorrhagic gastroenteritis after it was fed putrid sea fish by a woman visitor who had brought it expressly for this purpose.

Some zoos allow the public to feed certain animals. This expedient, however, does not seem to be a practical solution to the problem. People continue to bring all sorts of foods and are careless as to which animals they are permitted to feed. A complete ban on feeding zoo animals by public, as decided by the International Union of Zoo Directors at Copenhagen (Appelman, op. cit.), seems to be the only real solution.

The third and the most serious danger lies in the possibility of direct harm or indirect damage to the animals by worrying, teasing, and frightening or exciting them. Innumerable cases are on record where people, from malice or morbid disposition, have deliberately maimed or killed valuable animals. Dobberstein (op. cit.) cited several examples of malicious poisoning among young bears in Berlin and other zoological gardens of East Germany. Hediger (op. cit.) reported the tragic death of the two-ton, giant sea-lion of the Hamburg Zoo in 1930. In the stomach of this victim was found a broken beer bottle which caused perforations and fatal peritonitis. On another occasion, the glamorous bill of a whale-billed stork (Balaeniceps rex) was smashed with a hammer. In still another case, an elephant at the Geneva Zoological Garden (now closed) was offered an apple in which several pins were concealed. Records show that no zoo in any part of the world is safe from such regrettable incidents.

The most nearly practical solution of the above problem, as it appears to me, lies with the zoo personnel. The people that visit zoological gardens must be taught a new attitude towards animals. A zoo fits into a niche in the community in a number of ways. Zoological gardens of today are not just a place of amusement, but a biological institution. People must be taught a truer, more positively biological attitude towards animals. The result depends largely on how it is presented to the public and how it fits into the existing educational program of the community. The modern zoological gardens, besides showing animals, have to show them in relation to time and space; not the animal as an object, but the animal as a subject.

PART II

EXPERIMENTAL ATHEROSCLEROSIS IN GROUP AND SOCIALLY ISOLATED JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

INTRODUCTION

Studies of the past decade have shown that social interactions in populations of mammals and birds have profound effects on behavioral phenomena, physiological processes, responses to drugs, and susceptibility to a variety of diseases. With respect to behavior, interpreted as a measure of emotionality, it has been consistently reported that group-living animals are less emotional, timid, or fearful than animals caged individually (Stern et al., 1960; Ader et al., 1963; Ader and Friedman, 1964). The data, however, are not so consistent with respect to resistance to disease. Relative to animals caged individually, the group-housed animal is more susceptible than the individually-housed animal to alloxan diabetes (Ader et al., 1963), whole-body X-irradiation (Ader and Hahn, 1963), trichinosis (Davis and Read, 1958), and immobilization-produced gastric ulcers (Stern et al., op. cit.). These observations, however, are contradicted by others who have shown that animals caged in groups are more resistant to spontaneously developed mammary tumors (Andervont, 1944; Muhlbock, 1951), convulsions (King et al., 1955), and conflict-induced gastrict erosions (Conger et al., 1958).

Over the past three decades, death records of the animals at the Philadelphia Zoological Garden indicate an increase in the occurrence of arteriosclerosis in mammals and birds. This increase is characterized by two distinct bounds. The first increase was observed following 1935, when the scientific, balanced diet was first introduced. Since then, the diet was held constant. Nonetheless, a second increase occurred with a change in distribution of the lesions (Ratcliffe and Cronin, 1958; Ratcliffe et al., 1960). This second increase was attributed to stress and anxiety as a result of changes in each animal's environment, namely, an increase in cage population, noise, and frequency of contact with man (Ratcliffe and Snyder, 1962).

Katz et al. (1958), emphasizing the nutritional-metabolic cholesterol-lipid-lipoprotein theory of atherosclerosis, have said that diet does play a decisive, but not an exclusive, role in the causation of this disease. Heredity, physical and metabolic activity, stress, endocrine function and various environmental factors may interact significantly with diet.

Experimental evidence of animals showing the influence of stress on atherosclerosis are few, and often conflicting. Sellers and You (1956) showed a positive effect of cold-stress in inducing changes in coronary arteries of rats. A similar result was reported by Schoenfeld (1960) in rats following prolonged swimming in cold water.

Uhley _et al_. (1957), on the other hand, reported no definite relationship between the behavior of his chicks (aggressive and passive groups) and the development of arterial disease. However, food intake, weight gain, and serum cholesterol levels of the aggressive and passive groups were not equal.

Cholesterol-fed cockerels, when subjected to continued anxiety by repeated electric shocks, gave no indication of weight gain, blood lipid level change, or the development of atherosclerosis (Joyner _et al_., 1961). Gunn _et al_. (1960) reported that rabbits fed on cholesterol excess diet, and receiving chronic electric stimulation of their diencephalon in the hypothalamic region, exhibited a greater degree of aortic and coronary atherosclerosis than those not receiving hypothalamic stimulation. They concluded that central nervous system mechanism does exist which, under certain circumstances, is capable of significantly influencing arterial atherosclerosis.

The development of atherosclerosis is a silent phenomenon. The disease presents itself only when arteries become incapacitated and complications are superimposed. For this reason it is important to recognize the sub-clinical or the developmental stage of the disease. This, however, itself raises a problem since direct examination of large arteries *in vivo* is not feasible.

Clinical studies on the role of psychological stresses in atherosclerosis are also difficult to carry out. The main reason for the difficulty lies in the lack of precise measure by which to characterize psychological stress, and to adapt such stress evaluation to the reaction of a given individual (Katz *et al.*, 1959).

The blood of mammals and birds has been recognized as a sensitive indicator of their internal conditions. The liquid menstruum circulates in a closed circulatory system, carrying with it the erythrocytes, the leucocytes, and platelets or thrombocytes. This menstruum also carries a mixture of electrolytes, amino acids, enzymes, hormones, non-cellular proteins and other basic organic and inorganic materials. The concentration of various proteins in the circulation represents the balance between filtration into the capillaries and return from the tissue spaces by way of the lymphatic channel.

In a healthy adult of a given species, the concentration of various serum proteins remains surprisingly constant. Repeated electrophoretic analyses show patterns in which not only the size of each major boundary, but also its shape, remains constant. Hence, the concentration of minor components within a major group must retain their ratios to each other. This consistency of an individual's pattern has been suggested to be under genetic control.

The determination of plasma protein levels as an aid in diagnosis of diseases has such theoretical possibilities that a vast amount of work has been expended on it (Ehrmantraut, 1958). Because of the size of the liver and its importance in protein metabolism, liver diseases change the plasma protein markedly.

Electrophoresis is used for determining the isoelectric point of proteins and also for the fractionation of protein mixtures. It is customarily done in veronal buffer solution at pH 8.6, at which the anodic migration is more pronounced. Serum albumin, whose isoelectric point is near pH 4.6, moves with the greatest velocity, the α-, β-, and γ- globulins with decreasing velocities. Quantitative evaluation of these fractions, with subsequent increase or decrease of one or more protein components of the serum, constitute the "stress" pattern described by Dunn and Pears (1961).

According to Selye and Heuser (1956) the mechanism of mammalian reaction to non-specific stress is as follows:

1. The stimulation is conveyed to the hypothalamus which causes the anterior pituitary to produce adrenocorticotropic hormone (ACTH).

2. ACTH reaches the adrenal cortex and thereby releases hormones which stimulate various organs, including gastric glands of the stomach.

3. Pepsin and hydrochloric acid produced in the stomach attack its contents and/or the stomach lining. Continuation of this condition leads to gastric ulcers and malfunction of both the liver and the kidney.

This study was undertaken in an attempt to evaluate some environmental influence on the development of diet-induced atherosclerosis in Coturnix quail (*Coturnix coturnix japonica*). It was hoped that these birds, being gregarious in nature, would be suitable subjects for the study of the effects of social isolation. The electrophoretic pattern of the serum protein was analyzed in every case as a measure of physiological change, and it was hoped to provide some diagnostic measure of the developmental stage of the disease.

MATERIALS AND METHODS

Acquisition and handling of chicks

Fertile Japanese Quail eggs, selected randomly from the stock at the Michigan State University Experimental Poultry Farm, were incubated in a "Jamesway 262" forced-draft type incubator. The chicks hatched on the eighteenth day of incubation, when they were removed from the incubator and placed in "Jamesway" backwarmer type of starting batteries. The chicks were reared on paper for the first seven days, with the heating temperature adjusted to 95° F. At this time, the paper was removed and the chicks were held in the battery until they were two weeks old. During this period, all the chicks were kept on standard chick starter ration. The birds were then marked with leg bands, individually weighed, and randomly divided into 36 groups (18 experimental and 18 control). The final distribution of groups was as follows: six groups each having 15 birds; six groups each having five birds; and 24 groups each having a single bird.

Housing

Each group finally was transfered to a partitioned pen occupying one square foot of floor space. This resulted in placing birds at concentrations of fifteen, five, and one bird per square foot.

The pen available to house the quail had twelve compartments, six on each side, with the heating unit placed at the center. This heating unit was raised 5.5 inches above the wire mesh floor. Four compartments on either side of the heating unit, excepting the top and the bottom shelves, were partitioned off using plywood. This prevented the birds, especially those that were individually isolated, from seeing other birds kept in adjacent pens. The finished pens measured 12 inches by 12 inches and contained 144 square inches of floor space per pen. A common water trough, with a partition made at the center, was used for all four corner pens on each shelf. This partition prevented the birds from mixing and also proved easier for cleaning the trough when desired. The troughs on the outer two sides of the pen were used as feeders for all birds.

Management

The experimental groups were kept on one side of the heating unit, while the controls were kept on the other side. Control groups were fed on a standard Japanese Quail Breeder ration, developed at the Michigan State University Experimental Poultry Farm (Table 1). The experimental groups

Table 1. Japanese Quail Breeder Ration

Ground yellow corn	38.25
Soybean oilmeal dehulled 50%	37.00
Alfalfa meal 17%	5.00
Dried whey	2.50
Meat and bone scraps 50%	2.50
Fish meal (Menhaden 60%)	2.50
Ground limestone	5.00
Dicalcium phosphate	1.50
Salt (iodized)	0.50
Vitamin premix (NOPCOSOL M-4)	0.25
Fat	5.00
	100.00

received the same ration except that their ration was fortified by adding 1 per cent of Nutritional Cholesterol by weight. In order to make the two rations exactly comparable with respect to their caloric value, the ration for the control birds was mixed with cottonseed oil 1 per cent by weight. Feed and water were kept before the various groups at all times.

Heating units at all levels were initially adjusted to 95° F. before the birds were placed in the experimental pens. This was decreased gradually at the rate of 5 degrees per week until the temperature reached 80 degrees F. The air temperature inside the pen ranged from 10 to 12 degrees below that recorded on the heater thermometer. The temperature inside the room ranged from 40 to 70 degrees F. during this experiment.

All groups received continuous lights on the 24-hour basis which provided 15-20 foot candles of light at the level of the feed and a minimum of 12 foot candles of light in all parts of the pen. The experimental period lasted from six to ten weeks. All the birds at the start of the experiment were 15 days old. This was the experimental design used in inducing atherosclerosis.

Handling of birds

Birds were little disturbed during the entire experimental period. The pen was set in one corner of the room which prevented the birds, at least on two sides, from seeing other experimental birds housed elsewhere in the same room. The remaining two sides were covered with thick brown paper from outside, obstructing the bird's view and preventing them from seeing other birds or the attendants working in the room. Food and water were checked every day, and the cleaning of the water troughs was done once or twice a week as needed.

Collection of materials

As initially planned, the first batch of birds was ready to sacrifice at the end of six weeks. This included one group of fifteen birds, one group of five birds, and four groups of isolated birds. Birds were taken in this fashion both from the experimental and the control groups. The second and the third batches were ready at the end of the eighth and tenth week respectively.

Birds were individually weighed and sacrificed by decapitation. Blood samples of 2-3 cc. were collected in separate, sterilized centrifuge tubes, marked individually for each bird. Samples were allowed to clot without agitation and then centrifuged for 10 minutes at 1,500 rpm, taking care to prevent haemolysis and subsequent foiling of the whole sample. The serum thus separated from the blood clot was finally transfered to small glass tubes marked individually for each specimen.

An electrophoretic run of the sample was made as soon as possible in order to avoid changes in the pattern due to storage. Storage conditions can produce changes in electrophoretic pattern but the agreement among investigators is equivocal. Meisner and Hickman (1962) reported that freezing does not influence the electrophoretic pattern. However, they experienced difficulties with samples that had been frozen.

After the sample was taken, a mid-ventral incision was made, each bird eviscerated, and the sex and degree of maturity were noted. Both the adrenal glands, situated anterior to the testis or the ovary, were then exposed, carefully detached from the surrounding tissue, and weighed separately on a light precision balance. All the weights were recorded to the nearest tenth of a milligram.

After these parameters were recorded, the heart and the big blood vessels were exposed. These were carefully examined for evidence of atherosclerotic lesions. Portions of suspected tissue were preserved for histopathological examination in a mixture of: commercial formalin five parts, 95 per cent ethyl alcohol 25 parts, glacial acetic acid one part, and distilled water 20 parts (FAA). The preserved tissues were later sectioned and examined under a compound microscope.

Electrophoretic method

Fractionation of samples of the serum was performed with Beckman Model R-101 Microzone Cell, using Gelman's sepraphore III cellulose polyacetate electrophoresis support membrane (2.5 X 12 cm). This membrane has uniform pore size and distribution and has several advantages over other media such as filter paper, starch, or agar. Some of these advantages are: application to small samples (0.25 ml), minimal absorption of proteins to this material, thereby eliminating the tailing of fractions common to paper electrophoresis; short running time; and ability to resolve samples into more fractions with reasonable clarity. Also, the transparency and the tensile strength of cellulose acetate after it has been cleared with ethanol-acetic acid make this medium ideal for scanning.

Barbital buffer (veronal) having a pH of 8.6 and ionic strength of 0.075 was used both for soaking the strips and filling up the Microzone cell. It consists of 1.66 gm barbituric acid (crystals), 12.76 gm of sodium diethyl barbitone (powder), and sufficient water to make one liter. Storage of the buffer solution was avoided as it is attacked by microorganisms altering the conductivity of the solution. Some authors recommend the addition of sufficient merthiolate to obtain a concentration of 1 : 100,000, to prevent spoilage. Sodium azide is also employed for this purpose.

The sepraphore membrane was soaked in the buffer by carefully floating it on the surface of the buffer solution. This allows the membrane to wet uniformly, from the bottom, sinking into the buffer quickly as wetting progresses. At least an hour was allowed for soaking. This produces a colloidal change, bringing the membrane back to its original gel structure. Insufficient soaking may result in the

streaking or smearing of seprations.

While membrane was being soaked, the Microzone cell was filled by tilting the fluid-leveling siphon to a horizontal position and pouring buffer into one end of the siphon tube. This was done until all the air bubbles were eliminated from inside the tube. The siphon was then slowly released until it returned back to its original position. Buffer was poured in only one compartment which, in turn, was automatically transfered to the other side by the siphon tube. The cell was filled to a point between the two lines marked "FLUID LEVEL." Enough time was given for the buffer to equilibrate in both the compartments. If the buffer level is not equal in both the compartments, capillary action will take place through the membrane pores. Such flowage will smear and distort the fractions and the strip will be ruined.

Mounting the membrane on the bridge

After the membrane was thoroughly soaked in the buffer solution, it was placed between two blotters using a cover glass forceps. The membrane should never be touched with bare hands, dry or wet. Blotters should remove only the shining excess surface fluid. All operations done with the membrane uncovered should be done as quickly as possible.

The strip was then quickly placed over the membrane mount and secured properly by means of movable tensioning membrane mount (Beckman Tech. Bull., RM-IM, 1963). The bridge, properly mounted with the membrane, was then placed in the cell, taking care not to splash any buffer onto the membrane when the siphon flipped up.

Sample application

This process should be done as quickly as possible to avoid unnecessary evaporation from the membrane surface while the upper lid is off and to minimize diffusion of samples in the membrane before current is applied. Samples were applied using a sample applicator, taking all the precautions mentioned in Beckman Bulletin Manual (op. cit.). The tip of the applicator was immediately ringed after each application with a strong jet of distilled water from a wash bottle and dried by gentle touch of blotters. This precaution was necessary to avoid mixing of one sample with another. Eight samples were run at a time.

Power to the cell was supplied at constant voltage (250 v.) using a Spinco Model RD-2 Duostat. The run was made for 20 minutes with a starting current of 3.5 ma to 5.8 ma, and an ending current of 4.5 to 9.6 ma. When the ending current went over 9.6 ma, the whole run was discarded.

Staining, cleaning, and mounting of strips in the plastic envelope

Various dyes may be used to locate the protein fractions on the strip. A solution of Ponceau "S" described by Smith (1960) was the stain of choice in this investigation. It consists of 0.2 per cent Ponceau "S" in 3 per cent trichlor-acetic acid. The uptake of this dyd, according to the above named investigator, is quantitative to the amount of protein on the strip.

At the end of the time allowed for the separation, the power was turned off, and the membrane removed. The wet strip was then laid over the surface of the stain, avoiding air bubbles trapped underneath and allowing the stain to soak into the strip from below. Seven to nine minutes was allowed in each case for staining. The excess stain was removed by gentle agitation for 3 minutes in each of the three trays containing 5 per cent glacial acetic acid. It is important to remove all background stain before quanti-tation procedures are employed.

The membrane, after its background was completely white, was laid over 95 per cent ethyl alcohol for dehydration for about one minute. The strip was then transfered to another tray containing 10 per cent glacial acetic acid in 95 per cent ethanol (10 cc of glacial acetic acid and 90 cc of 95 per cent ethanol). The membrane was left in this solution for approximately 30 seconds and finally spread over a clear glass plate, taking precautions not to trap any air bubble. Excess fluid was removed by tilting the glass plate and squeezing the membrane gently and evenly by the smooth edge of the plastic strip holder. The glass plate with the membrane was then dried in a pre-heated oven (100 to 110 degrees C.) until all the smell of acetic acid was gone. The membrane was then carefully peeled off the plate and slipped inside the plastic strip holder for storage and later scanning.

Evaluation of the separated serum proteins

The strips were scanned in a Spinco Analytrol Model RD using R-102 Microzone Scanning Attachment. The analytrol draws the curves for each component and the integrator automatically indicates the exact area under each curve. Both these functions are performed at the same time in a single step.

While scanning cellulose acetate strips stained with Ponceau "S" stain, a "B-2" cam or so-called "optical density cam" was used. The slit width of 0.5 mm, and a height of 5 mm was used throughout the study. The original 500 mμ front interference filter used for scanning paper strips was replaced by 520 mμ interference filter. Strips were inserted in the carriage by properly fixing them inside the strip holder plastic covers. These transparent covers did not interfere with the scanning process and ensured safety of the membrane against the drive wheel. All strips were scanned on an approximately equal distance between the centers of the first and last peaks on the chart. This was obtained by adjusting the "SPEED CONTROL" knob at 45 in every case. The percentage of each individual component for statistical analysis was calculated as described in Beckman Bulletin RIM-5.

RESULTS

A total of 160 Coturnix quail (75 males and 85 females) used in this investigation were individually examined. These specimens were randomly selected and are presumed to be representative of the population. Also, the birds thrived well under the entire experimental period. Only three casualties were observed: one, a single isolated bird, died due to accidental entanglement in the wire mesh of the cage; and two probably were victims of fighting. The first group was started again with a fresh specimen taken from the reserve. The latter two birds, however, which died near the end of the experiment and although replaced by fresh birds for the rest of the period, were treated exactly like other specimens. Their data did not show any difference from that of the other birds of their own group and are therefore included in the final analysis.

A heterogeneous group of 16 birds were housed separately in one complete shelf of the pen, without any space limitation (875 square inches of space). These birds were kept throughout the experiment on the basic Japanese Quail Breeder Ration (page 89). The data obtained from these birds are assumed to be normal for the species and therefore are used for all comparisons in this investigation.

Body weight and adrenal weight

The mean body weights of the three population densities are shown in Table 2. The mean body weight of the birds kept in the group of 15 and isolated for 10 weeks was significantly smaller than the mean body weight of the normal birds (P = .05). This difference, however, was not consistent with other three stages of isolation (six, eight, and ten weeks). At the start of the experiment (two weeks of age), the mean weight of the females was not significantly different from the mean weight of the males. However, at six, eight, and ten weeks of isolation the mean weight of the females was significantly greater than the mean weight of the males (P = .05).

Table 2 further shows the mean weight of the adrenal glands (mg) calculated for each 100 gram of quail's body weight. The mean weight of the adrenals in the group of 15 and in individually caged birds at eight and ten weeks of isolation was always higher than the mean weight of the adrenals of the normal birds. These differences are statistically significant at the five per cent level. The

Table 2. Body Weight and Adrenal Weight Data for Isolated and Group Raised Japanese Quail

Treatment	Number of Quail	Body Weight (gm)	Adrenal Weight (mg) per 100 g Quail Weight	Coefficient of Variation
Six Week Isolation				
Group of fifteen	30	113.46 ± 3.24	10.41 ± 0.57	1.01
Group of five	10	119.78 ± 4.39	9.62 ± 1.12	3.69
Single birds	8	109.90 ± 6.06	11.70 ± 1.27	3.82
Eight Week Isolation				
Group of fifteen	30	117.37 ± 3.07	11.33 ± 0.56[1]	0.89
Group of five	10	120.77 ± 5.33	10.04 ± 1.11	3.50
Single birds	8	112.24 ± 4.52	12.96 ± 0.44[1]	1.21
Ten Week Isolation				
Group of fifteen	30	104.10 ± 2.23[2]	12.68 ± 0.53[3]	0.76
Group of five	10	121.01 ± 5.27	9.58 ± 0.65	2.15
Single birds	8	119.84 ± 8.17	11.39 ± 0.79[3]	2.47

[1]Significantly greater than the normal at 5 per cent level

[2]Significantly smaller than the normal at 5 per cent level

[3]Significantly different from the normal at 5 per cent level

birds caged in the group of five, however, did not show any difference; neither in their body weights nor in the weights of their adrenal glands.

Electrophoretic pattern of the serum protein

Normal birds

Serum samples from each specimen were run for electrophoretic pattern determination as described earlier. In most cases, at least seven distinct fractions were obtained. The two sub-fractions of the α-globulin (α_1 and α_2), in some cases, however, did not separate with distinct clarity. In the final analysis, therefore, these two sub-fractions were lumped together. Few specimens, on the other hand, revealed a distinct band of a fast moving protein fraction (pre-albumin). This was observed both in the normal and in the treated birds. The quantity of this fraction, however, was so small that it was not considered for statistical analysis.

A striking difference in the gamma globulin fraction of the male and the female bird was also noticeable in this investigation. This was found consistent throughout the experiment both in the normal and in the treated birds. In the normal female birds, this fraction represented 8.50 $\pm$ 0.54 per cent of the total serum protein as against 4.44 $\pm$

1.11 per cent of the serum proteins of the males. This difference is significantly greater at the five per cent level. The curve, as appeared on the graph after scanning (Figure 1), is traced and shown in Figure 2.

A greater quantity of this fraction in the females was balanced by a reduced quantity of their albumin fraction. Albumin was 36.46 ± 0.81 per cent of the total serum proteins in the females as compared to 46.93 ± 2.48 per cent of the males. This difference, again, is significant statistically (P = .05). Table 3 shows the difference in the electrophoretic pattern of the serum proteins of the normal male and female Coturnix quail. Other globulin fractions, however, did not show marked sex differences. The last column of Table 3 represents the percentage fractions of total serum proteins of male and female birds combined. These pooled values are used for all other comparisons in this investigation.

Birds kept on cholesterol-mix diet

The electrophoretic pattern of the serum protein of birds kept on cholesterol-mix diet shows a different picture from that of normal birds. A slight decrease in the albumin and in the alpha-globulin fraction was almost consistent. Differences in these fractions, however, were not

Figure 1. Analytrol showing a scanned graph.

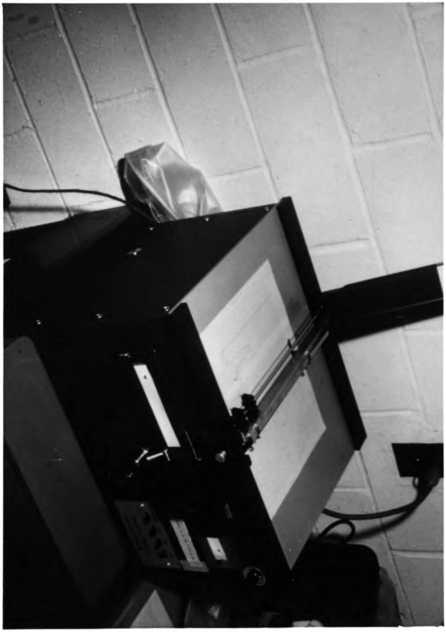
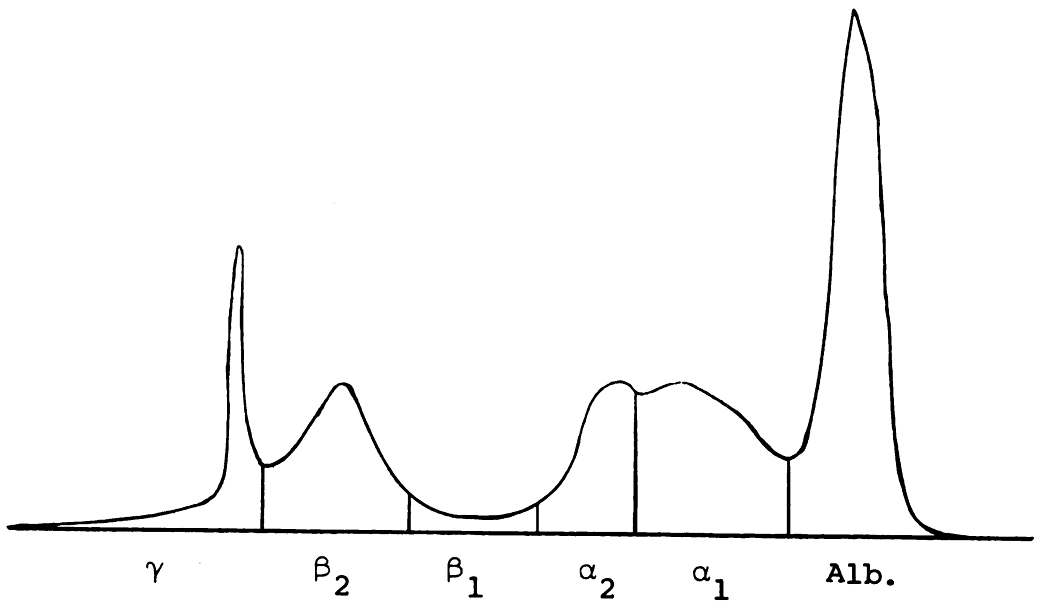
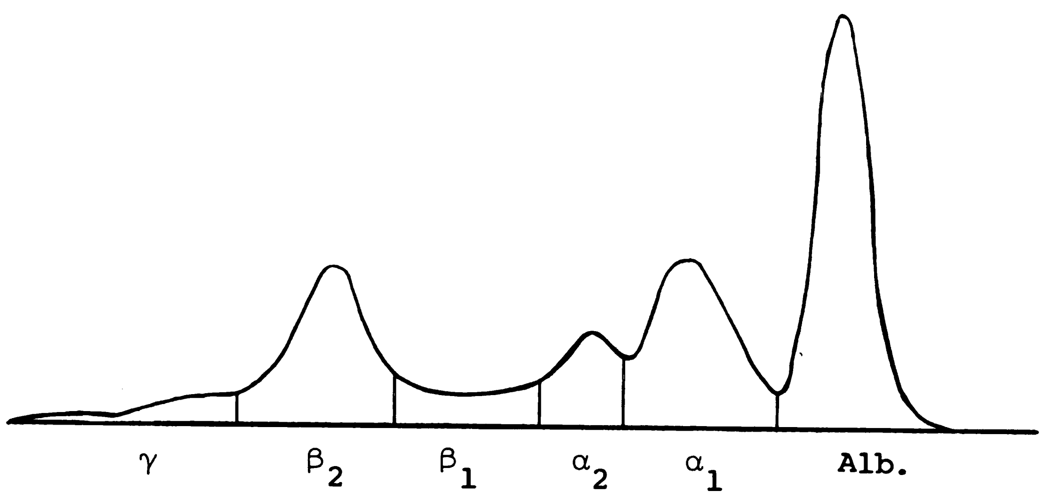


Figure 2. Electrophoretic pattern of the serum of a normal female Coturnix quail (Coturnix coturnix japonica). Notice the sharp peak in the region of γ_i globulin.

Figure 3. Electrophoretic pattern of the serum of a normal male Coturnix quail (Coturnix coturnix japonica).



γ
β2
β1
α2
α1
Alb.



γ
β2
β1
α2
α1
Alb.

Table 3. Summary of Electrophoretic Pattern of Serum Protein of Normal Male and Female Japanese Quail

Fractions	Male	Female	Male and Female Combined
Albumin	46.93 ± 2.48	36.86 ± 0.81[1]	39.38 ± 1.39
Alpha-globulin	27.19 ± 1.81	33.37 ± 1.52	31.83 ± 1.39
$beta_1$-globulin	4.65 ± 0.39	5.93 ± 1.00	5.54 ± 0.76
$beta_2$-globulin	16.80 ± 1.42	15.36 ± 0.67	15.72 ± 0.61
$beta_1$ + $beta_2$-globulin	21.44 ± 1.43	21.21 ± 1.03	21.26 ± 0.83
$gamma_1$ + $gamma_2$-globulin	4.44 ± 1.11	8.50 ± 0.54[1]	7.48 ± 0.65

[1]Significantly different from the males at 5 per cent level

statistically significant. The most striking difference observed was in the beta-globulin fraction. There was a consistent increase in this fraction at the density of 15 birds per cage and in birds caged singly at all stages of isolation (six, eight, and ten weeks). The only group that deviated from this general trend was the individual birds isolated for eight weeks. All these differences are significant statistically at the five per cent level. A general trend toward decline was also observed in the gamma-globulin fraction. These differences are shown in Table 4.

<u>Birds kept on non-cholesterol diet</u>

The electrophoretic pattern of the serum protein of birds kept on cholesterol-free diet (Table 5) showed little or no difference from that of the normal birds (Table 3). The only difference noticed was in the beta and gamma globulin fractions which showed a tendency toward decline. This reduction of the gamma-globulin was significant at least in two groups of birds at the population density of five and fifteen and at six and eight weeks of isolation ($P = .05$). The significant decrease in the beta-globulin was also found in the same group of five birds isolated for eight weeks, and one group of birds caged singly and isolated for ten weeks ($P = .05$). A striking difference,

Table 4. Summary of Electrophoretic Pattern of Serum Protein of Japanese Quail Kept on Cholesterol Diet

Treatment	Albumin	Globulins				
		alpha	$beta_1$	$beta_2$	$beta_1$ + $beta_2$	$gamma_1$ + $gamma_2$
6 Wk. Isolation						
Group of 15	35.75±1.43	33.13±1.05	5.84±0.41	19.18±1.44	25.68±1.31[1]	5.25±0.70
Group of 5	39.51±2.81	31.84±2.02	5.24±1.00	17.16±0.57	22.40±0.94	5.74±1.51
Single Birds	37.59±3.87	35.47±1.27	4.96±0.54	14.78±1.85	39.48±1.95[2]	6.64±2.98
8 Wk. Isolation						
Group of 15	38.89±1.19	28.80±0.83	5.73±0.47	19.61±0.90	25.35±0.97[1]	6.11±0.62
Group of 5	43.45±1.52	27.60±2.19	5.62±1.52	16.25±0.56	21.87±1.55	6.04±1.49
Single Birds	38.25±3.97	34.20±0.12	4.77±0.91	15.41±1.45	20.29±1.50	7.27±2.83
10 Wk. Isolation						
Group of 15	38.38±1.54	27.56±1.12	5.49±0.44	22.12±1.00	27.61±0.98[2]	6.31±2.19
Group of 5	35.83±3.31	31.97±1.63	3.24±0.39	20.24±2.32	23.48±2.31	8.72±0.92
Single Birds	37.72±4.09	32.71±3.39	7.72±2.15	18.65±1.94	26.37±0.66[2]	3.19±1.01[1]

[1]Significantly different from the normal at 5 per cent level

[2]Significantly greater than the normal at 5 per cent level

Table 5. Summary of Electrophoretic Pattern of Serum Protein of Japanese Quail Kept on Non-cholesterol Diet

Treatment	Albumin	Globulins				
		alpha	$beta_1$	$beta_2$	$beta_1$ + $beta_2$	$gamma_1$ + $gamma_2$
6 Wk. Isolation						
Group of 15	38.53±1.50	31.99±1.24	4.12±0.26	19.56±0.72	23.82±0.86	5.16±0.48[1]
Group of 5	38.88±3.15	29.13±0.82	4.78±0.58	18.04±1.02	22.82±1.16	9.17±2.53
Single Birds	41.48±2.73	28.54±2.18	4.94±0.43	16.88±1.16	21.81±1.38	6.46±1.33
8 Wk. Isolation						
Group of 15	38.57±1.02	32.20±1.19	4.28±0.30	18.08±0.68	22.36±0.70	6.17±0.57
Group of 5	44.42±2.85	34.88±3.86	3.08±0.40	13.72±0.93	16.80±0.88[1]	3.55±0.61[2]
Single Birds	38.77±4.49	33.73±0.72	4.55±0.73	18.23±2.53	22.76±2.09	4.40±1.62
10 Wk. Isolation						
Group of 15	38.01±1.50	32.20±0.94	5.37±0.44	16.74±0.78	22.12±1.00	7.34±1.18
Group of 5	41.89±1.45	30.13±1.85	5.20±0.51	16.22±1.15	21.41±1.44	6.57±1.97
Single Birds	41.68±1.50	33.57±1.45	4.76±1.51	12.96±0.56	17.72±0.96[1]	7.04±1.84

[1]Significantly different from the normal at 5 per cent level

[2]Significantly smaller than the normal at 5 per cent level

however, was evident when the electrophoretic pattern of the whole group was compared with the electrophoretic pattern of the serum protein of the birds kept on cholesterol-mix diet (Table 4). On the whole, the general increase of the beta-globulin fraction in the cholesterol-fed birds was represented by a consistent decline in the beta-globulin fraction of birds kept on non-cholesterol diet.

Incidence of disease in the experimental and control birds

The birds subjected to group isolation showed striking differences in atherogenesis. Diet-induced atherosclerosis was significantly enhanced in the birds subjected to group isolation, especially at the population density of 15 and in socially isolated birds caged singly. Out of 45 birds in the three experimental groups (kept on cholesterol-mix diet at the density of 15) 18 developed the disease; two at six, five at eight, and eleven at ten weeks of isolation. Among the socially isolated birds, six out of twelve developed the disease within eight and ten weeks. One of these birds, however, belonged to socially isolated group kept on cholesterol-free diet. At the population density of five, kept on cholesterol-mix diet, only one developed the lesions at ten weeks isolation. These differences are significant statistically at 0.1 per cent level (chi-square = 59.79 with 17 degrees of freedom). Sex, however, did not seem to effect atherogenesis in this investigation. Out of 45 birds developing arterial lesions, 15 were males and 10 females. This difference is not significant statistically.

Figures 4, 5, and 6 show the formation of atheromatous plaques in the aorta of Coturnix quail. Three distinct stages were recognized which were distinguished on gross, naked eye examination.

a) Lipid deposit: Superficial yellow or greyish-yellow lesions on the intima visible without a microscope.

b) Fibrous plaques: Circumscribed thickening of the intima producing a firm, grey or glistening prominence.

c) Atheroma: A plaque in which fatty softening predominated. In the stained sections, the fatty material was dissolved by the preservative (FAA) and represented empty vacuoles.

<u>Behavioral differences</u>

In addition to the above parametric observations, interesting changes in the behavior of birds isolated singly were noticed. For the first two weeks, they produced more noises than other birds caged in the group of 15 and five. Their calls invariably were replied to by other birds

Figures 4 and 5. Formation of atherosclerotic plaque in the lumen of aorta. Top picture (Fig. 4) shows the cross section of a normal aorta. Bottom picture (Fig. 5) shows a big atheromatous plaque in the lumen. Deposits within the intima are hardened and the lumen has been narrowed considerably. Hematoxylin-eosin. 40 X.

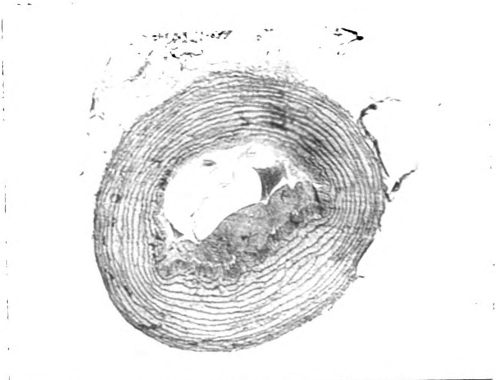


Figure 6. An advanced case of atheromatous plaque in the aorta. Note the endothelial lining and a much thickened intima infiltered with lipid deposits. Hematoxylin-eosin. 290 X.

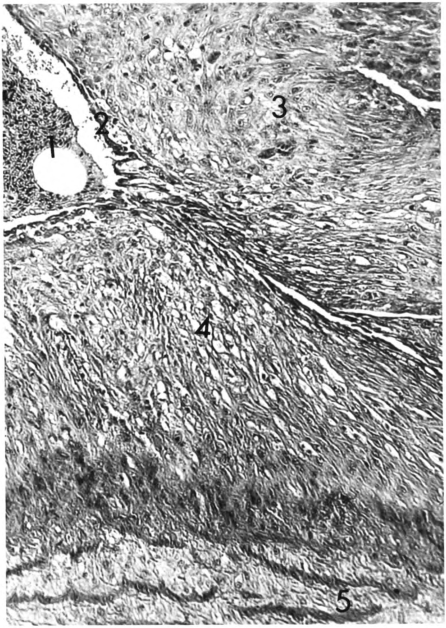
1. Lumen with blood cells

2. Endothelial lining

3. Thickened intima

4. Lipid vacuoles

5. Media or the muscular layer



1
2
3
4
5

isolated singly. Later, these birds developed neurotic behavior marked by an exceedingly strong tendency to be over active, interrupted only by short intervals. They scratched in their feeding troughs and watering bottles with an untiring persistence, flapping their wings and scattering the food without actually eating. When I cleaned their pens or refilled the water bottles, they actively resisted and violently attacked with extreme excitability. The birds kept in the group of 15, on the other hand, were more timid and either crouched or tried to escape while being handled.

DISCUSSION

Body weight and adrenal weight

The well established pituitary-adrenal relationship of mammals described elsewhere (page 86) is not so well defined in birds. Evidence from recent studies indicates a relative autonomy of the adrenocortical tissue of birds (Flickinger, 1959). Nonetheless, in this experiment, the adrenals of Coturnix quail kept at high density levels have been heavier than the adrenals of birds kept at low density. Siegel (1959; 1960), in the case of his chicks, attributed this difference to adrenocortical hyperplasia.

Social order, especially among the cocks, seemed to be more important in very crowded pens. Hens were usually subordinate to cocks. This was evident from direct observation. These birds were poorly feathered and their plumage was frayed and dirty. However, they did not show a greater number of injuries and their health appeared to be equivalent to that of the birds in less dense populations.

Failure to show significant difference either in the body weight, or in the weights of their adrenal glands at six weeks of isolation can be explained by the fact that the birds had not yet reached sexual maturity. The social hierarchy in the males probably becomes more rigidly established only after they have attained sexual maturity (about 8 weeks). It was because of this sexual maturity and subsequent social hierarchy that a significant correlation of adrenal weights was established in the latter two groups at eight and ten weeks of isolation.

The occurrence of even larger adrenals in the socially isolated birds suggests that smell, sound, or even sight reactions as a whole may be important in eliciting adrenal responses. This was evident from the changed behavior of these birds which were greatly excited at the call of other birds kept in different parts of the pen. These facts, rather than the social interactions between the members of the group, seem to be the possible explanation for larger adrenals in the birds caged singly as compared to the birds kept at the density of 15.

Failure to obtain a consistent difference in the body weights of the Coturnix quail kept at higher density, or even in socially isolated ones, suggests their remarkable adjustment to crowding. In the light of this observation, lipid depletion in the cortical tissue, or even direct comparison of corticosteron in the circulating blood would give a better estimate of a "stress" situation in these birds.

A significant increase in the body weight of females at all density levels, and at all stages of isolation, however, was expected. This occurred with Coturnix quail housed under various environmental conditions (Stanford, 1957; Wilson *et al.*, 1961).

The electrophoretic pattern of serum protein

From the electrophoretic analysis of the various serum proteins of the normal and experimental birds it is obvious that some variation in the distribution of the blood proteins occurs from individual to individual. This variation appears as differences in mobilities, in the number and relative proportions of components, and in the failure of some sera to show good resolution of various fractions.

In many of the sera studied it was rather difficult to ascertain the amount of particular globulin present, primarily because they were not sufficiently separated. In other words, differences in their mobility in an electrical field were too small for good resolution. This was particularly true in the case of alpha and gamma globulins, in which evidences of the presence of these proteins were sometimes seen in the form of a sharp peak superimposed on a mass of some other protein fraction. No explanation for

this difficulty could be given at present as within the same electrophoretic run samples which did separate well and those which did not were found. Due to poor resolution, the areas of fractions α_1- and α_2-, and β_1- and β_2-, were lumped together for each specimen to allow a better comparison of the data. These groupings may have masked some significant changes within these fractions, but no alternative was available.

Certain specimens, on the other hand, showed the presence of protein which migrated with a mobility somewhat higher than that of albumin. These faster moving components (pre-albumin) did not seem to occur constantly, but appeared in various serum samples in a random fashion, in the males and females taken from control as well as experimental groups. No biological activity can yet be attributed to this protein, although binding of thyroxine by a pre-albumin has been demonstrated in man (Tata, 1959). Random presence of pre-albunin in very small quantity has also been reported in the plasma of other avian species like white Leghorn, bronze turkeys, ring-necked pheasants, pigeons, and muscovy ducks (Deutsch and Goodloe, 1945). There are no studies, however, to my knowledge, on electrophoresis of serum protein of Coturnix quail, other than the one presented here.

Also, it seems to present the first report on the effects of social isolation in experimental atherogenesis in this animal.

Gamma-globulin in the Coturnix quail, like human gamma-globulin, migrated in a peculiar way. Because of electro-endosmosis and other unexplained phenomena at the end of electrophoresis, the band stretched from the origin toward the cathode end, forming a wide band. A second peak, however, consistently appeared in the females in the region of γ_1. Differences in this particular globulin were so pronounced that a mere visual inspection of the pattern was enough to predict sex with almost 100 per cent accuracy.

Antibodies in the sera usually migrate with a mobility near that of gamma-globulin, but no electrophoretic differences can be detected between physiologically occurring gamma-globulin and that containing immune bodies. It has been suggested, however, that all gamma-globulins are antibodies (Anker, 1960). This basis is supported by the fact that in animals raised under sterile conditions the gamma-globulin concentration is almost negligible. In human beings, hyperfunction of the adrenal gland, or those treated with ACTH or cortisone, have low levels of gamma-globulin possibly due to lysis of lymphatic tissue (Vaughan _et al._, 1951). If this is true also for birds, then it might explain the higher gamma-globulin fraction in the normal Coturnix quail kept in unlimited space free from stress and reduction (although not consistent) in birds kept at the density of 15 or caged singly due to crowding or social stress.

The most striking difference, as expected, however, was found in the beta-globulin fractions of normal and experimental birds kept on cholesterol-mix diet at the density of 15 or caged singly. These globulins often were concerned with the lipid present in the serum, as indicated by the movement of turbidity with these fractions. This was further confirmed by pre-staining the serum lipoproteins with Sudan Black-B and then comparing the blue-band zones of lipoproteins with other protein fractions stained in Ponceau "S." In the case of Coturnix quail studied, this turbidity migrated on the fast side of globulin complex and seemed to be associated predominantly with the beta-globulin and less so with alpha and gamma globulins. In humans, beta-lipoproteins form approximately 5 per cent of the plasma proteins and contain about 70 per cent of the plasma lipids (Haurowitz, 1963). In the present study, quantitative analysis of various lipoproteins was not done, but the stained patterns in Sudan Black indicated that at least 60-70 per cent of the lipids were bound up with beta-globulins.

In the light of the above observations it is apparent that environmental factors, which might alter the psychological state of quail, can influence the development of dietary-induced atherogenesis. The high incidence of disease in birds kept at a population density of 15 per square foot and in socially isolated birds kept on equal floor space, accompanied by a significant increase in their beta-globulin fraction could alone justify the above observation. However, the mechanism underlying the effect of such environmental influence on atherogenesis is not clear. Decreased physical activity in the birds kept at higher density level have to be considered. But equal or even greater susceptibility to the disease in over active, singly caged birds makes physical activity per se an unlikely explanation. It is possible that psychological stress in these birds might have influenced the cholesterol metabolism by release of cortisone which is known to elevate the blood cholesterol levels.

Further study might be directed toward isolating the types of environmental changes which can influence atherogenesis and the exact mechanism involved.

SUMMARY

In view of previous findings, it seems that crowding, beyond certain limits, does affect the adrenal function. In Japanese quail, kept in the group of 15 per square foot of space, adrenals were significantly heavier than the adrenals of birds kept in identical situation in the group of five. Even larger adrenals were found in socially isolated birds caged singly. Stimulation of adrenal glands correlated well with the amount of social interaction, or intolerance of individuals towards others of their own group, at higher population levels (15 birds per cage). This was evident by direct observation and also by the amount of injuries present and the loss of their body plumage. Social hierarchy in males developed only after they had attained sexual maturity (about 8 weeks). After this stage, the adrenals of birds kept in the group of fifteen were significantly heavier than the adrenals of birds kept five together.

Olfactory, acoustic, and even visual stimuli seem to evoke adrenal responses in socially isolated animals. This was supposed to be the only reason for heavier adrenals found in Coturnix quail caged singly in this investigation. The exact neural mechanism, and nature of the controlling

process or processes involved, is still unknown.

Coturnix quail are endowed with a remarkable power for adjustment to crowding. Despite significant changes in the gross weight of adrenals, no significant difference was found in their body weights.

A highly unnatural situation in the form of social isolation or intense crowding imposed on Coturnix quail in this investigation augmented experimentally induced atherogenesis. Incidence of the disease was significantly enhanced in birds fed on cholesterol-mix diet and kept either in the group of 15 or caged singly in complete social isolation. During the entire experimental period, only one bird out of 15, kept on atherogenic diet and not subjected to crowding or to social isolation, developed the disease. This suggests that, besides diet, there are multiple interacting factors to influence atherogenesis. Isolation of these environmental factors, and the exact mechanism involved, is the subject for further research.

The electrophoretic pattern of serum protein seems promising as an index to an individual's physiological state. Increased amounts of beta-globulin components in the birds kept on the atherogenic diet and subjected to crowding or to social isolation seem to be a reliable indication of the altered nutritional-metabolic cholesterol-lipid-lipoprotein state of an individual. A significant increase in this component, therefore, could be taken as a reliable guide for the developmental stage of the disease.

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