A MORPHOLOGIC AND RADIOAUTOGRAPHIC STUDY OF THE PROSIMIAN, GALAGO SENEGALENSIS

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ABSTRACT

A MORPHOLOGIC AND RADIOAUTOGRAPHIC STUDY OF THE PROSIMIAN, GALAGO SENEGALENSIS

By

Michel Fouad Nassar

A morphologic study of the adrenals and urogenital tract of the female Galago senegalensis was carried out.

Tritium labeled thymidine in a dose of 4 uc/gm. was injected subcutaneously to four female Galagos that were sacrificed 2 hours, 1, 2 and 4 days after the injection. A fifth female Galago received a dose of 10 uc/gm. tritiated thymidine and was sacrificed 8 days after the injection.

Thirty-six different tissues were selected for radioautographic investigation.

The radioactive index, and the number of silver grains per nucleus were calculated for the different tissues at the various time intervals.

The turnover time was also calculated from the radioactive index of the various tissues of the animals that were sacrificed two hours and one day after the injection. The duration of the DNA synthesis was estimated to be four hours.

Michel Nassar

The various tissues were classified as to their turnover rate into fast, moderate, slow, sporadic replacement, growth only and no growth by mitosis. The merits of each case were discussed and a comparison of the turnover rates of some tissues of the Galago with those of the rat, mouse and human was made.

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By

Michel Fouad Nassar

A THESIS

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To those who were the cause of my
existence, and the reason of my
success.
To my Parents,
I dedicate this work.

ACKNOWLEDGEMENTS

No words can be put in sentences to express my appreciation and deep gratitude to Dr. Bruce E. Walker, whose patience, understanding and most valuable assistance and advice made this task possible. All that I have accomplished during my graduate career I owe to Dr. John H. McNeill, without whose encouragement and advice, this would have been impossible. My deep appreciation and gratitude also to Dr. Robert Echt and Dr. Charles Taban for their unswerving patience, cooperation and advice.

Many thanks are due to Mrs. Joanne Burwick for supervising all the laboratory work and for her precious technical advice and assistance.

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To my colleagues the graduate students and all the laboratory technicians, many thanks for their help and assistance. I am grateful to Mrs. Mary Grace for the excellent job she did in typing this dissertation.

Men are not worried by things,
but by their ideas about things.
When we meet with difficulties,
become anxious or troubled,
let us not blame others, but rather
ourselves, that is:

Our ideas about things, because everywhere man blames nature and fate, yet his fate is mostly but the echo of his character and passions, his mistakes and weaknesses.

M.F.N.

THE AUTHOR IS A FELLOW OF THE LEBANESE
NATIONAL COUNCIL OF SCIENTIFIC RESEARCH

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ABBREVIATIONS

RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
ATP	Adenosine triphosphate
TTP	Thymidine triphosphate
H&E	Hematoxylin and Eosin
PAS	Periodic Acid Schiff
A	Adenine
G	Guanine
C	Cytosine
T	Thymine
u	μ

PREFACE

A preface provides a writer with an opportunity to express to the reader the sort of things he may say to a friend who picks up his manuscript. These may include reflections about the nature of his work, perhaps a capsule summary of the contents, some incidental information of a background nature, and, in effort to avoid misunderstandings, some statements about the limits and boundaries of the work.

The elucidation of immediate experience is the sole justification of any thought, and the starting point for thought is the analytic observation of components of this experience.

Many of the concepts and ideas that we usually employ are part of our professional equipment and come as a result of professional training. However, some ideas, and these are difficult to acknowledge specifically, we may borrow almost imperceptibly from the intellectual climate in which we live and work. We do so almost without knowing it, often in the process of "talking shop". In view of this it is impossible to acknowledge one's total intellectual indebtedness, or even to acknowledge the most important instances, for these may well be the instances in imperceptible borrowing.

I have clearly left myself open to the twin charges of overgeneralization and underdocumentation. To the former I would reply that any theory is built on a limited number of observations, on the other hand I am very conscious that many of my statements must be considered hypotheses which remain to be tested.

I feel at this stage that the paucity of testable hypotheses has become an even more serious bottleneck in studying cell turnover than the shortage of data. Hypotheses beget data, and I certainly hope that some of my propositions will lend themselves, and be subjected, to empirical research.

INTRODUCTION

The vastly increasing knowledge about the basic principles of the medical sciences is an important stimulus to search for more sophisticated information about the basic unit of tissue structure, namely the cell. Essentially, all the functions of the living organism are expressed in the qualities of the single cell. For more than a century now, a voluminous collection of data has been gathered on the phenomenon of growth regulation, cellular differentiation and migration. New facts are reported daily in the scientific journals to contribute to the intellectual progress only in so far as they can be assembled into an organized conceptual perspective. The discovery of common denominators among the various systems of knowledge permits the conversion of miscellaneous data and their interpretation into a well integrated hypothesis which, to be useful, must be expressed in specific terms which can be proved to be right or wrong.

Inasmuch as different kinds of cells exhibit varying rates of proliferation and migration, the generality is often expressed that mitotic activity is inversely proportional to the degree of cellular specialization. The nature of the proliferative activity indulged in by various kinds of cells has long been recognized as a basis for tissue classification.

The basic information on cell dynamics has been derived primarily from rodents and no systemic survey has been made of a primate.

The purpose of this research project is to collect baseline information on the rate of cell turnover, proliferation and migration in the various tissues of a primate.

The lesser bush baby, Galago senegalensis, a prosimian primate, provides an excellent laboratory tool for the study of cell migration and turnover in.a variety of tissues. The use of radioactive thymidine in these cell turnover studies in the Galago senegalensis is valuable because:

1) The average body weight of the adult male Galago is about 250 grams; and that of the female is about 225 grams. Thus, they are small in size, easy to handle and do not require a large dose of expensive radioactive compounds or drugs.
2) Since the Galago is closer evolutionary to the human it would be more accurate to extrapolate the experimental data obtained on the Galago to the human.
3) The Galago is much cheaper to house and feed, and less dangerous to handle than the Rhesus monkey.

The collection of this baseline information is fundamental to the understanding of the systemic study of reproduction in the Galago. The phylogenetic relation of the Galago to man which is somewhat removed but not too distant offers the behaviorists an opportunity to study the phylogenetic development of several patterns of life, most notable of which is reproduction.

The field of animal husbandry is very important to the reproductive biology of primates because it provides information that can be related to man. As a matter of fact, the reproductive behavior is a key issue and very important to understand, because it occupies a central position in the lives of all mammals, having adaptive significance for the survival of the species. It follows from this that if behavior has evolved under the influence of natural selection, then behavioral differences between closely related species are keys to an understanding of the survival of these species.

The information provided from this project can also serve as a cornerstone in the further investigation of RNA and protein turnover in the cells of various tissues examined and can lead to a better control of breeding by the use of drugs, hormones or any other kind of contraceptive. It also can serve as a baseline for experimental teratology and the effect of hormones on reproduction and the consequences of hormonal imbalance in the animal and the human with the appreciation of the wide spectrum of developmental phenomena which it embraces.

Because of these facts I saw it necessary to make a thorough and detailed description of the gross and microscopic anatomy of the female urogenital system of the Galago, and to study the nature of cell migration and turnover in thirty-six tissues by using tritium labelled thymidine for radioautographic techniques.

Most of the research on cell kinetics by the use of radioactive DNA precursors that was published until now was done on the rat and the mouse. Very little had been done on the monkey and the human. Nothing has been reported for the Galago.

The recognition of the experimental animal's role as a surrogate for man is, of course, neither new nor unique. In fact, as one looks at the entire question of the employment of non-primate animals in medical research it becomes more and more difficult to justify their use insofar as one wants to relate the findings directly to man. The classical laboratory animals, rats, cats, dogs, guinea pigs, etc., have been going their separate evolutionary ways from man for a very long time. Is it not a bit presumptuous to think that the changes that have occurred over this span of time will not affect the validity of our extrapolations? Is it not a bit more reasonable to use, where possible, an animal that is more closely related to man in time?

As such, we can make a useful comparison between the data available on cell turnover in the rodents,man, and those obtained from our experiments. These data, if analyzed thoroughly, should prove useful for the understanding of cell population dynamics and also lead to a better understanding of function.

The major advantage of these experiments is that they may provide a basis of information which will enable one to distinguish between the roles of tissue mass and tissue function in regulating growth. Analysis of the data obtained from these experiments will reduce the problem of cell turnover to a relatively simple set of alternatives, namely, whether their regulation is by means of stimulation, inhibition or a balance of both and as such we will learn more about the intercellular communication by which the incidence of mitosis is controlled.

Control mechanisms have to evolve along with other physiological attributes of the organs whose functions and growth they govern. Since function is the raison d'etre of morphology it is very logical to expect some physiological factors to be favored by evolution as a primary objective for a feedback mechanism that is responsible for regulating cellular proliferation and growth in post embryonic organisms to be the key that will open the door for future research in the field of experimental teratology.

Warburton in 1955 wrote that a morphologic property of a structure is important only if it affects its function. If this were not the case there would be no way of avoiding the accumulation of masses of non-functional cells. Structures without functions persist during evolution only at the risk of reducing the general efficiency of the organism. Therefore it is improbable that cell turnover mechanisms in Galago tissues become so independent of functional considerations that they don't have sufficient selective advantage to survive as a base for further structure-function correlation studies.

The approach to the problem of studying cell turnover rate, proliferation, and migration with radioactive thymidine is not without its disadvantages.

If we consider the animal itself at first, we find out that the Galagos are very difficult to study because of their nocturnal and arboreal habits. This fact poses a serious hindrance. Making an artificial environment which duplicates the natural one is a difficult task and it requires the scientist to adjust his thinking to the unusual perception of the nocturnal animal where the emphasis is placed mostly on smell, hearing, touch vs. color, manual precision, spatial judgement and stereoscopic vision.

When we realize the fact that the Galagos have not been intensively studied in their native habitat, and most of our information comes from studies on the captive animals, it becomes apparent that our reliable knowledge is very limited in this area. All of the major studies have been concerned with improving the breeding of these expensive animals and self sustaining the captive colonies for long term research. With only one exception, these studies have not produced any direct benefit to man, in terms of combating disease. This exception was the use of Galagos in life cycle studies of the yellow fever virus.

Considering secondly the cellular basis we find out that it necessitates, for example, the accurate and precise definition of what a particular organ's function is, which is something that is not always readily apparent in all cases, especially in the case of the Galago because of the lack of information about the various anatomical and physiological processes in this animal. Also, many cells and organs perform more than one function and as such it becomes necessary to know whether the regulation of the cellular process is governed by one or more of these functions.

Yet the advantages of this experimental design soars much higher than its disadvantages at the level of obtaining information that is amenable to experimental testing and accordingly will provide the rationale for numerous investigations in future experiments to study cellular differentiation and growth regulation to improve the status of our being.

This survey starts from the position that the catalytic activity of ideas is at least as important for the continuation of the process of scientific discovery as is the tested body of skills.

REVIEW OF LITERATURE

I. The Experimental Animal: Galago senegalensis (Figure 1)

A. Taxonomy:

There is considerable disagreement as to the taxonomic classification of primates; the major reason for this is the lack of a precise definition of a primate (Hill, W.C.O., 1953).

In 1873, Mivart defined a primate as an "Unguicilate, claviculate placental mammal, with orbits encircled by bone, three kinds of teeth, at least at one time of life, brain always with a posterior lobe and calcarine fissure, the innermost digit of at least one paw of extremities opposable, hallux with a flat nail or none, a well-developed caecum; penis pendulous, testes scrotal; and always two pectoral mammae".

These characteristics are not the only prerogatives of primates and most of them are shared by other orders.

In 1929 Wood Jones wrote that there is no single character in the definition of Mivart which constitutes a peculiarity of primates: for a primate animal may only be diagnosed by possessing the aggregate of them all.

In 1932, J.P. Hill stated that the outcome of a survey of the development in representatives of the several subdivisions of the Order, he distinguished four well-defined developmental stages or grades within which he designated the Lemuroid, the Tarsioid, the Pithecoid and the Anthropoid stages.

These coincide only in part with the subdivisions recognized by the systematists. The Lemuroid stage is characterized developmentally by a combination of generalized or primitive features such as are met within the development of lower mammals, together with certain other features which are regarded as progressive and anticipatory of conditions which become fully manifested in the Tarsioid grade.

The Galagos are usually considered in two genera:

Galago, containing the majority of species, and Euoticus, the needle-clawed Galagos, destinguished by the extended, pointed nails of certain digits as opposed to the flattened ones of the Galago (Crandall, L.S., 1964).

The Galagidae are defined by W.C.O. Hill (1953) as: "long tailed Larisoidea with greatly elongated pelvic limbs, including a specialized, elongated tarso-metatarsal region, with large greatly mobile ears standing out from the surrounding fur; very active; progressing by saltation".

There are five main types or groups of Galagos presently existing in Africa, but it is difficult to decide whether they represent five nominal species or whether they form genera or subgenera (Butler, 1964).

W.C.O. Hill (1953) compromises in the manner shown in the table below:

Classification of living Galagos

Family: Galagidae

Genus: Galago (typical galagos)

Species:
1) Galago crassicaudatus, also known as grand or thick-tailed Galago.
2) Galago senegalensis, or the lesser galago or the bushbaby.
3) Galago alleni, also called Allen's galago.

Genus: Euoticus (needle clawed galagos)

Species: Euoticus elegantulus, also known as the needle clawed galago.

Genus: Galagoides (Dwarf galagos)

Species: Galagoides demidovii also known as Demidoff's galago, or dwarf galago or bushbaby.

The Galago senegalensis have **nine** sub-species, the Galago crassicaudatus have ten sub-species (Napier and Napier, 1967).

B. Geographical Distribution and Habitat:

The habitat of the Galagos is Africa. They are found over most of the forested and woodland savannah areas, south of the Sahara from Senegal in the west to the Abbyssinia plateau in the east and south to Natal (Hill, W.C.O., 1953).

Species variations exist with regard to specific details of the habitats. The Galago senegalensis senegalensis prefers dry, open bush country and grass areas and spends its days nesting in tree holes that may be as low as 4 or 5 feet off the ground. Sauer and Sauer (1963) reported temperatures varying from 17.5° F. to 117.5° F. in southwest Africa where the galago senegalensis moholi thrives.

C. Diet:

The bushbabies are omnivorous, for they eat fruits, eggs, vegetables, flying and ground insects.

Doyle and Bekker, 1967, attest the fact that Galagos spend some time searching for food on the ground, despite the fact that most of their food is found on trees. This belief is supported by Haddow and Ellice (1964), who examined the stomachs of 103 recently sacrificed bushbabies. In 69 stomachs, dung beetles were found which, together with the incidence of other ground living insects, suggests a considerable amount of time spent in foraging for food on the ground.

D. Morphology and External Characteristics:

All primates in fact show various kinds of adaptations for living in trees. The anatomical variations can be used to distinguish the major primate groups and to identify the variations between the different prosimian families. Some features are important to characterize the Galagos as a group. Such anatomical variations are obvious in the shape and structure of the hands, feet, brain, eyes, ears, teeth and jaw bones.

The Galago senegalensis and Galago alleni are smaller in size than the Galago crassicaudatus. The male is usually slightly heavier than the female.

The average weight of a male Galago senegalensis is about 300 gm. and that of a female is about 229 gm., while that of a Galago crassicaudatus male is about 1241 gm. and that of a female is about 1034 gm. (Napier and Napier, 1967).

Doyle and Bekker (1967) reported that the weight of infants at birth varies from 8 to 13 gm., and the body length of the mature adult varies from six inches to seven inches from nose tip to the base of the tail.

The neck is short, the fur is soft and wooly and the color is a mottled slate grey and silver with a light brown wash on the dorsal surface of the posterior half of the body. The ventral surface is white with a slate grey on the deeper portions of the fur, seen only when the fur is separated. The length of the tail varies between 8.5 to 10 inches (Lowther, 1940). The Galagos have large round eyes bulging forward, and giving a conspicuously rounded appearance to the surface. The membraneous ears are large and almost naked on the inner and outer surfaces except at the rim where there is a sparse covering of fur (Lowther, 1940). The hands and feet are pentadactyl, the digits bear nails and there are six well-defined palmar pads. This anatomical structure shows a progressive adaptation for the animal to be able to climb by grasping instead of using digging claws; this has a special significance for it permits a greater security in supporting the body while climbing.

The long hind legs, elongated tarsus and massive vasti of these animals have been assumed to be adaptations advantageous to the remarkable saltatory gait that they have (Hall-Craggs, 1963).

The thumb is pseudo-opposable and the skin between the palmar pads is smooth. The index finger function is uncommitted and variable, and when the hand is inactive the fingers are flexed over the thumb.

The female has two pairs of mammary glands. The anterior pair are so laterally placed that each lies practically on a line with the inner border of the arm. The inguinal pair lie closer to the median line (Lowther, 1940).

E. Behaviour:

The Galagos are typically primates in the development and use of the fore-limb as a hand but are anomalous as primates in being strongly nocturnal (Luck, 1963). This feature is, anatomically speaking, most typically revealed in their eyes, which have all the hallmarks of the thoroughgoing nocturnalist: a pure rod retina, a large anterior segment of the eye, a large lens and a slit pupil.

The difficulty of studying these animals in their natural habitat cannot be underestimated. In spite of the fact that they are wholly nocturnal in their habits there are a few reports on their activity during the day. Seldom have groups or troops been seen or reported. Three or four may move around together in a kind of unit. The largest number ever seen sleeping in one nest during the day has been five and that was in an artificial nest provided by human observers. At twilight the animals would generally go their separate ways through the trees (Buettner-Janush, 1964).

The Galago, essentially a hopping and jumping animal, normally perches in high places. Although it uses all four feet for climbing and usually for perching, it does not normally run or walk on all fours. The structure of the posterior limbs is responsible for the characteristic hopping and leaping motions (Lowther, 1940).

The Galago is an unusually clean animal. When its hands are soiled by food or foreign substance, they are carefully licked clean. General grooming involves the frequent use of the procumbent scraper, the tongue, and the occasional use of the claw which serves as a scratcher for such spots, otherwise inaccessible, as behind the ear (Lowther, 1940).

An outstanding Galago peculiarity is the ability not only to move the ear as a whole forward, laterally or upward, but also to fold the pinna along the ridges somewhat like an accordion, throwing its outer tip back over the upper transverse ridge. The ears are perhaps the most expressive feature, since they move singly or in unison in the direction of the least unexpected sound (Lowther, 1940).

Hill, W.C.O. (1953) distinguished the primate species on the basis of the teeth and shape of the jaw bones.

However, there is a general dentition structure for all prosimians with moderate variations. The toothcomb used for grooming and the canines which are fairly large in size reflect the omnivorous diet and the predominant need for structure adaptation to eat insects and plants.

The Galagos show excellent manual precision and spatial judgement. This characterized them evolutionary for the use of the forelimb as a hand.

F. Reproductive Biology:

1. Sexual Behaviour and Characteristics:

There are many discrepancies in the literature available about the Galago's reproductive physiology and behaviour, yet enough is known to present these facts as ranges and approximations.

Perhaps future studies and experience with breeding colonies over a long period of time will help to identify the variations due to the true species variations in contrast to those due to man's imposed artificial environment.

There is general agreement in the literature that the Galago senegalensis has a restricted season of sexual activity resulting in two pregnancies per year (Haddow and Ellice, 1964).

Butler (1960) indicated that the Galago senegalensis appears to have an estrous cycle lasting four to six weeks and evidence suggests that it exhibits a post-partum estrous. The actual length of estrous is unknown. Manley (1965) observed four females over 32 calendar months and found a mean estrous cycle of 31.7 days. He found immediate postpartum estrous only in a case of a primiparous female which ate her offspring at birth and who conceived again three days later. Doyle (1970) confirmed the immediate postpartum estrous.

Sauer and Sauer (1963), reported that estrous is accompanied by a white vaginal discharge lasting seven consecutive days.

Lowther (1940) and Sauer and Sauer (1963) estimated the gestation period at about 120 days for the Galago senegalensis moholi.

Manley (1965) gauged the gestation period in the Galago senegalensis senegalensis at 144-146 days in four females.

Doyle _et al_ (1967) observed both copulation and partuition four times in two females of the Galago senegalensis moholi; birth occurred 122-125 days from the first day of estrous on which copulation occurred.

Sexual maturity for the female Galago is reached in 8-9 months, but for the male it takes a whole year. The length of the reproductive life is not known but the whole life span of the Galagos varies between 8-11 years (Buettner-Janush, 1964).

Doyle and Bekker (1967) designed a facility for naturalistic studies of the lesser bushbaby and made extensive observations concerning courtship, mating partuition and sexual behavior.

The male's sexual interest in the female remains at a fairly constant level throughout the year. During a

50-minute observation period, Doyle, Pelletier and Bekker (1967) reported that the male will approach the female from one to nine times to examine her genitalia.

Courtship is always initiated by the male and is probably released by olfactory cues.

Every time the male approaches the female, the occasion will usually be followed or accompanied by the male "urine marking", a form of behavior given by some authors that in the male may be correlated with a sexual function (Sauer and Sauer, 1963), but Lowther (1940) and Hill (1953) disagreed.

Briefly, urine marking involves placing the cupped hand directly under the urethral opening and depositing into it a few drops of urine. The sole of the foot on the same side, which is already in a raised position to facilitate the placing of the hand under the urethral opening, is then methodically wiped with the moist hand from one to four times. Females urine mark in exactly the same way as males though far less often and have never been observed to urine mark in a sexual situation.

Mating behavior fluctuates, with periods of intense sexual activity consisting of much chasing and many mountings of short durations between 10-50 seconds, and the rear copulatory approach is consistently used. Intromission and ejaculation occur when the mounting lasts as long as 120 seconds (Doyle, Pelletier and Bekker, 1967).

The pattern of partuition consists of three distinct stages. The first stage is characterized by intense activity in jumping about the cage from one nest box to the other. The second stage begins as the first stage subsides. It is characterized by restlessness, intense self grooming and nest making. The third stage begins with the delivery of the first infant and is heralded by a short period of intense genital grooming. Delivery of the first infant takes about 30 seconds (Doyle, Pelletier and Bekker, 1967).

The birth of twins among the Galagos seems to be a common occurrence, although single births do occur (Lowther, 1940).

W.C.O. Hill (1953) stated that a single offspring is the general rule, but twinning appears to be regular in the Galagidae.

The importance of removing the male Galago from the cage when the female approaches partuition cannot be overestimated. In two cases, where the male parent was allowed to remain in the cage the infants were killed by the male within a short time after birth (Buettner-Janush, 1964). Doyle (1970) disagrees with this idea.

Between 10 and 11 weeks the infants become fully independent of their mothers. The only maternal activity which does not change is grooming, which is fairly intense throughout and, in fact, never ceases (Doyle, Anderson and Bearder, 1969).

2. Histological Structure - Function Correlations:

The most outstanding contribution to our knowledge on the reproductive anatomy and physiology of the Galago senegalensis senegalensis comes from Butler.

The problem whether oogenesis is continuous throughout the reproductive life of a mammal as is spermatogenesis is controversial (Zuckerman, 1960).

The most striking observations are those of Gerard (1920, 1932), who compared the ovary of a suckling female infant of a Galago senegalensis moholi and a Galagoides demidovii with that of their mothers. He examined 18 ovaries of pregnant, lactating and non-pregnant adult females and found histological appearances indicative of active oogenesis in 16, that is mitotic proliferation of the germinal epithelium and nuclei in the prophases of the heterotypic division.

In 1960, Herlant reaffirmed the presence of histological stages of oogenesis in adult ovaries in both pregnant and non-pregnant Galago senegalensis females.

Zuckerman (1960) concluded by stating: "Our information is less extensive than one might desire, but for the few species for which we have information the evidence is overwhelmingly in favour of the hypothesis that oogenesis, unlike spermatogenesis, ceases when reproductive life begins".

Butler (1960) divided the follicles of the ovary of the Galago into four types ranging in diameter from 200 u to 700 u, and labelled them as:

Type I: 200-400 u in diameter, solid follicles with no antrum formation.

Type II: 300-400 u in diameter, a few small, but discrete, antra.

Type III: 200-400 u, the antra are large and beginning to coalesce, or coalesced to form a single large antrum.

Type IV: 300-700 u, follicles of the same structure as Type III. He proposed that these are new follicles.

He also measured the diameter of the uterine cornu and found it to be 2.5 m.m., while the length of the cornua is between 4.0 - 4.5 m.m. The endometrium reaches the thickness of 500 u in some places.

In 1967, Butler reported that he examined the ovaries histologically of one captive and two wild animals shortly after ovulation. He found that each had one recently ruptured Graafian follicle with a central haemorrhage and early luteinization of the granulosa cells. The liberated ovum was found in one animal in the proximal end of the right uterine tube and contained male and female pronuclei.

Large numbers of Graafian follicles up to 600 u in diameter with antra were seen in the ovaries, and others showed varying degrees of atresia such as basophil droplets among the granulosa cells and in the follicular fluid, shrunken ova, abnormal polar spindles, etc. There were many solid follicles, up to 300 u in diameter, with many granulosa cells in mitosis and no signs of atresia. One ovary contained a subsurface corpus luteum, 800 u in diameter, with many degenerate luteal cells.

The endometrium was divided into a basal glandular zone and a subepithelial zone of edematous stroma. The uterine epithelial cells were 30 to 40 u tall and formed a pseudo-stratified epithelium with three to four rows of nuclei. A few cells showed mitotic figures. The uterine gland cells were 12 to 13 u tall and a few were in mitosis. The gland lumina contained a little coagulated secretion.

At estrus, the vaginal orifice was widely open and the labia and clitoris were red and swollen. The redness and swelling was present for only 24 to 48 hours. The vagina had a transverse diameter of 11.0 m.m. and an antero-posterior diameter of 7.0 m.m.

In general, the Senegal galago follows the typical pattern of the mammalian estrus cycle, ovulation occurs spontaneously in isolated females, and as a rule, only one follicle ruptures at each estrus (Butler, 1967).

Another outstanding feature of the non-pregnant cycle of the Senegal galago is the unusually long life of the corpus luteum. Butler (1967) reported that by 14 days after ovulation it was 500-770 u in diameter and its cells showed no signs of degeneration. By 26 days it was 1350 u in diameter and some cells showed cytoplasmic vacuolation indicative of degeneration.

The Senegal galago does not menstruate in spite of the fact that it has a dual arterial supply, like that of the higher primates and man which do menstruate (Butler, 1967).

Kanagasuntheram and Verzin (1964) studied the pattern of innervation of the female reproductive organs of the lesser bush baby and reported the presence of ganglionic tissue around the middle third of the vagina. Whether these paravaginal ganglia belong to the sympathetic, or the parasympathetic, system, or both, is not revealed. The endometrium is richly innervated and nerve fibers penetrate as far as the epithelial layer, while the myometrium of the uterine horns is innervated by fine fibers with bulbous or pointed terminations. The ovaries have a well-developed cortical plexus of nerves and fibers that penetrate the peripheral cells of the follicle.

Ahmed and Kanagasunterham (1965) studied the mammary gland histologically and found that the skin overlying the glandular tissue is composed of thinly keratinized epithelium consisting of two to three layers of cells. Few sebaceous glands were seen in the skin but no sweat glands. The glandular tissue was found to spread through the underlying area, although there was no visible external swelling to indicate its presence. Strands of striped muscle fibers were seen between the lobules of the glands. Most of the alveoli of the glands were partially or completely collapsed, but the alveoli that retained their lumina contained little or no secretion. Desquamated epithelial cells were frequently seen within the lumina of the alveoli and those of the ducts. The ducts, in contrast to the alveoli, were distended and lined by flattened epithelial cells. They contained large quantities of secretion and a few leukocytes. The connective tissue separating the lobules was distinct but there were no fat cells within the interlobular connective tissue. Each nipple has two ducts which pass from the base of the nipple towards its apex where the openings of the ducts are situated. The glandular tissue is found deep to the base of the nipple as well as in the skin around it.

Another organ that is very well studied in Galago senegalensis is the skin. Yasuda et al (1961) reported that the epidermis of the general body surface is thin with a malpighian layer 3 or 4 cells deep and the basal cells have distinct cytoplasmic denticles that grow into the dermis. The stratum granulosum contains sparse keratohyaline granules and the stratum corneum is 8 to 10 layers thick. The dermis is composed mostly of a reticular layer. There are few capillaries under the epidermis and around the cutaneous appendages. The hair follicles grow in small groups of 3 to 6 and the sebaceous glands are small but none is well vascularized. Apocrine sweat glands are found over the general body surface. These small glands open directly to the surface near the pilary canal. These glands are probably not functional, except on the lips, eyelids, perianal skin, vulva and scrotum.

The skin of the lesser Galago is relatively unspecialized. It has some features which are characteristic of primates, but with the exception of the hair groups, the skin is apparently very primitive.

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II. Cell Proliferation, Migration and Turnover

A. Tissue Classification:

Various kinds of cells show varying types of proliferative activities and this served as a basis for tissue classification.

Bizzozero (1894) was the original contributor along this line who proposed the existence of three categories of tissues according to whether they:

1) multiply throughout life,
2) proliferate only in growing individuals but seldom in adults,
or 3) fail to exhibit mitosis altogether once they have differentiated.

Many years later, Cowdry (1942) suggested a similar classification of tissues, but he referred to them as:

1) vegetative intermitotic cells,
2) reversible post-mitotic cells,
and 3) fixed post-mitotic cells.

Subsequent technical refinements, based primarily on the use of tritium labelled thymidine, have led to a number of recent modifications of this fundamental system of classification (Leblond and Walker, 1956; O'Steen and Walker, 1960; Messier and Leblond, 1960; Schultze and Oehlert, 1960).

Radioautographic techniques allowed for a more precise distinction to be made between "renewing" and "expanding" cell populations, as was defined by Messier and Leblond (1960), than was previously possible by counting mitotic figures.

Cells labelled with radioactive thymidine can be traced for prolonged periods of time; while a mitotic figure can be detected only during the brief interval of its existence. In a renewing tissue, the label will disappear eventually as the cells are lost or destroyed, while in an expanding system the labelled cells will persist and can only lose their radioactivity by dilution during subsequent divisions. As such, there are two essential differences between the renewing and expanding tissues, namely, whether or not cells are regularly lost, and whether new cells are derived from special germinative tissues or from the population at large.

Static tissues represent a special case of expanding systems in which growth by cell division is restricted to early stages of development, though it may continue by cellular hypertrophy during later periods of time. However, there are occasional reports of mitosis in neurons (Altman, 1962) and muscles (Messier and Leblond, 1960).

Leblond and Walker (1956) classified the cell populations of the adult organism, on the basis of mitotic activity. They divided them into three groups:

1) The cell populations in which no mitoses have been detected. That is, the various types of cells encountered in the nervous system, sense organs and adrenal medulla. The number of cells in these structures remains constant once the adult stage is reached, and further growth can occur only by cell enlargement.

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2) The cell populations in which mitoses are infrequent, for instance, with a daily mitotic activity below the range of 0.8-1.5 per cent in male rats weighing 200-250 grams -- a mitotic rate just sufficient to bring about an increase in cell number proportional to the increase in body weight, and, therefore, contributing only to growth.

3) The cell populations in which mitoses are abundant (for instance, with a daily mitotic rate exceeding 1.5% in rats weighing 200-250 gm.). Such mitotic activity indicates the occurrance of more new cells than are required for growth and, hence, renewal must take place.

Leblond (1964) defined the static cell populations in 7 to 90 day-old rats as homogeneous groups of cells in which mitotic activity can be detected even with colchicine or H^3-thymidine, so that the total DNA content remains constant. He then defined the expanding cell populations as homogeneous groups of cells in the 7- to 90-day-old rats, in which scattered mitosis can be detected with colchicine or H^3-thymidine, in numbers accounting for the increase in total DNA content. Under these conditions the life of each cell or its progeny lasts as long as that of the individual in whom the cell is present.

Leblond (1964) also defined renewing cell populations in the same group of animals as homogeneous groups of cells in which abundant mitoses can be detected with colchicine or H^3-thymidine, in numbers exceeding those required by the increase in total DNA content, as the high cell production is largely balanced by a cell loss.

Compiled from the data of Messier and Leblond (1960), Osteen and Walker (1960), and Schultze and Oehlert (1960), the histological systems were divided into the three populations defined above. Renewal systems include:

Epidermis and its derivatives,
Endodermal Epithelium,
Endometrium,
Transitional epithelium,
Gonadal germinative cells,
Haemopoietic tissues, and
Skeletal tissues (in part).

Expanding cell populations are found in liver, kidney, exocrine glands, endocrine glands, lens, connective tissue proper and skeletal tissue (in part).

Striated muscle, neurons and neural retina belong to the static cell population.

B. Biosynthesis of DNA:

Analysis of DNA from many different sources, performed originally by Chargaff and Davidson (1955), showed that the contents of purines (adenine and guanine) always equaled the contents of pyrimidines (cytosine and thymine). Furthermore, the bases with an amino group in position 6 (A and C) were equal in total amount to the bases having a

6-Keto group (G and T). On the basis of these findings and of the x-ray structure data, Watson and Crick proposed their double-stranded helical model for DNA in which each adenine was paired with a thymine base and each guanine with a cytosine.

A most appealing feature of the Watson-Crick structure for DNA was that it suggested a very reasonable mechanism for replicating a DNA molecule with preservation of its information content.

During the replication of DNA, the sequence of nucleotides in the parent molecule must be accurately reproduced in the daughter molecules, since the genetic information contained in the sequence would otherwise be lost or distorted. It is likely, therefore, that an enzyme system will exist that can synthesize double-stranded polydeoxy-ribonucleotides with Watson-Crick base pairing but that also uses a primer DNA and reproduces the nucleotide sequence present in that primer DNA. It is generally believed that semiconservative replication occurs; this implies that the two strands of the primer DNA molecule will separate at least locally where the enzyme is acting. A new single strand of DNA is then built up by the enzyme on each separate strand (Ingram, 1966). The polymerization of the nucleotides into the helical configuration of the DNA molecule is catalyzed by the enzyme DNA polymerase.

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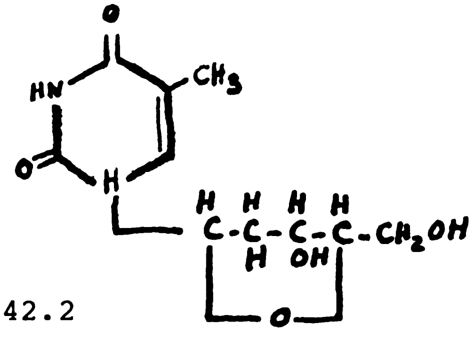
C. Thymidine Metabolism and Cell Kinetics:

The synthesis of tritium labelled thymidine which was first reported by Taylor _et al_ in 1957, and the introduction of radioautography for the detection of the subcellular location of labelled molecules, made it very valuable for experimental work.

Tritiation was achieved by means of a catalytic exchange between thymidine and tritiated acetic acid. The thymine molecule has four sites at which H^3 atoms can be substituted for hydrogen. These are positions 1, 3, and 6 and the methyl group, but not all of these are equally stable (Cleaver, 1967). Hydrogen atoms at positions 1 and 3 are labile, and H^3 may be lost from these sites by exchange with hydrogen atoms in aqueous solution. After catalytic exchange, treatment with a weak alkali removes the H^3 from the labile positions and the only remaining H^3 atoms are those in the methyl group and at position 6, which are firmly bound. The locations of H^3 in the radioactive thymidine molecule are consequently only in the methyl group and 6, no H^3 being bound at position 3 (Verley and Hunebelle, 1957).

Thymidine-methyl-H^3



Molecular weight - 242.2

Later, Pastore and Friedkin (1962) discovered that the H^3 atom was located exclusively in the methyl group.

Thymidine itself does not occur naturally on the main intracellular pathways that lead to DNA synthesis but is introduced into them by a single phosphorylation step to thymidine monophosphate. Although thymidine is not essential for DNA synthesis except in organisms which cannot make their own thymidine monophosphate, it is incorporated rapidly into DNA in most organisms (Cleaver, 1967).

The incorporation of thymidine into DNA proceeds by a sequence of phosphorylation steps throughthymidine monophosphate, thymidine diphosphate and thymidine triphosphate followed by the assembly of TTP, together with the other nucleoside triphosphates, into DNA. The enzymes involved in the phosphorylation steps are known as thymidine kinase, thymidine monophosphate kinase, and thymidine diphosphate kinase, respectively, though the latter two are commonly discussed under the combined term of thymidylate kinase (Grav and Smelli, 1964).

When thymidine is added to a biological system the time course of phosphorylation depends in detail on the precise nature of that system. Of the three phosphorylating enzymes involved in the incorporation of thymidine into DNA, only thymidine kinase has been studied in detail up the present times.

In addition to the substrate, the thymidine kinase reaction has an absolute requirement for ATP and Mg^{++} ions. The role of ATP in the reaction may not only be to give a phosphate group, but also to act as an activator, so that two molecules of ATP are required for each molecule of thymidine and thymidine kinase (Okazaki and Kornberg, 1964). DNA is relatively stable in the cell nucleus except during cell division, so that an injection of labelled thymidine should be found in cells forming DNA in preparation for division at the time of the injection. Once the cells are labelled they should remain so for long periods of time depending on the interval before the labelled cells again divide (MacDonald and Mallory, 1959). The time for the completion of DNA synthesis is between 6 to 8 hours (Thorell, 1955).

A considerable amount of injected H^3-thymidine is retained in the body, the amount retained in any particular tissue is a small fraction of the amount supplied. In the rat for example, only about 1% of the injected H^3-thymidine is incorporated into DNA in the regenerating liver and the breakdown products are detectable in the acid soluble fraction of the liver within 2 minutes of the injection (Chang and Looney, 1965).

The time for which thymidine is available as a precursor of DNA after its injection depends on the type of experimental system utilized.

Tritium-labelled thymidine initially is uniformly distributed throughout the body and is either promptly incorporated into DNA or degraded (Cronkite et al, 1959). In animals, the breakdown and excretion of injected thymidine results in a short availability time of only a few hours

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(Cleaver, 1967). The effective availability time of radioactive thymidine is less than one hour during which time it is either degraded or incorporated into DNA (Cronkite *et al*, 1959). If it is assumed that thymine bases are all derived from an external medium, thymidine will be used up at a rate of about $1.7X10^{-17}$ moles/cell/min. (Cleaver, 1967).

There is no evidence for or against an active transport mechanism of H^3-thymidine into the cells synthesizing DNA. However, the evidence that H^3-thymidine is concentrated by some mechanism is conclusive. Cronkite *et al*,(1959) stated that active transport is a possibility but they were more inclined to believe that thymidine diffuses into the cells.

Pelc and Appleton,(1965) stated that thymidine becomes available to most tissues directly after injection and diffuses rapidly from the blood vessels at a rate of 2u/sec.

The concentration of thymidine that becomes available for DNA synthesis may not be identical for every tissue. The availability depends on the distribution of blood vessles, membrane permeability, etc.

Labelling of the proliferative cells of the bone marrow was nearly complete within ten minutes after injection, and thereafter the label appeared to remain in these cells or their progeny for their life span, diluted only by successive mitoses (Rubini,Cronkite and Bond, 1960).

When thymidine is supplied by feeding, it remains available for DNA synthesis for a longer period than after in

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injection, and incorporation may continue for at least two hours. This is due to the time required for assimilation from the gastrointestinal tract. The labelled thymidine may be incorporated preferentially into DNA by cells in the epithelium of the gastrointestinal tract (Rubini et al, 1961), although this has not always been observed (Field et al, 1969).

After a subcutaneous injection, thymidine remains in the body for 30 minutes (Cronkite et al, 1959). Some incorporation of label into DNA may be detected from several days to a week after a single injection of H^3-thymidine (Bryant, 1962, Steel, 1966). This is due to the degradation of labelled DNA from cells which die during this period.

Following an intravenous or intraperitoneal injection most of the incorporation into DNA occurs during the first 30 to 60 minutes (Hughes et al, 1958, Rubini et al, 1960).

Plasma clearance of H^3-thymidine commences in the first circulation time and becomes exponential following apparent equilibrium with total body water (Rubini et al, 1960).

The rate of clearance from the body is slightly faster following intravenous injection than intraperitoneal injection (Hughes et al, 1968). This rapid plasma clearance was associated with incorporation into newly formed DNA of proliferating cells as early as one minute from injection time (Rubini et al, 1960).

The concentration of labelled thymidine declines in a fashion that follows a curve described as the sum of two exponential components with different half lives. In man the two component half lives are one minute and two minutes (Rubini et al, 1960). In the mouse the half lives are about 1 to 2 minutes and 25 minutes (Staroscik et al, 1964), and in the rat during liver regeneration about 8 and 30 minutes (Chang and Looney, 1965).

Degradation steps of thymidine consist of the cleavage of the glycosidic bond to form thymine and deoxyribose-1-P by thymidine phosphorylase (Klein, 1935). In mammalian tissues the phosphorylase is found in the order of decreasing activity in the intestinal mucosa, liver, bone marrow, kidney, spleen, lung and heart and much less activity is found in the brain and muscle (Friedkin and Roberts, 1954). The major pathway for degradation is through thymine, dihydrothymine, β-ureidoisobutyric acid, β-aminoisobutyric acid to water and carbon dioxide (Armstrong et al, 1963).

About one-third of the labelled thymidine is catabolized to tritiated water within a few hours after injection (Rubini et al, 1960).

In conclusion, it is likely that DNA synthesis is controlled by other factors than merely the activity of the enzymes which lead to the synthesis of TTP. The pathway involving thymidine kinase and thymidylate kinase can be considered as an important example of a controlled pathway for the synthesis of one of the precursors of DNA rather than a control mechanism for DNA synthesis itself.

III. Replication of DNA and the Cell Cycle:

By the introduction of quantitative cytological techniques and radioactive precursors, it became more feasible to distinguish between the replication of the chromosomes and their segregation at mitosis.

Howard and Pelc (1953) suggested that the cell cycle could be represented in terms of progression around a clock face, and the various phases of the cycle represented as sectors on that face.

The phases were labeled as M and S to designate mitosis and DNA synthesis respectively and G_1 and G_2 to represent the gaps during which no DNA synthesis occurs, although RNA and protein synthesis occurs throughout both gaps and the S phase.

Bertalanffy in 1964 reported that the cells of a tissue undergoing cell renewal pass through four principal phases, and he described them as:

1) Cell formation is by mitosis in most tissues of fairly undifferentiated cell comprising the generative cell fraction. This phase is known as the M (mitotic) phase. In most differentiated cells forms the maturing cell fraction apparently has lost the ability to divide under normal circumstances.

   The duration of morphologic mitosis differs between cell types. It seems as though most mitotic durations fall within a range of 40 to 70 minutes, although durations of several hours were reported for some tissues.

2) The phases of differentiation and metabolic activity are combined as the G_1 phase or the first long post-mitotic gap.

Different cell populations manifest a great range in the duration of the G_1 phase. While it may be very brief with the cells of intestinal cypts (e.g., 18 hours) it may occupy several weeks with those of mesothelia and urinary tract epithelium. The cells of non-proliferating populations can be conceived as being sustained in a perpetual G_1 phase.

In tissue cultures the duration of the G_1 phase can be between 1.5 and 60 hours (Cleaver, 1967).

3) Cells of renewing populations invariably pass sooner or later into the S phase, or phase of DNA synthesis.

In cells destined to divide, DNA synthesis ensues in an augmentation of the DNA content from the diploid to a tetraploid amount of DNA (Lajatha, 1956). The duration of the S phase is fairly constant lasting from 6 to 8 hours with many mammalian cell populations (Cameron and Greulich, 1963). Painter and Drew (1959) reported that the duration of DNA synthesis in a cell preparing for division is 5-8 hours. Basing his judgement on his work on the epithelium of the esophageal mucosa, ileum and colon of the mouse, Cameron in 1961 found the time interval to be 7 1/2 hours. In the rat's Ileum, the DNA synthesis time is 5.2 hours (Warburton, 1961).

4) The S phase is followed by a brief period, the second premitotic gap or G_2 phase, intervening between completion of DNA synthesis and the onset of morphologically descernible mitosis. The G_2 phase ranges from less than one hour to about four hours in different cell populations Defendi and Manson, 1963).
From the G_2 phase, cells pass gradually into the first stage of the M phase, that is, into morphologic prophase.

The great latitude of the duration of the G_1 phase in particular is responsible for the dissimilar life span of various cell types, and thus for the different turnover or renewal times of cell population (Bertanlanffy, 1964). Modifications of environmental factors such as pH and nutritional conditions appear to affect mostly the length of the G_1 period (Defendi and Manson, 1963). Not only does the mean duration of G_1 vary considerably from one cell type to another, but within one particular system it is the phase in which most of the variation between individual cells occurs (Sisken and Morasca, 1965).

Tritiated thymidine is incorporated solely into the DNA molecule. The label is thus acquired only by cells that are in the S phase at the time H^3-thymidine is available. Once the label has been incorporated it remains firmly bound within cells, and is diluted only by subsequent division of the labelled cells. The tritium atoms are distributed at each division about equally to the two daughter cells (Bertalanffy, 1964).

The label becomes halved at each successive division and although it does not disappear completely, will cease after a few divisions to be demonstrable in radioautographs exposed for moderate periods (Quastler and Sherman, 1959).

During exposure, the tritium atoms of thymidine in the newly synthesized DNA decay to helium by the emission of nuclear electrons (Johnson, Haymaker and Rubini, 1960). These low energy electrons travel only a short distance through the photographic emulsion, 90 per cent of them less than 3 u. When cells are viewed from above, the majority of reduced silver grains are consequently super-imposed above the nucleus (Johnson, Rubini <u>et al</u>, 1960).

IV. Radioautography:

The term radioautography is used to describe the method by which a radioautogram is obtained. In a radioautogram, a radiation source within a structure, such as a tissue section or a single cell, is localized by a photographic film with optimally fine grains in close contact to the structure (Feinendegen, 1967). The photographic emulsion, owing to its high amplification,is the most sensitive device to detect radioactivity.

Fitzgerald <u>et al</u>, in 1951, used tritium with its low energy β-radiation as a label and brought radioautographic resolution to the level of single cells.

The ionizing radiation interacts with a photographic emulsion which consists of gelatin into which are embedded silver halide crystals, such as silver bromide. When the electrons are set free a negative charge is trapped in the crystal and held first at the sensitivity specks of ionized silver (Webb, 1964). The sensitivity specks consists mainly of silver sulfide deposits on the surface of the silver bromide crystals and thus a latent image is produced. The crystals become susceptible to the developers which are reducing agents to convert more silver ions to metallic silver which constitutes the final image. Such reducing agents are, for example, hydroquinone, aminophenols or ferro-oxalate, in solutions of various pH. For radioautographs prepared with liquid emulsions, 3 minutes are usually sufficient to develop all latent images. The temperature at which development is carried out is between 16^{o} and 20^{o}C.

Following the ordinary method of chemical development, the emulsion is rinsed with water or 1-3% acetic acid for a few seconds. The non-developed crystals are made water-soluble and are removed by the process of fixation. The emulsion becomes translucent during the procedure. Fixation proceeds by complex formation of the silver halide with sodium or ammonium thiosulfate in aqueous solution of 15% at a pH of approximately 6 (Feinendegen, 1967). The length of the fixation is less crucial than the timing of development. It may continue twice as long as the time used for development without affecting the final image, which remains stable over a period of many years.

Biological specimens can be treated by any histochemical technique prior to radioautography, as long as the specimen can be thoroughly freed from oxidizing or reducing chemicals before application of the film (Pearse, 1960). Staining with periodic acid, for example, must be carried out prior to mounting the nuclear track emulsion since it involves hydrolysis. While hematoxylin and eosin do not leach nor cause chemographic effects, they are used after the completion of the photographic processing of the radioautograms (Kopriwa and Leblond, 1962).

Radioautograms are stained like other ordinary histological preparations in slide racks, which are submerged into the staining solutions in a conventional glass dish. The staining can be carried out at room temperatures.

The stained radioautograms can be mounted with a cover slip for protection. The mounting medium can be Canada balsam or Permount properly diluted and used.

Only the technically adequate radioautograms should be used for evaluation. They should be neither overexposed nor underexposed, and the background should be low. There should be no difficulty in correlating the specimen with the appropriate image in the emulsion. It is usually not difficult to distinguish in the light microscope a silver grain from artifacts, small air locks, stain particles, or dirt in the emulsion.

Grain counting is facilitated by a calibrated grid or a micrometer device mounted in the ocular of the microscope. Also, sizes of objects and nuclei can be measured.

It is often sufficient to determine whether a structure in the specimen is labelled or not without counting individual grains over the labelled structure. This type of evaluation is useful for studying cellular proliferation with radioactive thymidine.

V. Cellular Kinetics in Animal Tissues:

Normally the mass of an organ or tissue is essentially constant. The turnover rate or number of cells produced per unit time varies with the turnover time and population size (Cronkite _et al_, 1959).

Turnover time is dependent upon the average life span of individual cells, the size of the total population and its compartmentalization; the latter may be both anatomical and functional. Upon completion of one or a series of multiplicative mitoses, differentiation into mature functioning elements occurs. These cellular systems can be divided into three basic compartments involving generation, maturation and function. The flow rate of cells across each hypothetical boundary may vary depending upon the generative cycle. However, the ultimate flow rate from the total generative and maturative compartments must equal the sum of death and utilization rates to maintain a relatively constant mass (Cronkite _et al,_ 1959).

Labelling the DNA of dividing cells with a radioactive nuclei acid precursor, and using radioautography to follow the labelled cells, is an ideal method to study the origin and migration of newly formed cells (Walker and Leblond, 1958).

Utilizing radioautography to identify the intracellular tritiated thymidine, it is possible, by means of repeated tissue sampling, to study the kinetics of cellular replication within a particular tissue or organ (Cole and McKalen, 1961). With certain limitations, one may determine that fraction of the total cell population is actively synthesizing DNA, and the location of these cells within the tissue studied.

Tissue classification by Leblond and Walker (1956), O'Steen and Walker (1960), Messier and Leblond (1960), and Schultze and Oehlert (1960), was based on radioautographic techniques.

No attempt will be made to review the work of each of these authors and to show each tissue to which of the cell population belongs. Mention has already been made under the heading "Tissue Classification" about the assortment of each tissue to the three types of cell populations.

A few articles by various authors will be reviewed in this chapter that have a special bearing on our research.

Starting with the nervous system, Sidman et al, (1959) defined the primitive ependymal layer as a pseudostratified columnar epithelium, within which nuclei of undifferentiated cells migrate to and fro in relation to the mitotic cycle. Smart in 1961 saw the subependymal layer of the brain as a collection of undifferentiated, mitotically active cells. It appears during embryonic development between the ependymal and mantle layers of the forebrain in the region of the lateral ventricles. It plays an important role in the histogenesis of the cerebral cortex and persists into adult life retaining its ability to form new cells.

In adult rats and mice, mitotic figures may be found in the subependymal layers, but there is no migration of labelled cells from these layers into the surrounding brain tissue (Smart, 1961). In the rest of the nervous system labelled cells were found to be rare or absent (Bryans, 1959, Smart and Leblond, 1961).

The general assumption has been that there is no glial cell proliferation in normal adult animals (Penfield, 1932, Leblond and Walker, 1956). After injection of H^3-thymidine labelled glia nuclei were found in the brains of adults of the following species:

mouse (Messier _et al_, 1958, Walker and Leblond, 1958)
cat (Koenig, 1958)
guinea pig, rat and moneky (Hain _et al_, 1960)
man (Johnson _et al_, 1960).

Hence in these species examined, DNA synthesis is taking place in some nuclei of neuroglia.

There is no subependymal layer in the brain of the Rhesus monkey and the glial cells remain in a resting state (Noetzel and Rox, 1964).

A low degree of mitotic activity continually takes place in the glial populations of adult rats (Hommes and Leblond, 1967).

In the peripheral nervous system Asbury (1967) reported that the per cent of Schwann cells proliferating was calculated to be 27% per day.

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Radioautographs of the jejunum and ileum of mice showed that the cells labelled in the crypts soon after injection of H^3-thymidine find their way up along the sides of the villi and are extruded into the intestinal lumen, presumably from the villus tip (Leblond and Messier, 1958). Rarely, reaction foci were seen over the parietal and zymogenic cells in the rat's stomach. The cells arising in the isthmus migrate along the walls of the pits to reach the free surface and drop into the lumen. Few mucous cells (neck) showed labelled nuclei immediately after injection, but the number of these reactions decreased with time suggesting a turnover of these cells too (Messier, 1960a).

Radioautographic results confirm the existence of a renewal of the colonic epithelium and indicate that the bulk of the epithelial cells are renewed approximately every 3 days. However, some of the cells at the base of the crypts seem to turn over at a much slower rate (Messier, 1960b).

Data on the rat and mouse intestine indicate a duration of the S phase from 6-8 hours and 10-14 hours for the whole cell cycle. The results of the migration rate experiments showed that the crypt cell stays at least 12 hours in the maturing cell compartment before entering the functional compartment of the villus (Galjaard and Bootsma, 1969).

Detailed measurements have been made of the normal rate of cell renewal in the stomach, ileum, colon and rectum of human subjects. The measurements reveal a mean generation time approaching at least one day for the epithelial cells of the tissues studied. The epithelial cells of the gastrointestinal tract are produced at a mean rate close to one cell per hundred cells per hour. This proliferation rate also determines the turnover rate (Lipkin *et al*, 1963).

Radioautographs of serial biopsies of rectal mucosa in a human subject following the incorporation *in vivo* of tritiated thymidine, indicate that the cells of the rectal epithelium are renewed every 6 to 8 days (Cole and McKalen, 1961).

In 1964 MacDonald *et al* reported that within one hour after injection of labelled thymidine into two human subjects, labelled cells were evident in the gastric pits, gland isthmuses and in the duodenal and rectal crypts. Parietal and chief cells were not labelled, but occasionally radioautographic reactions were seen over mucous neck cells deep in the gland. Migration from gastric pits to the surface usually took 4 to 6 days, although cells from a few pits reached the surface in 36 hours. Duodenal cells migrated from the crypt mitotic zones up the villi to be extruded from the crypt tips in 5 to 6 days. Rectal epithelial cells reached the surface in about 5 to 6 days.

In these subjects the duodenal and rectal epithelial migration time was 5 to 6 days which is 2 to 3 times that reported in rodents.

Messier and Leblond (1960) reported that in the rat and mouse, the adrenal cortex had a slowly expanding cell population which showed none of the characteristics of active cell migration.

Walker and Rennels (1961) reported a study of mature female mice which had been injected during proestrus with a large does of H^3-thymidine (600uc/mouse) and then been killed at intervals up to three weeks. They found a considerable incorporation of label into cells of the glomerulosa-fasiculata region, but these labelled cells did not leave the periphery of the cortex in the time period studied. They suggested, therefore, that the escalator theory be abandoned.

Brenner (1963), studying injured adrenals, reported that labelled cells were initially distributed at the periphery of the cortex and scattered between the glomerulosa and fasiculata zones. Within four to six weeks heavily labelled cells were found deep within the cortex indicating that centripetal migration had occurred. The upper fasiculata was judged to be a region of maximum cell turnover from which cells had migrated centripetally, but migration of cells from the glomerulosa seemed to be minimal. This investigation was best interpreted as providing an illustration of the potential of the mouse adrenal cortex to expand its population by means of centripetal migration of mitotically active cells subsequent to the acute stress of Carbon tetrachloride administration.

Ford and Young (1963) showed that cell proliferation was greatest in the zona glomerulosa, dropped off progressively deeper in the cortex and was negligible in the inner fasiculata and reticularis. They concluded that growth of the cortex was achieved through peripheral addition of new cells produced by mitosis in the outermost regions of the gland.

Seki et al (1969) showed that mitosis in the adrenal cortex was localized at the intermediate zone and the rate of mitosis was 0.6% of parenchymal cells. Incorporation of H^3-thymidine was also limited in the same zone and the rate of labelled cells was 0.8% of the parenchymal cells.

Leblond and Walker, (1956) showed that the vaginal epithelium is a good example of the cellular renewal system in which cell production is balanced by cell loss.

Walker (1960a and 1960b) reported that the divergent fates of stem cells and differentiating cells can be associated with division. He also stated that when the basal cells of the stratified squamous epithelium divide, one cell remains behind as a basal cell while the other moves away and develops into a spinous layer cell. Husbands and Walker (1963) then showed that this was true differentiation and suggested that this takes place when new cells are formed from stem cells or in association with mitosis.

Walker (1960a) found that the vaginal epithelial cells in the cycling mouse migrate in a wave from the basal layer rather than moving individually. However, Leblond et al (1964) demonstrated random movement of cells from the basal layer of esophageal epithelium.

Peckham (1962) concluded that the differentiating cells in the vaginal epithelium do not begin to move out of the basal position until they have passed through mitosis and have reached the post mitotic resting phase.

MATERIALS AND METHODS

The Galagos used in this study belonged to a colony of Galago senegalensis that has been established in the Department of Anatomy at Michigan State University.

The colony consists of ten females and eleven males, housed in the Center for Laboratory Animal Resources. The animals were purchased from the International Animal Exchange Firm in Ferndale - Detroit, Michigan, at the cost of $75 per Galago.

The colony is housed in a ventilated room measuring 7X8X8 feet. Each two or three Galagos are placed in a wood-framed, wire cage that measures 17X45X14 inches. The cages are equipped with removable dropping trays for ease of cleaning. Each cage contains a nesting box that measures 7 1/2 X 3 1/2 X 7 1/2 inches, and has openings at each end plus a central partition. The top of the nesting box is hinged. The cage provides an area of approximately 3500 inches per animal. The temperature of the room is maintained at 30°C., and the humidity at 50%.

The average adult body weight is approximately 250 grams for males and 235 grams for females. The weight charts of the animals, show that their weights are kept nearly constant with very small range changes between 2-5 grams over a period of six months.

Ten Galagos from a recently bought colony are fed K/d dietary animal food, provided by: Riviana Foods Inc., Topeka, Kansas. The rest of the animals are fed pulverized Purina monkey chow blended with bananas. 0.6 c.c. of A.D.C. pediatric vitamins (Parke-Davis) are added to the diet per cage. Water is provided ad libitum.

The normal rectal temperature is 101°F as measured by a rectal probe. The animal room is illuminated with a white light at night, and a 60 Watt Ken-Rad red light during the day. The lighting cycle is automatically controlled by a timer which provides the room with a 12-hour day and a 12-hour night.

Identification of each animal is accomplished by an ear tatoo. Some of the Galagos are very tame and pet-like, but since they possess a powerful jaw with an effective set of canines, it is much safer to wear thick protective gloves when handling them.

Seven female Galagos were used for this study. The first two animals, which died from plugging of tubules of the kidneys with oxalate crystals, four months before the beginning of this study were preserved in 10% formalin, and used to study the histology of the various tissues. The rest were injected with radioactive thymidine before they were sacrificed.

The weight of four of the animals used ranged between 225 and 280 grams. Only one weighed 375 grams (Table 1).

Table 1

Weights (over a period of 15 months) of the Galagos that were sacrificed 2 hours, 2 days and 4 days after the labeled thymidine injection

Date	2 hours wt. in gms.	2 days wt. in gms.	4 days wt. in gms.
July 3/68	168.6	151.3	148.5
8	181.5	164.2	171.6
11	182.9	163.5	164.0
15	185.6	164.8	165.0
18	187.5	169.4	168.0
22	186.0	171.9	177.0
25	193.5	176.3	173.5
29	193.5	184.0	176.8
Aug. 1/68	199.5	188.2	179.5
5	186.8	193.5	182.0
8	204.3	195.5	189.3
12	200.8	194.0	189.0
15	204.5	197.5	185.0
19	203.5	200.5	192.3
21	202.0	198.0	185.0
29	204.0	203.4	201.0
Sept. 3/68	212.5	214.3	206.8
5	212.6	212.0	208.1
9	215.4	221.0	214.8
12	218.6	215.9	212.8
16	220.6	---	212.2
19	227.3	---	---
Oct. 7/68	239.3	233.2	---
11	240.5	242.0	---
14	243.5	239.5	224.8
17	240.0	242.4	228.2
22	254.5	245.0	230.0
24	254.0	242.2	225.4
28	258.4	243.6	228.8
31	261.4	241.8	229.0
Nov. 4/68	266.3	243.0	231.4
7	271.8	244.0	234.7
13	276.2	248.3	233.5
18	276.4	247.0	232.5
21	288.5	256.3	237.8
25	290.4	258.7	239.5
27	292.4	259.4	239.4

Table 1 (cont'd.)

Date		2 hours wt. in gms.	2 days wt. in gms.	4 days wt. in gms.
Dec.	2/68	293.4	260.0	238.6
	5	300.0	265.5	245.2
	9	299.5	264.0	246.7
	12	304.2	267.5	244.3
	16	302.2	260.2	243.6
	19	300.8	262.8	240.5
	26	314.1	269.2	251.0
Jan.	2/69	315.5	270.2	248.8
	6	320.5	268.4	249.4
	9	318.0	268.2	243.5
	14	322.0	275.5	249.8
	17	324.3	275.6	253.2
	20	325.0	274.6	257.0
	23	324.2	275.1	252.5
	27	320.6	273.4	255.2
	30	323.7	277.4	253.9
*Feb.	2/69	324.0	270.6	252.4
	6	329.1	270.6	253.6
	10	327.4	268.6	253.0
	13	330.0	273.1	257.3
	17	332.4	275.0	258.4
	20	322.5	271.6	247.8
	24	333.5	274.2	260.5
	27	336.2	279.6	259.6
March	3/69	332.1	278.4	265.8
	10	333.0	282.2	264.5
	17	328.0	277.5	263.8
	20	321.5	274.2	253.8
	24	331.0	283.0	266.4
	26	341.3	280.0	273.4
	31	328.3	288.0	270.6
April	3/69	334.3	283.5	272.4
	8	339.4	285.3	272.0
	14	347.8	284.8	278.6
	28	333.8	261.5	278.5
May	13/69	360.0	250.8	277.0
	19	362.1	239.5	281.5
	26	356.5	238.5	279.4
June	4/69	339.5	234.5	269.0
	12	345.0	248.8	274.3
	20	351.0	246.5	263.0
	27	354.1	260.0	270.0

Table 1 (cont'd.)

Date		2 hours wt. in gms.	2 days wt. in gms.	4 days wt. in gms.
July	7/69	345.2	266.0	270.4
	23	346.5	275.0	272.4
	30	349.5	277.0	266.5
Aug.	11/69	352.5	274.0	257.0
	22	359.0	277.0	245.0
Sept.	2/69	367.5	274.0	241.5
	24	374.0	272.8	242.5
Oct.	3/69	375.0 (Sacrificed)	---	---
Dec.	16/69		283.0	268.5
Jan.	5/70		273.4 (Sacrificed)	---
Feb.	20/70			281.0 (Sacrificed)

Explanation of symbols:

--- : figures are not available

*Feb. 2/69 : Beginning of the second 8 months period (see discussion)

The animals were weighed, the external genitalia checked for any signs of estrus, then injected with thymidine-methyl-H^3 subcutaneously.

The molecular weight of radioactive thymidine was 242.2; the radioactivity was 5.0 millicuries per 5.0 milliliters and the specific activity was 20 curies per millimole. This was supplied by the New England Nuclear Corp. in vials with a concentration of 0.60 milligrams in 5.0 milliliters sterile water or 70% ethanol solution. The radiochemical purity of the compound is greater than 98.3% and contains less than 0.25% thymine-methyl-H^3 riboside as determined by paper chromatography.

Each of the first four animals was given a dose of 400 microcurie per 100 grams body weight of thymidine-methyl-H^3 subcutaneously. The first animal was sacrificed two hours after the injection. The other three were isolated and kept for 24 hours without food prior to their sacrifice at 1, 2, and 4 days after the injection. The last animal was given a dose of 1,000 microcuries per 100 grams body weight and was sacrificed 8 days after the time of the injection.

Anesthesia:

At the time designated for sacrifice, the animal was held firmly with thick protective gloves and shaved with a hair clipper.

Sodium thiamylal (Surital $^{(R)}$), an ultrashort acting barbiturate, was used in a 5% concentration.

The dose used was 25 mg/kg. body weight. The volume of the anesthetic was mixed with an equal volume of

sterilized destilled water or normal saline in a syringe. A 27 gauge 1/2 inch needle, was used to infuse the anesthetic via the left or right saphenous vein. When the anesthetic was fresh and the entire dose infused in the blood, the animal was under surgical anesthesia after one minute. In previous experiments performed to test the effectiveness and duration of sodium thiamylal, it was found that with a dose of 25 mg/kg., 5% solution, the animal could remain under surgical anesthesia for at least one hour.

In one case, two doses were given subcutaneously unintentionally in both legs and after 45 minutes, the animal was still alert. A third dose was given intravenously, and the animal was completely anesthetized in 30 seconds.

Dripping blood from the saphenous vein was used to make thin blood smears on slides that were previously cleaned and prepared for this purpose.

In one animal, the external genitalia were checked and the vagina was found open. Pap smears were taken before the injection of thymidine-H^3, and also four days later at the time of sacrifice. In all the other animals, the vagina was closed with no signs of estrus.

An incision through the skin and the long muscles of the back of the thigh was made to reach the head of the femur. The femur was broken and bone marrow was sucked up by means of a syringe and a 20 gauge needle for bone marrow smears. In the last animal sacrificed, ethylene diamine tetra acetic acid (EDTA), was used to wet the syringe before extracting the bone marrow.

The abdominal cavity was then opened, retractors placed on each side, and a small piece of the spleen was cut and used for spleen imprints placed on clean glass slides. After this procedure, the animal was ready for perfusion.

Perfusion:

A midline incision was made over the sternum, from the xiphoid to the manubrium. The left ribs were cut at the costosternal junctions and the thoracic cavity was exposed and the ribs retracted.

0.25 c.c. heparin (1,000 units/c.c.) were then injected via cardiac puncture into the left ventricle and allowed to circulate for one minute.

The perfusion apparatus consisted of a 250 c.c. solution bottle with a drip meter and plastic tubing. A screw clamp was placed over the plastic tubing to control the rate of flow. A needle adapter was connected to the end of the plastic tube. A 20 gauge needle was used with the perfusion apparatus, and inserted into the left ventricle towards the aorta. The clamp was released and perfusion with 10% formalin (60-70 drops/minute) began until the animal started to show spastic movements. The inferior vena cava or the right or left femoral vein was usually cut for an outlet and the perfusion continued until the fluid became clear (about 20 minutes) and after 75-100 c.c. of formalin had been used.

Samples of tissues from the following organs were then cut and placed in small tubes containing 10% formalin for further processing after 24 hours:

Brain, pituitary gland, spinal cord, spinal ganglia, sciatic nerve, tongue, tonsils, parotid gland, submandibular gland, sublingual gland, thyroid gland, thymus, cardiac muscle, skeletal muscle, lung, stomach, duodenum, jejunum, ileum, colon, liver, spleen, pancreas, mesenteric lymph nodes, adrenal glands, kidneys, ureters, urinary bladder, urethra, ovary, oviducts, uterus, vagina, mammary gland, integuement and bone marrow.

The rest of the animal was placed in a labeled jar containing 10% formalin and saved.

Colored pictures for slides and black and white pictures for prints were taken, before and after the perfusion, of the whole animal with organs in situ, and for some isolated structures.

Tissue Processing:

The brain was removed from the cranium and the dura mater was stripped off. A mid-saggital section was made with a sharp knife through the longitudinal fissure, between the two cerebral hemispheres and passing through the cerebellum and medulla.

The right half was used for saggital sections, while the left side was cut in cross-sections at the levels of the frontal, temporal and occipital lobes.

The pituitary was removed from the sella turcica and placed in 10% formalin. The uterus and vagina were dissected and removed completely from the animals. In two animals a saggital section was made passing between the two cornua and continuing down through the saggital axis of the vagina, separating the organ into right and left parts. Each part was sectioned saggitally. In the remaining animals, the genital tract was cut in cross-sections at various levels and each section and level were marked.

The standard procedure for tissue fixation was followed and can be summarized as:

> The tissues were placed in 70% alcohol for two hours, with 4 drops of eosin added as a tincture.
>
> Two changes in 95% alcohol followed with one hour interval for each change. Then, three changes in 100% alcohol, each for an interval of 40 minutes, were followed by three changes in toluene for half an hour each.
>
> The tissues were immersed in three changes, forty minutes each, of toluene and paraffin (1:1). This was followed by paraffin under vacuum and two changes for 40 minutes each.
>
> The tissues were then embedded in paraplast, after being oriented in the manner desired for sectioning.

The paraffin blocks were sectioned at 7u. Ten slides were prepared for each tissue, for radioautographs according to Walker's modification (1959) of the method of Messier and Leblond (1957). The procedure is summarized as follows:

> Sections were placed on albuminized slides, floated with 2% formalin, straightened and drained. Slides were then placed in carriers and allowed to dry in a warming oven at 37°C. for 24 hours. Deparaffinization was accomplished with three changes of xylol for five minutes each, and the xylol was removed by two changes of absolute alcohol for three minutes each, followed by one change in 95%, 80%, and 70% alcohol for one minute each. The slide rack was then put in distilled water for five minutes before being transferred to the warming oven to dry for at least one hour.
>
> The slide distribution was as follows:
>
> slide #1: was stored as a reserve slide.
>
> slide #2: stained with hematoxylin and eosin (H&E) and used to study the normal histology of the tissue.
>
> slide #3: coated with the emulsion, developed after one week and stained with H&E.
>
> slide #4: coated with the emulsion, developed after two weeks and stained with H&E.
>
> slide #5: stained with Periodic Acid Schiff,

coated with the emulsion, developed after two weeks and counter-stained with H&E.

slide #6: stained with Aldehyde Fuchsin (Gomori, 1950), coated with the emulsion and developed after two weeks.

slide #7: coated with the emulsion, developed after three weeks and stained with H&E.

slide #8: coated with the emulsion, developed after four weeks and stained with H&E.

slide #9: coated with the emulsion, developed after eight weeks and stained with H&E.

slide #10: stained with Hematoxylin-Phloxine-Alcian Blue-Orange G differential stain for prekeratin, keratin and mucin (Dane and Herman, 1963).

Slides were coated in the dark room with Kodak NTB-2 gel emulsion at 40°C. and allowed to dry for five hours, before being placed in light-proof bakelite boxes containing Drierite and sealed with masking tape.

The slides were then placed in the refrigerator at 60°C. and were exposed at the various time intervals mentioned above.

Using Kodak Microdol-X liquid developer, slides were developed for five minutes. They were rinsed in distilled water and cleared for five minutes in Kodak Acid Fixer. After rinsing in distilled water for at least five minutes, they were stained with H&E or as described above.

Using permount as a mounting medium, cover slips were placed on the slides. Excess mounting medium was removed, after allowing the slides to air-dry for at least two days, by immersing them for one minute in toluene. The slides were then scraped with a razor blade, labelled with India ink and were then ready for observation.

Histologic and Radioautographic Observations:

1) Selection of cell types:

In this study thirty-six different tissues were selected. Some of them, for example, liver parenchymal cells, granulosa cells of the ovary, islets of Langerhans, skeletal muscle cells are treated as separate cell types. In others, the cell populations were selected as to spatial distribution. For example, glomerulosa cells of the adrenal cortex were set apart as a population from the cells of the fasiculata or reticularis. In other cases, cells composing the alveoli of certain glands, such as the salivary glands, were considered as a population different from the ductal epithelium of that gland. All the neurons in the cerebrum and all the nucleated cells of the bone marrow were arbitrarily lumped together as a population.

A listing of all cell types investigated will follow later.

2) Estimation of background and counting of labelled nuclei:

In all the radioautographs some of the cell nuclei had incorporated the tritiated thymidine, and these cells were identified by the presence of reduced silver grains in the film overlying the nuclei. Only the nuclei that showed five or more silver grains were considered as labelled cells. The background count under the conditions of this experiment was insignificant, and labelled and unlabelled cells were clearly distinguishable.

3) Measurement conversions:

The ocular grid and the micrometer were calibrated to measure length and area of an oil immersion, high dry, medium and low power, fields.

In the oil immersion field (97X), each 100 divisions in the micrometer measured 50u. The total area of the grid was 0.0024 mm^2.

In the high dry field (45X), each 100 divisions in the micrometer were equivalent to 0.12 mm., while the grid area was 0.0132 mm^2.

In the medium power field (10X), each 100 divisions in the micrometer measured 0.50 mm. and the total area of the grid was 0.25 mm^2.

The low power field was not used for any measurements.

RESULTS

An attempt will be made to describe the gross anatomy of the adrenal glands and the urogenital system of the female Galago senegalensis (Figures 1 and 2).

The histological features and the radioautographic data for each tissue studied will be described afterwards.

I. Adrenal Glands:

The adrenal glands are a pair of small soft yellowish-brown bodies that bear the same relationship to the kidneys, in both size and position (Figure 2).

They are flat and bilobed, placed cranial and medial to the upper pole of each kidney, on either side of the vertebral column, on the posterior abdominal wall behind the peritoneum (Figure 3).

The two glands are assymetrical with respect to morphology, position and relationships on either side.

The left adrenal is 0.92 cm. long, 0.35 cm. in width and 0.20 cm. in thickness and weighs about 0.11 gms. (Table 2). It is in the shape of a flattened pyramid with the base of the pyramid resting on the kidney. It is markedly constricted in its central portion with the cranial portion long and narrow while the caudal portion is flat and broad. It lies medially to the cranial pole of the kidney and is separated from the postcava and aorta by fascia and fatty tissue.

The anterior surface of the left adrenal is related superiorly to the cardiac portion of the stomach and the upper extremity of the spleen. Inferiorly it is related to the pancreas and it is devoid of peritoneum at this area. The posterior surface lies against the diaphragm, lateral to the aorta.

The right adrenal lies between the surface of the cranial pole of the right kidney and the lateral aspect of the inferior vena cava. It is shaped like a flattened wedge, with 0.8 cm. in length, 0.55 cm. in width and 0.20 cm. in thickness and weighs about 0.09 gms. (Table 2).

Each adrenal is enclosed in a fibrous capsule of fascia and is invested by loose connective tissue and fascia of the same structure as the one around the kidney.

A longitudinal section of the adrenal can distinguish the cortex and medulla grossly by the color and consistency of the two parts (Figure 4). The shape of the medulla conforms to that of the gland, the cortex is firm in structure and yellowish in appearance while the medulla is soft and brownish.

The arteries supplying the adrenals are small and several in number, arising from the dorsal aorta, inferior phrenics and renals on either side. The venous drainage comprises small venules and a large vein on either side (Figure 3).

II. The Kidneys:

The kidneys are built on the typical lorisoid plan. The right is placed cranial to the left and close to the right crus of the diaphragm (Figure 5).

A smooth thin fibrous capsule covers the surface of each kidney. They are bean-shaped with the caudal end closer to the median plane than the cranial end and slightly bulkier. Except for the difference in position and size the two kidneys are similar.

The anterior surface looks forwards and sidewards, while the posterior surface looks backwards and medially. The cranial and caudal portions are joined by a lateral border that is convex and by a medial border that is slightly concave and which is indented by an oval vertical slit, the hilum which transmits the ureter and renal vessels.

Both kidneys lie on the upper part of the posterior wall of the abdomen where they are anchored by a fibrous network behind the peritoneum, one on either side of the aorta and postcava (Figure 3).

In one animal, out of five examined, the kidneys and all the other visceral organs were not retroperitoneal.

The renal fascia encloses each kidney together with the supra renal glands and some renal fat.

The left kidney weighs about 1.02 gms. and measures about 1.90 cm. in length and 1.30 cm. in width and 0.50 cm. in thickness at the hilum.

The right kidney weighs about 0.95 gms. and measures about 1.70 cm. in length, 1.10 cm. in width and 0.70 cm. in thickness at the hilum (Table 2).

The hilum of the kidney is a small slit-like opening at the medial concave border where the ureter and renal vessels pass through it. The rim is sharp and smooth and the renal vein is the largest of the entering structures and is located ventral to the renal artery.

The relations as can be seen at the hilum are: the ureter is most caudal, the artery is most cranial and the vein is in between.

A cross-section of the kidney shows distinctive structures. The cortex and medulla can be detected by the unaided eye. Several calyces open into the pelvis. Arciform arteries and veins are located in a boundary zone between the cortex and medulla and can be easily shown (Figure 6). Four lobes can be seen in a longitudinal section.

III. Ureter:

It is a whitish fibromuscular tube that begins in the renal sinus as a dilatation, and issues from the hilum descending along the medial border of the kidney, where at its lower end it narrows to become the ureter proper (Figure 2 and 3).

The left ureter measures about 4.5 cm. in length, the right ureter is 0.25 - 0.50 cm. longer. The diameter is 0.50 - 1.0 mm. and is slightly larger at the pelvis.

Folds of mucosa lining the lumen can be seen easily with a magnifying glass.

The ureters pass down and medially to enter the pelvis by crossing over the origin of the external iliac artery. Dorsally they lie on the psoas muscles and the peritoneum covers them on the ventral side. In their lower course the ovarian vessels are closely related (Figure 3).

The ureters pass under and behind the uterus to pierce the bladder obliquely between the two layers of peritoneum that form the lateral ligament of the bladder at its dorso-lateral surface.

The blood supply is mainly from the renal artery.

IV. Urinary Bladder:

The size of the bladder and its position depends on the amount of urine it contains. When empty it has an apex which lies behind the upper margin of the pubic symphysis (Figure 5). It crowds the uterus and the rectum and is firmly anchored to the anterior abdominal wall by small strands of fibers (Figure 7). The base looks backwards and it is separated from the rectum by the vagina and fascia. The neck is the lowest part of the bladder where the urethra begins. It is in contact with the small intestine dorsally and with the descending colon in the female, craniolateral to the divergence of the uterine horns from the body of the uterus. Two lateral ligaments connect the lateral surfaces of the bladder to the pelvic wall. They are nearly triangular in shapeand they blend with the broad ligament of the uterus.

Blood vessels to and from the bladder can be well seen on the anterior side of the lateral ligaments as branches of the urogenital blood vessels.

V. The Female Urethra:

The female urethra is very peculiar in the Galago in that it is dilatable, about 1.9 cm. long and curves downwards and forwards from the neck of the bladder, in the shape of the letter s, during its course (Figure 8). It passes below and behind the pubic symphysis, through the pelvic fascia and gradually expands in diameter to form a small vestibule before it reaches the vulva and then perforates the whole length of the peniform clitoris.

The Female Genital Organs:

The ovaries, oviducts, uterus, vagina, clitoris and vulva constitute the female genital organs. The ovaries lie on the side wall of the pelvis, between the ureter and broad ligament (Figure 3).

The dorso-lateral walls of the abdominal wall and the lateral walls of the pelvis are connected to the ovaries, oviducts and uterus by paired double folds of peritoneum, the right and left broad ligaments.

The mesosalpinx is formed by a double fold of the peritoneum, and as a portion of the broad ligament between the uterine tube and the attachment of the ovary. The ovary is enclosed within it and so is the entire oviduct.

The round ligament attaches to the surface of the uterus anterolaterally and far caudally. It consists of connective tissue and is somewhat flattened.

The mesovarium is continuous with the mesometrium which begins at the cranial border of the uterine horn and continues caudally to reflect onto the bladder and the colon. It extends over the uterine horns, the uterus proper to attach along the abdominal and pelvic walls.

The uterine vessels and nerves run between the peritoneal layers of the mesometrium. The uterus does not lie in the mid-saggital plane of the body. In some animals it was found pushed to the left and the rectum lies on the right (Figure 3), while in others, it was to the right and the rectum lies on the left (Figure 9).

The Ovaries:

Paired, and each is about the size of a lentil (Figure 10). They are oval or fusiform in shape and rest upon the posterior layer of the broad ligament on the side wall of the pelvis (Figures 3, 5 and 9). The left ovary weighs about 9 milligrams (Figure 11), and the right ovary weighs about 7 milligrams (Figure 12). Each is connected with the broad ligament by the mesovarium and between its layers, the blood vessels and nerves pass to the ovary. Each ovary is placed at a variable distance from the uterus. The external appearance of a few of the ovaries examined showed black, large bulges on the surface that were suggestive of a corpora lutea (Figure 12 and 13). Histologic examination of these bodies confirmed the suggestion.

The ovarian arteries branch from the dorsal aorta and the uterine arteries. The veins form a plexus from which the ovarian vein ascends to join the inferior vena cava on the right side and the renal vein on the left side (Figures 2 and 3).

The Oviducts:

Each fallopian tube is very much convoluted and opens into the anterior angle of the uterine cavity, while the cranial end opens into the peritoneal cavity (Figures 2, 5 and 9). The location of each oviduct is between the peritoneal layers of the mesosalpinx. The abdominal end of the oviduct is the funnel-shaped infundubulum which has a number of small finger-like projections, the fimbrae.

The blood supply is from the uterine and ovarian vessels.

In one specimen examined, the left fallopian tube was tortuous and convoluted. The right tube was smoother in structure compared to the left and it bifurcated into two tubes running side by side, starting from the fimbriated ampulla, down to the uterine horns (cornua), where they joined. This anomaly was confirmed histologically.

The Uterus:

The uterus of the Galago is bicornuated with a small corpus and relatively long, conical cornua (Figure 11). The length of each cornu varies from 4.0 to 7.0 m.m. There is a well-marked intercornual ligament between the two cornua. It is slightly flattened anteroposteriorly and connects the oviducts to the vagina. It lies between the rectum and the bladder, obliquely placed and overhanging the bladder (Figures 15 and 16). The uterus is in line with the relatively long vagina (Figure 17), and the cervix is single and very small. The presence of the cervix was confirmed histologically.

The vesical surface is nearly flat and in relation to the bladder and small intestines, while dorsally it is in relation with the descending colon, Psoas muscles and ureters (Figure 15).

Vessels arising from the anterior surface of the aorta follow a long independent course to the ovaries and uterus. Branches from the urogenital artery also supply the uterus. The veins follow the course of the arteries, they are very tortuous in their course between the peritoneal layers of the broad ligament.

The Vagina:

It is a musculomembranous, relatively thin-walled cavity extending from the uterus to the orifice of the vagina (Figure 18). It is flattened anteroposteriorly and its greatest diameter runs transversely (Figure 17). Cranially it is limited by the very short cervix. The mucosa forms low longitudinal ridges and folds in the inside.

It descends in the pelvis in front of the rectum and behind the bladder (Figures 15 and 16). The dorsal and ventral peritoneal coverings of the vagina fuse laterally to become a part of the broad ligament. The long urethra is connected ventrally to the lower half of the vagina by means of loose connective tissue. The vaginal blood vessels and nerves and the ureters are lateral to the lower half of the vagina.

The vestibule is a funnel-shaped recess. It opens externally close to the anus, under the tail, thus connecting the vagina to the external genital opening.

The vagina is supplied by the vaginal and uterine arteries.

The clitoris is long and pendulous, and measures 0.8 cm. in length and has a slit-like urethral opening on the ventral aspect near its tip. The undersurface is marked by a median raphe as far back as the vulval opening. The vulva consists of thin labia majora, haired to their edges,with a slit-like opening directed forwards and obliquely (Figure 19).

Table 2

Dimensions of the adrenals, kidneys and ovaries of the Galago

(weights in gm., other dimensions in cm.)

		Galago Weight					
Organ	Dimension	375	239	273	281	224	Average
Adrenal (left)	weight	0.11	0.10	0.13	0.09	0.12	0.11
	length	0.89	0.90	1.00	0.85	0.95	0.92
	width	0.35	0.25	0.40	0.30	0.46	0.35
	thickness	0.30	0.19	0.20	0.10	0.22	0.20
Adrenal (right)	weight	0.07	0.09	0.11	0.06	0.12	0.09
	length	0.80	0.90	0.85	0.70	0.75	0.80
	width	0.50	0.55	0.52	0.58	0.60	0.55
	thickness	0.25	0.20	0.28	0.15	0.12	0.20
Kidney (left)	weight	1.03	1.02	1.01	1.06	1.00	1.02
	length	1.90	2.05	1.85	1.90	1.84	1.90
	width	1.25	1.44	1.30	1.24	1.31	1.30
	thickness	0.42	0.50	0.66	0.45	0.50	0.50
Kidney (right)	weight	0.85	1.05	0.88	0.95	1.02	0.95
	length	1.82	1.60	1.70	1.68	1.70	1.70
	width	1.18	1.02	1.05	1.10	1.15	1.10
	thickness	0.70	0.55	0.85	0.80	0.60	0.70
Ovary (left)	weight	0.008	0.009	0.010	0.011	0.007	0.009
Ovary (right)	weight	0.007	0.009	0.007	0.008	0.007	0.007

Histologic and Radioautographic Results:

Central Nervous System:

Histologic study of brain tissue was done on a sagittal section and on cross-sections through the frontal, temporal and cerebellar regions.

In one brain the folliculus was isolated intact and it was coiled like the outer shell of a snail.

In principle the nervous tissue of the Galago is similar to that of the human. No significant difference was noted. The neuronal cell bodies are found in the grey matter. The fibers are found in both the grey and the white matter.

The diameter of the nucleus of the neuron in the frontal region of the brain is 5-10 u., and that of a sybependymal cell nucleus is about 5u. The nucleus of the choroid plexus cell is circular with a diameter of 5.5 u. In the cerebellar cortex, the diameter of the nucleus of the outer molecular layer cell is 5 u., and that of the granular layer cell 4.2 u. The Purkinjie cells have large nuclei, 8.3 u. in diameter.

Each radioautographic slide of the brain sections, with the three types of stains used, namely H&E, PAS and aldehyde fuchsin, was examined and scanned thoroughly for radioactive nuclei. The results are summarized in Tables 3 and 4, and the grain count on the subependymal layer nuclei in Table 17.

Spinal Cord:

Cross-sections of the spinal cord from the thoracic region were used for this study. The diameter of such sections was about 2.4 m.m. The histologic organization is the same as that of the human spinal cord. The cells in the grey and white matter were considered as one cell population.

At the outer margin of the section, in the pia mater covering, there were scattered cells with darkly stained round nuclei and a diameter of about 5 u. Their cytoplasm was scanty and they resembled lymphocytes. Their radioactive index and turnover time is reported in Tables 3 and 4.

Dorsal Root Ganglion:

Each ganglion cell has a round eccentric nucleus, with a single compact nucleolus. The diameter of the nucleus is about 12 u.

A single row of supporting or satellite cells surround the cell body and the diameter of their nuclei is about 4 u.

None of the nuclei of the ganglion cells in all the sections, at the different time intervals examined, showed any radioactivity (Table 4).

Peripheral Nerve:

A cross-section through the sciatic nerve was used for this study.

A section of the nerve from the Galago that was sacrificed two hours after the injection of radioactive thymidine was elliptical in shape with a longitudinal axis of 0.85 m.m. and a transverse axis 0.32 m.m. Only two radioactive connective tissue nuclei were noted in it.

The section of the nerve from the Galago that was sacrificed one day after the injection was circular with a diameter of 0.48 m.m. Only one connective tissue nucleus was labeled in it.

No radioactive nuclei were noted in any of the sections of the nerves of the Galagos that were sacrificed 2, 4 and 8 days after the injection, and were circular with diameters of 0.7, 0.8 and 0.6 m.m. respectively.

Table 3

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

Central Nervous System

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area (mm^2)	Estimated cell population
Cerebrum	Neurons	2 hours	38	12	0.49	456
		1 day	20	44	0.26	880
		2 days	40	19	0.52	760
		4 days	20	26	0.26	520
		8 days	25	56	0.32	1400
	Choroid plexus	2 hours	26	16	0.33	416
		1 day	30	17	0.39	510
		2 days	26	21	0.33	546
		4 days	22	22	0.28	585
		8 days	51	12	0.66	612
Cerebellum	Outer molecular layer	2 hours	150	15	1.98	2250
		1 day	20	25	0.26	500
		2 days	120	67	1.56	8040
		4 days	20	99	0.26	1980
		8 days	50	16	0.65	800

Table 3 (cont'd.)

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area(mm^2)	Estimated cell population
	Granular layer	2 hours	225	107	2.97	24075
		1 day	125	75	1.62	9375
		2 days	200	59	2.64	11800
		4 days	20	134	0.26	2680
		8 days	100	20	1.32	2000
Spinal cord	White and grey matter nuclei	2 hours	241	28	3.13	6748
		1 day	250	21	3.25	3500
		2 days	218	14	2.83	4578
		4 days	291	17	3.78	4947
		8 days	100	19	1.32	1900

Table 4

Frequency of labeled nuclei in various cell populations of the central nervous system. Turnover Time is based on an extrapolation of labeling index at 2 hours and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Cerebrum	neurons	2 hrs.	0	456	0	∞
		1 day	0	880	0	
		2 days	0	760	0	
		4 days	0	520	0	
		8 days	0	1400	0	
	subependymal	2 hrs.	5	100	5	3.3
		1 day	4	100	4	8.3
		2 days	2	100	2	
		4 days	3	100	3	
		8 days	3	100	3	
	choroid plexus	2 hrs.	1	416	0.23	72.4
		1 day	0	510	0	
		2 days	0	546	0	
		4 days	0	484	0	
		8 days	0	612	0	
Cerebellum	Outer molecular layer	2 hrs.	0	2250	0	∞
		1 day	0	500	0	
		2 days	0	8040	0	
		4 days	0	1980	0	
		8 days	0	800	0	
	purkinje	2 hrs.	0	100	0	∞
		1 day	0	100	0	
		2 days	0	100	0	
		4 days	0	100	0	
		8 days	0	100	0	
	Granular	2 hrs.	0	24075	0	∞
		1 day	0	9375	0	
		2 days	0	11800	0	
		4 days	0	2680	0	
		8 days	0	2000	0	

Table 4

Organ	Cell population	Time interval	Radio-active nuclei	Total nuclei	% of radio-active nuclei	Turn-over time (days)
Spinal cord	Central canal epith.	2 hrs.	0	48	0	∞
		1 day	0	51	0	
		2 days	0	52	0	
		4 days	0	45	0	
		8 days	0	51	0	
	White & grey matter nuclei	2 hrs.	0	6748	0	∞
		1 day	0	3500	0	
		2 days	0	4578	0	
		4 days	0	4947	0	
		8 days	0	1900	0	
	Lymphoid nuclei	2 hrs.	7	38	18.4	0.90 (21.6 hrs.)
		1 day	2	14	14.2	2.3
		2 days	4	44	9.0	
		4 days	0	16	0	
		8 days	0	23	0	
Spinal ganglion	Satellite cells	2 hrs.	2	200	1.0	16.6
		1 day	0	200	0	
		2 days	0	200	0	
		4 days	1	200	0.50	
		8 days	0	200	0	

The Endocrine System:

Pituitary gland:

In the anterior lobe, a row of four or five nuclei form a sinusoid-like arrangement with a lumen in the center. There is no endothelial lining in them. The colloid is PAS positive. Radioactive nuclei were infrequent (Tables 5 and 6).

Thyroid gland:

All the cells lining the lumen of the follicle were identified as follicular cells, the rest were considered non-follicular. The thyroid gland of the Galago is similar to that of the human, histologically. Radioactive nuclei were rare (Tables 5 and 6).

Pancreas:

The histologic structure of the pancreas of the Galago is the same as that of the human. Radioactive nuclei were uncommon in both the exocrine and endocrine portions of the gland (Tables 5 and 6).

Adrenal gland:

The adrenal gland of one Galago that died of kidney infection showed a cell layer between the innermost region of the zona reticularis and the medulla that is characteristically different from the normal parenchymal cells of the reticularis. It is suggested that the change in the morphology of the cell might be due to stress factors. This animal was not used in the radioautographic experiments.

In general the adrenal gland of the Galago is similar histologically to that of the human. Frequency of radioactive nuclei was variable, according to the area of the gland studied (Tables 5, 6 and 17).

Table 5

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

Endocrine system

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area (mm^2)	Estimated cell population
Pituitary	anterior lobe	2 hrs.	24	36	0.31	864
		1 day	30	35	0.39	1050
		2 days	53	42	0.69	2226
		4 days	191	48	2.48	9168
		8 days	107	32	1.39	3424
	posterio lobe	2 hrs.	87	32	1.13	2784
		1 day	80	21	1.04	1680
		2 days	87	40	1.13	3480
		4 days	191	18	2.48	3438
		8 days	51	16	0.66	816
Thyroid	follicular & non-follicular	2 hrs.	30	168	0.39	5040
		1 day	20	133	0.26	2660
		2 days	20	141	0.26	2820
		4 days	20	120	0.26	2400
		8 days	20	161	0.26	3220
Pancreas	islets	2 hrs.	20	34	0.26	680
		1 day	20	34	0.26	680
		2 days	20	43	0.26	860
		4 days	20	37	0.26	740
		8 days	20	38	0.26	760
	exocrine cells	2 hrs.	50	28	0.65	1400
		1 day	50	21	0.65	1050
		2 days	50	23	0.65	1150
		4 days	50	22	0.65	1100
		8 days	50	24	0.65	1200
Adrenal	glomerulosa	2 hrs.	5	114	0.065	570
		1 day	5	131	0.065	655
		2 days	5	181	0.065	905
		4 days	5	157	0.065	785
		8 days	10	108	0.13	1080

Table 5 (cont'd.)

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area (mm^2)	Estimated cell population
Adrenal	fasiculata	2 hrs.	5	101	0.065	505
		1 day	5	76	0.065	380
		2 days	5	137	0.065	685
		4 days	5	136	0.065	680
		8 days	10	72	0.13	720
	reticularis	2 hrs.	5	102	0.065	510
		1 day	5	84	0.065	520
		2 days	5	167	0.065	835
		4 days	5	199	0.065	995
		8 days	10	78	0.13	780
	Medulla	2 hrs.	8	57	0.104	456
		1 day	5	157	0.065	785
		2 days	8	67	0.104	536
		4 days	10	91	0.13	910
		8 days	10	62	0.13	620

Table 6

Frequency of labeled nuclei in various cell populations of the endocrine system. Turnover time is based on extrapolation of labeling index at 2 hours and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (Days)
Pituitary	anterior lobe	2 hrs.	4	864	0.46	36.2
		1 day	6	1050	0.57	58.4
		2 days	1	2226	0.04	
		4 days	2	9168	0.02	
		8 days	1	3424	0.02	
	posterior lobe	2 hrs.	2	2784	0.07	238.0
		1 day	6	1680	0.35	95.2
		2 days	0	3480	0	
		4 days	0	3438	0	
		8 days	0	816	0	
Thyroid	follicular	2 hrs.	3	5040	0.05	333.3
		1 day	4	2660	0.15	222.2
		2 days	0	2820	0	
		4 days	0	2400	0	
		8 days	0	3220	0	
	non-follicular	2 hrs.	14	5040	0.27	61.7
		1 day	5	2660	0.18	185.1
		2 days	2	2820	0.07	
		4 days	0	2400	0	
		8 days	0	3220	0	
Pancreas	islets	2 hrs.	1	680	0.14	119.0
		1 day	2	680	0.29	114.9
		2 days	2	860	0.23	
		4 days	0	740	0	
		8 days	0	760	0	
	exocrine cells	2 hrs.	3	1400	0.21	79.3
		1 day	10	1050	0.95	35.0
		2 days	2	1150	0.17	
		4 days	1	1100	0.09	
		8 days	1	1200	0.08	

Table 6 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (Days)
Adrenal	glomerulosa	2 hrs.	14	570	2.4	6.9
		1 day	12	655	1.8	18.5
		2 days	5	905	0.5	
		4 days	7	785	0.8	
		8 days	12	1080	1.1	
	fasiculata	2 hrs.	3	505	0.5	33.3
		1 day	4	380	1.05	31.7
		2 days	2	685	0.29	
		4 days	1	680	0.14	
		8 days	7	720	0.97	
	reticularis	2 hrs.	2	510	0.39	42.7
		1 day	2	420	0.47	70.9
		2 days	1	835	0.11	
		4 days	0	995	0	
		8 days	0	780	0	
	medulla	2 hrs.	8	456	1.74	9.5
		1 day	3	785	0.38	87.7
		2 days	1	536	0.18	
		4 days	1	910	0.10	
		8 days	0	620	0	

The Digestive System:

The tongue:

Three types of papillae were noted in sections of the tongue, namely filliform, fungiform and circumvallate. Taste buds were also seen. The labeled nuclei were frequent in the three layers of the stratified squamous epithelium that lined the undersurface of the tongue (Tables 8 and 9). Their relative frequency in each layer varied in a systematic manner with time.

The submandibular gland contains both serous and mucous acini. A large number of ducts are seen within the gland substance. Radioactive nuclei were rare according to the estimated number of nuclei per unit area studied (Tables 7 and 8).

The sublingual gland is purely mucous in the Galago, since it stains turquoise blue with the quadruple stain. The gland from the animal that was sacrificed 2 hours after the H^3-thymidine injection was not retrieved. Radioactive nuclei were rare, as in the other salivary glands (Tables 7 and 8).

The parotid gland is a purely serous gland in the Galago. Excretory ducts are also present. Radioactive nuclei were rare according to the estimated number of nuclei per unit area studied (Tables 7 and 8).

The thickness of the muscularis mucosae of the stomach was 14 u. The outer muscular wall was 0.66 mm. thick. The average depth of three gastric pits was 0.10 mm. in the fundus and 0.12 mm. in the pylorus.

A pattern of cell migration lumenwards was noted in all the sections of the stomach. In a section from the fundus of the stomach of the animal killed 2 hours after injection, the majority of labeled nuclei were localized in the isthmus. The total distance from the muscularis mucosae to the tip of the gastric pit was 1.56 mm., the concentration of the radioactive cells was 1.44 mm. luminal from the muscularis mucosae, or 92% of the total distance. In the animal that was killed one day after the injection, the total distance was 0.115 mm. and the concentration of labeled nuclei was 0.10 mm. from the muscularis mucosae or 87% of the total length. In the animal that was killed two days after the injection, the total distance was 1.46 mm. and the concentration of labeled nuclei was 1.44 mm. from the muscularis mucosae or 99% of the total length. The majority of the radioactive cells in the animal that was killed four days after the injection were located in the surface epithelium and some were seen in the lumen. Frequency of labeled nuclei was variable between animals (Table 8 and 17).

In the duodenum, the average langth of five villi was 0.69 mm. and the depth of the crypt was 0.12 mm. The thickness of the outer muscular wall was 0.07 mm.

Paneth cells lie at the bottom of the crypts, their cytoplasm contains eosinophilic granules. In all the sections, at the various time intervals, none of them showed any radioactivity.

In the villus core the type of cells present were not differentiated for the purpose of tabulating radioactive cells, but plasma cell nuclei, lymphocytes connective tissue nuclei and smooth muscle nuclei were recognised to be present.

Frequency of labeled nuclei followed a decreasing pattern with time in the crypt and an increasing pattern in the villus core (Tables 8 and 17).

A pattern of cell migration from the base of the crypt lumenwards was obvious (Table 9).

Jejunum:

Goblet cells were abundant in this portion of the small intestine. The length of the villi was variable and ranged from 0.25 to 1.0 mm. with an average of 0.58 mm. (average of 7 villi), and the depth of the crypt 0.10 mm. The thickness of the outer muscular wall was 0.10 mm.

None of the paneth cells, in all the sections at the various time intervals, showed any radioactivity.

Frequency of labeled nuclei followed a decreasing pattern with time in the crypt and an increasing pattern in the villus core (Tables 8 and 17).

A pattern of cell migration from the base of the crypt towards the lumen was obvious (Table 9).

Ileum:

Peyers patches were very prominent in the ileum. The length of the villi varied from 0.25 to 0.75 mm., with an average of 0.43 mm. (average of 5 villi). The thickness of the outer muscular wall was 0.075 mm.

None of the Paneth cells showed any radioactivity at the various time intervals.

Some villi showed eroded epithelium at the tip. Labeled nuclei were noted in the lumen, of the four days animal ileum.

Frequency of labeled nuclei followed the same pattern as the duodenum and jejunum (Tables 8 and 17).

Colon:

Sections from the transverse colon were used for this study. The mucosa is folded to form villus-like extensions towards the lumen. They are leaf-like, long and broad. Their length vary from 0.05 to 1.0 mm. with an average length of 0.39 mm. (average of 5 villi). The thickness of the outer muscular wall varies from 0.20 to 0.30 mm. Goblet cells are very prominent.

Frequency of labeled nuclei decreased with time in the crypt, but was variable in the villus core (Tables 8 and 17). Migration could be demonstrated (Table 9).

Liver:

The liver of the Galago is similar histologically to that of the human.

The Parenchymal cell nucleus was round with a distinct nucleolus and measured 6.25 u.

Kupffer cells and other cells lining the sinusoids were all grouped together as endothelial cells. Portal triads are well seen in all the sections.

Frequency of radioactive nuclei decreased with time in the Parenchymal cell population, but was variable in the endothelial and bile duct epithelium (Tables 8 and 17).

Table 7

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

The Digestive System

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area mm^2	Estimated total population
Sublingual gland	alveolar	2 hrs.	---	---	---	---
		1 day	20	28	0.26	560
		2 days	20	21	0.26	420
		4 days	20	29	0.26	580
		8 days	20	26	0.26	520
Submandibular	alveolar	2 hrs.	49	16	0.64	784
		1 day	56	21	0.73	1076
		2 days	62	15	0.81	930
		4 days	90	22	1.17	1980
		8 days	50	18	0.65	900
Parotid	alveolar	2 hrs.	31	21	0.40	651
		1 day	65	20	0.85	1300
		2 days	40	23	0.52	920
		4 days	80	18	1.04	1440
		8 days	50	27	0.65	1350

Table 8

Frequency of labeled nuclei in various cell populations of the digestive system. Turnover time is based on an extrapolation of labeling index at 2 hrs. and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Tongue	basal	2 hrs.	24	100	24.0	0.70 (16.8 hrs)
		1 day	15	100	15.0	1.1
		2 days	12	100	12.0	1.3
		4 days	5	100	5.0	3.3
		8 days	1	100	1.0	16.6
	spinous	2 hrs.	1	100	1.0	16.6
		1 day	15	100	15.0	1.1
		2 days	18	100	18.0	0.92 (22 hrs)
		4 days	19	100	19.0	0.87 (19.7 hrs)
		8 days	12	100	12.0	1.3
	surface	2 hrs.	0	100	0	
		1 day	0	100	0	
		2 days	2	100	2.0	8.3
		4 days	5	100	5.0	3.3
		8 days	4	100	4.0	4.1
Sublingual	alveolar	2 hrs.	---	---	---	---
		1 day	4	560	0.71	46.9
		2 days	0	420	0	
		4 days	0	580	0	
		8 days	0	520	0	
Submandibular	alveolar	2 hrs.	1	784	0.12	138.8
		1 day	0	1076	0	
		2 days	0	930	0	
		4 days	2	1980	0.10	
		8 days	1	900	0.11	
	duct epithelium	2 hrs.	0	41	0	∞
		1 day	0	212	0	
		2 days	0	173	0	
		4 days	1	364	0.27	
		8 days	0	218	0	

Table 8 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Parotid	alveolar	2 hrs.	2	651	0.30	55.5
		1 day	2	1300	0.15	222.2
		2 days	3	920	0.32	
		4 days	0	1440	0	
		8 days	0	1350	0	
	duct epithelium	2 hrs.	0	24	0	∞
		1 day	0	78	0	
		2 days	0	102	0	
		4 days	0	149	0	
		8 days	0	164	0	
Stomach	surface epithelium (Fundus)	2 hrs.	3	200	1.5	11.1
		1 day	6	200	3.0	11.1
		2 days	7	200	3.5	
		4 days	5	200	2.5	
		8 days	1	200	0.5	
	isthmus	2 hrs.	14	100	14.0	1.2
		1 day	9	100	9.0	3.7
		2 days	10	100	10.0	
		4 days	3	100	3.0	
		8 days	0	100	0	
	surface epithelium (Pylorus)	2 hrs.	--	---	--	--
		1 day	3	200	1.5	22.2
		2 days	10	200	5.0	
		4 days	7	200	3.5	
		8 days	0	200	0	
	pyloric glands	2 hrs.	--	---	--	--
		1 day	4	100	4.0	8.3
		2 days	4	100	4.0	
		4 days	2	100	2.0	
		8 days	0	100	0	
Duodenum	crypt	2 hrs.	24	100	24.0	0.70 (16.8 hrs)
		1 day	18	100	18.0	1.8
		2 days	15	100	15.0	
		4 days	11	100	11.0	
		8 days	2	100	2.0	

Table 8 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
	villus core	2 hrs.	8	100	8.0	2.0
		1 day	10	100	10.0	3.3
		2 days	13	100	13.0	
		4 days	17	100	17.0	
		8 days	7	100	7.0	
Jejunum	crypt	2 hrs.	22	100	22.0	0.75 (18 hrs)
		1 day	16	100	16.0	2.0
		2 days	7	100	7.0	
		4 days	5	100	5.0	
		8 days	1	100	1.0	
	villus core	2 hrs.	3	100	3.0	5.0
		1 day	5	100	5.0	6.6
		2 days	8	100	8.0	
		4 days	19	100	19.0	
		8 days	7	100	7.0	
Ileum	crypt	2 hrs.	15	100	15.0	1.1
		1 day	14	100	14.0	2.3
		2 days	10	100	10.0	
		4 days	2	100	2.0	
		8 days	1	100	1.0	
	villus core	2 hrs.	7	100	7.0	2.4
		1 day	9	100	9.0	3.7
		2 days	16	100	16.0	
		4 days	11	100	11.0	
		8 days	6	100	6.0	
Colon	crypt	2 hrs.	24	100	24.0	0.70 (16.8 hrs)
		1 day	19	100	19.0	1.7
		2 days	9	100	8.0	
		4 days	6	100	6.0	
		8 days	2	100	2.0	
	villus core	2 hrs.	15	100	15.0	1.1
		1 day	7	100	7.0	4.7
		2 days	6	100	6.0	
		4 days	7	100	7.0	
		8 days	6	100	6.0	

Table 8 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Liver	parenchymal	2 hrs.	7	200	3.5	4.7
		1 day	5	200	2.5	13.3
		2 days	2	200	1.0	
		4 days	2	200	1.0	
		8 days	0	200	0	
	endothelium	2 hrs.	5	200	2.5	6.6
		1 day	4	200	2.0	16.6
		2 days	1	200	0.5	
		4 days	0	200	0	
		8 days	2	200	1.0	
	bile duct epithelium	2 hrs.	2	36	5.5	3.0
		1 day	3	31	9.6	3.4
		2 days	0	21	0	
		4 days	0	44	0	
		8 days	0	21	0	

Table 9

Pattern of migration of labeled nuclei lumenwards in the small intestine and the colon

Organ	Time interval	Length of villus mm	Conc. of radioactive nuclei from base	% of total length
Duodenum	2 hrs.	0.69	base	---
	1 day	0.96	0.24	25
	2 days	0.65	0.24	37
	4 days	0.38	0.28	73
	8 days	0.63	0.58	92
Jejunum	2 hrs.	0.84	base	---
	1 day	0.72	0.09	12.5
	2 days	0.45	0.12	27
	4 days	0.50	0.30	60
	8 days	0.48	0.43	90
Ileum	2 hrs.	0.30	base	---
	1 day	0.50	0.17	35
	2 days	0.35	0.15	43
	4 days	0.25	0.22	90
	8 days	0.65	surface epith	100
Colon	2 hrs.	0.44	base	---
	1 day	0.24	0.06	25
	2 days	0.50	0.30	60
	4 days	0.12	0.10	80
	8 days	0.20	surface epith	100

Muscle Cells:

None of the cardiac muscle fiber nuclei or the skeletal muscle fiber nuclei showed any radioactivity (Tables 10 and 11).

None of the smooth muscle fiber nuclei in the uterine cornu, stomach and small intestines of all the animals injected with tritiated thymidine, at various time intervals showed any radioactivity.

Lung:

Scattered reactions were seen in the parenchymal tissue of the lung, which decreased in number with time (Tables 10 and 11).

Radioactive nuclei were rare in the bronchial epithelium (Table 11).

Bone Marrow:

All nucleated marrow cells were considered as a single population without separation of various cell types except for the megakaryocytes, which were counted separately.

Bone marrow from the animal that was sacrificed two hours after the injection of radioactive thymidine was not retrieved. The frequency of radioactive nuclei was high in both the megakaryocytes and the other cells, and decreased with time (Tables 11 and 17).

The Integument:

A section from the skin of the back, and the skin of the mammary gland were used to study the labeling of cells in the integument. The labeled nuclei were frequent in the three layers of the stratified squamous epithelium. Their relative frequency in each layer varied in a systematic manner with time (Table 11).

Table 10

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area mm^2	Estimated total population
Heart	cardiac muscle	2 hrs.	50	11	0.65	550
		1 day	50	8	0.65	400
		2 days	50	10	0.65	500
		4 days	50	13	0.65	650
		8 days	50	11	0.65	550
Quadriceps	skeletal muscle	2 hrs.	80	16	1.04	1280
		1 day	80	10	1.04	800
		2 days	80	17	1.04	1360
		4 days	80	13	1.04	1040
		8 days	80	19	1.04	1520
Lung	parenchymal tissue	2 hrs.	15	26	0.195	390
		1 day	15	31	0.195	465
		2 days	15	28	0.195	420
		4 days	15	33	0.195	495
		8 days	15	23	0.195	345

Table 11

Frequency of labeled nuclei in various cell populations. Turnover time is based on an extrapolation of labeling index at 2 hours and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Cardiac	muscle	2 hrs.	0	550	0	∞
		1 day	0	400	0	
		2 days	0	500	0	
		4 days	0	650	0	
		8 days	0	550	0	
Skeletal	muscle	2 hrs.	0	1280	0	∞
		1 day	0	800	0	
		2 days	0	1360	0	
		4 days	0	1040	0	
		8 days	0	1520	0	
Lung	parenchymal tissue	2 hrs.	8	390	2.05	8.1
		1 day	7	465	1.50	22.2
		2 days	1	420	0.23	
		4 days	2	495	0.40	
		8 days	1	345	0.28	
	bronchial epithelium	2 hrs.	1	200	0.50	33.3
		1 day	0	200	0	
		2 days	0	200	0	
		4 days	0	200	0	
		8 days	0	200	0	
Bone	megakaryocytes	2 hrs.	--	---	---	----
		1 day	4	50	8.0	4.1
		2 days	2	50	4.0	
		4 days	1	50	2.0	
		8 days	1	50	2.0	
	other cells	2 hrs.	--	---	---	----
		1 day	14	100	14.0	2.3
		2 days	9	100	9.0	
		4 days	2	100	2.0	
		8 days	0	100	0	
Skin	basal	2 hrs.	8	100	8.0	2.0
		1 day	5	100	5.0	3.3
		2 days	3	100	3.0	5.5
		4 days	2	100	2.0	8.3
		8 days	0	100	0	

Table 11 (Cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
	spinous	2 hrs.	3	100	3.0	5.5
		1 day	5	100	5.0	3.3
		2 days	4	100	4.0	4.1
		4 days	5	100	5.0	3.3
		8 days	4	100	4.0	4.1
	surface	2 hrs.	0	100	0	∞
		1 day	0	100	0	
		2 days	1	100	1.0	16.6
		4 days	4	100	4.0	4.1
		8 days	2	100	2.0	8.3

Lymphoid organs:

The capsule and trabeculae of the spleen of the Galago consist of dense collagenous connective tissue, elastic fibers and smooth muscle fibers. Most cells with a large dark nucleus and thin cytoplasm are seen in the spleen.

Lymph nodules with a germinal center were noted in each tonsil examined. The tonsils of the animal that was sacrificed two hours after the injection of labeled thymidine were not isolated, but were lost in the process of dissection.

The cortex of the thymus contained densely packed lymphocytes, but the medulla was not as tightly packed with cells. Hassal's corpuscles were different in morphology from those in the human thymus by being more concentric in layers. Sinusoid-like structures were noted with 4 or 5 nuclei in a row around a lumen, in the substance of the gland. No radioactivity was noted in Hassal's corpuscles at the various time intervals after the injection of radioactive thymidine.

The cortex of the mesenteric lymph node had a greater concentration of cells than the medulla. The node from the animal that was sacrificed two days after the injection of labeled thymidine was a hemolymph node with pools of erythrocytes in it.

The frequency of labeled nuclei in all the lymphoid organs examined decreased with time (Table 12). The average grain count per nucleus also decreased with time (Table 17).

Table 12

Frequency of labeled nuclei in various cell populations. Turnover time is based on an extrapolation of labeling index at 2 hours and 1 day

Lymphoid Organs

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Spleen	lymph nodule	2 hrs.	22	200	11.0	1.5
		1 day	19	200	9.5	3.5
		2 days	14	200	7.0	
		4 days	5	200	2.5	
		8 days	3	200	1.5	
	nuclear aggregation	2 hrs.	19	200	9.5	1.7
		1 day	15	200	7.5	4.4
		2 days	7	200	3.5	
		4 days	4	200	2.0	
		8 days	1	200	0.5	
	open area	2 hrs.	17	200	8.5	1.9
		1 day	9	200	4.5	7.4
		2 days	7	200	3.5	
		4 days	2	200	1.0	
		8 days	0	200	0	
Tonsils	germinal center	2 hrs.	--	---	---	---
		1 day	48	200	24.0	1.3
		2 days	32	200	16.0	
		4 days	18	200	9.0	
		8 days	2	200	1.0	
	cortex	2 hrs.	--	---	---	---
		1 day	21	200	10.5	3.1
		2 days	12	200	6.0	
		4 days	4	200	2.0	
		8 days	2	200	1.0	
Thymus	medulla	2 hrs.	9	200	4.5	3.7
		1 day	8	200	4.0	8.3
		2 days	6	200	3.0	
		4 days	5	200	2.5	
		8 days	4	200	2.0	

Table 12 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
	cortex	2 hrs.	11	200	5.5	3.0
		1 day	8	200	4.0	8.3
		2 days	8	200	4.0	
		4 days	6	200	3.0	
		8 days	4	200	2.0	
Mesent. lymph node	cortex	2 hrs.	12	100	12.0	1.4
		1 day	9	100	9.0	3.7
		2 days	5	100	5.0	
		4 days	1	100	1.0	
		8 days	1	100	1.0	
	subcapsular sinus	2 hrs.	7	100	7.0	2.3
		1 day	5	100	5.0	6.6
		2 days	5	100	5.0	
		4 days	3	100	3.0	
		8 days	1	100	1.0	
	medullary cords	2 hrs.	6	100	6.0	2.8
		1 day	4	100	4.0	8.3
		2 days	2	100	2.0	
		4 days	2	100	2.0	
		8 days	2	100	2.0	
	medullary sinus	2 hrs.	10	100	10.0	1.6
		1 day	7	100	7.0	4.7
		2 days	4	100	4.0	
		4 days	2	100	2.0	
		8 days	0	100	0	

The Urinary System:

The Galago's kidney is similar to the human kidney in that it is multi-lobar, in contrast to the rat's and rabbit's kidneys which are unilobar. Two out of seven kidneys examined from seven different Galagos showed an unidentified glandular tissue at the hilus. The cells were vacuolated, stained red with H & E, with a centrally placed round, dark nucleus 10 u in diameter. The nucleolus is well shown also. It is suggested to be a steroid type secreting gland. The Galagos that had this type of gland were not used in the radioactive thymidine injection experiments.

Frequency of radioactive nuclei was variable, according to the area of the gland studied (Tables 13, 14 and 17).

The average thickness of the outer muscular wall of five ureters was 48 u.

Radioactive nuclei were rare (Table 14).

The thickness of the mucosa of the urinary bladder adapts to changes in the bladder volume and the degree of its distension. Two types of epithelia were considered. The thick epithelium, produced when a part of the mucosa was in the contracted state, had three layers of cells, namely, basal, middle and surface layers. The thin type epithelium, produced when a part of the mucosa is distended, could not be clearly differentiated into cell layers. The bladder of the animal that was sacrificed 8 days after the injection of labeled thymidine was completely distended.

Radioactive nuclei were rare in all the bladders examined, except in a certain area in the bladder of the Galago that was sacrificed four days after the injection of labeled thymidine (Tables 14 and 17).

The urethra that runs through the clitoris was used for the radioactive cell count. Frequency of labeled nuclei was variable according to the cell layer studied (Tables 13 and 14).

Table 13

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

Urinary System

Organ	Cell population	Time interval	# of grids	# of nuclei per grid	Total area (mm^2)	Estimated cell population
Kidney	Medulla	2 hrs.	30	21	0.39	630
		1 day	30	28	0.39	840
		2 days	30	23	0.39	690
		4 days	30	19	0.39	570
		8 days	20	34	0.26	680
Urethra	Basal	2 hrs.	20	9	0.26	180
		1 day	20	10	0.26	200
		2 days	20	12	0.26	240
		4 days	20	8	0.26	160
		8 days	10	12	0.13	120
	Middle	2 hrs.	20	26	0.26	520
		1 day	20	29	0.26	580
		2 days	20	16	0.26	320
		4 days	20	10	0.26	200
		8 days	10	44	0.13	440
	Surface	2 hrs.	20	7	0.26	140
		1 day	20	6	0.26	120
		2 days	20	10	0.26	200
		4 days	20	11	0.26	220
		8 days	10	9	0.13	90

Table 14

Frequency of labeled nuclei in various cell populations of the urinary system. Turnover time is based on an extrapolation of labeling index at 2 hours and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (Days)
Kidney	glomerular tuft	2 hrs.	7	529	1.30	12.8
		1 day	3	533	0.56	59.5
		2 days	2	465	0.40	
		4 days	1	431	0.33	
		8 days	0	364	0	
	parietal bowman	2 hrs.	5	76	6.60	2.5
		1 day	2	95	2.10	15.8
		2 days	0	57	0	
		4 days	2	62	3.22	
		8 days	0	101	0	
	proximal convoluted	2 hrs.	6	84	7.14	2.3
		1 day	2	86	2.32	14.3
		2 days	0	79	0	
		4 days	0	103	0	
		8 days	0	107	0	
	distal convoluted	2 hrs.	5	172	2.90	5.7
		1 day	3	167	1.79	18.6
		2 days	2	121	1.65	
		4 days	3	131	2.29	
		8 days	0	135	0	
	medulla collecting tubules	2 hrs.	3	630	0.47	35.4
		1 day	2	840	0.23	144.9
		2 days	2	690	0.28	
		4 days	5	570	0.35	
		8 days	0	680	0	
	medullary ducts	2 hrs.	2	630	0.31	53.7
		1 day	2	840	0.23	144.9
		2 days	3	690	0.43	
		4 days	2	570	0.35	
		8 days	0	600	0	

Table 14 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (Days)
Kidney	thick section Henle's loop	2 hrs.	2	630	0.31	53.7
		1 day	2	840	0.23	144.9
		2 days	0	690	0	
		4 days	3	570	0.52	
		8 days	2	680	0.29	
	thin section Henle's loop	2 hrs.	1	630	0.15	111.1
		1 day	1	840	0.11	303.0
		2 days	0	690	0	
		4 days	1	570	0.17	
		8 days	0	680	0	
Ureter	basal	2 hrs.	1	132	0.75	22.2
		1 day	0	142	0	
		2 days	0	72	0	
		4 days	0	63	0	
		8 days	0	103	0	
	middle	2 hrs.	3	319	0.94	17.7
		1 day	3	646	0.46	72.4
		2 days	0	304	0	
		4 days	0	182	0	
		8 days	0	211	0	
	surface	2 hrs.	0	72	0	∞
		1 day	0	128	0	
		2 days	0	48	0	
		4 days	0	42	0	
		8 days	0	69	0	
Urinary bladder	thin epith.	2 hrs.	0	1400	0	
		1 day	1	1240	0.08	416.6
		2 days	1	1170	0.08	
		4 days	0	1350	0	
		8 days	0	480	0	
	thick epith. basal	2 hrs.	1	100	1.0	16.6
		1 day	0	100	0	
		2 days	0	100	0	
		4 days	14	100	14.0	
		8 days	--	---	----	

Table 14 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (Days)
Urinary bladder	middle	2 hrs.	0	100	0	∞
		1 day	0	100	0	
		2 days	2	100	2.0	
		4 days	9	100	9.0	
		8 days	--	---	---	
	surface	2 hrs.	0	100	0	∞
		1 day	0	100	0	
		2 days	0	100	0	
		4 days	2	100	2.0	
		8 days	--	---	---	
Urethra	basal	2 hrs.	12	180	6.66	2.5
		1 day	8	200	4.0	8.3
		2 days	1	240	0.41	
		4 days	0	160	0	
		8 days	1	120	0.83	
	middle	2 hrs.	1	520	0.19	87.7
		1 day	2	580	0.34	91.1
		2 days	7	320	2.18	
		4 days	1	200	0.50	
		8 days	2	440	0.45	
	surface	2 hrs.	0	140	0	∞
		1 day	0	120	0	
		2 days	0	200	0	
		4 days	6	220	2.72	
		8 days	-	90	0	

The Female Genital System:

The ovary:

The follicles were divided into four types depending on their diameter. The theca externa was considered the outer limit for measurement.

Follicle I had a diameter range from 0.01 to 0.20 mm., follicle II, 0.21 to 0.30 mm., follicle III 0.31 to 0.50 mm., and follicle IV more than 0.51 mm. A follicle as large as the follicle IV category was found only in the animal killed 2 hours after injection.

The cell population of both thecas interna and externa of the follicles from the ovaries of the Galagos that were sacrificed two hours and two days after the radioactive thymidine injection were not differentiated. In the rest of the animals they were considered separately.

The ovaries of the animals that were sacrificed 1 day and 8 days after the thymidine injection did not have any corpora lutea.

The frequency of labelled nuclei varied with time according to the cell population considered (Tables 15, 16 and 17).

The fallopian tube:

The general histology is the same as that of the human. A mitotic figure in the Anaphase stage was noted in one section.

The right fallopian tube from the Galago that was sacrificed two hours after the thymidine injection bifurcated into two tubes, wrapped together by connective tissue fibers and running side by side from the fimbriated ampulla down to the uterine cornu, where they joined. Their histologic structure was similar.

The average thickness of the muscular wall of three fallopian tubes was 68 u.

Radioactive nuclei were infrequent (Tables 15 and 16).

The uterine cornua:

The uterus of the Galago is bicornuated. The average thickness of the myometrium of three uteri was 0.27 mm., and that of the endometrium 0.35 mm.

The frequency of labelled nuclei varied with time in the different cell populations considered (Tables 15, 16 and 17).

A sagittal section between the two cornua of the uterus and through the vagina revealed the presence of a cervix. Its location is at the junction of the end of the uterine cornua and the beginning of the vagina.

Projections that are villus like or rugae structures are seen in the vaginal canal. Close to the cervix the length of each projection is about 0.54 mm., and the width of its base is about 0.14 mm. At the middle of the vaginal canal, the projections become shorter and broader and the length becomes 0.42 mm. and the width at the base 0.36 mm. Towards the end of the vagina and close to the external opening the projections flatten out to reach a length of about 0.07 mm. and later become one cell layer overlying the connective tissue and the muscular wall.

The length of the epithelium covering the cervix of the animal that was sacrificed four days after thymidine injection was 0.009 mm. Among 100 cells of the epithelial covering, only one nucleus was labelled.

The vagina:

Radioactive nuclei were frequent in the three layers of the stratified squamous epithelium. Their relative frequency in each layer varied in a systematic manner with time (Tables 16 and 17).

The mammary gland:

Stratified squamous epithelium overlay the loose connective tissue that had lactiferous ducts, fat cells and a layer of muscle fibers underneath. Mammary ducts ran through the nipple. The cells of the ducts were identified as basal or luminal, depending on whether they lined the lumen or not. The length of the duct epithelium was measured and the number of cells was estimated per unit length.

A section of the mammary gland of the Galago that was sacrificed two hours after thymidine injection had both lactiferous and mammary ducts. Along a total length of 1.17 mm. of lactiferous duct epithelium, estimated to contain 376 nuclei, only 7 were radioactive. Two of them were luminal and five basal. 0.98 mm. of mammary duct epithelium, estimated to contain 1018 nuclei, 31 nuclei were labelled, 26 basal and 5 luminal.

The section of the mammary gland from the Galago that was sacrificed one day after thymidine injection, did not show any glandular tissue.

2.03 mm. of lactiferous duct epithelium from the mammary gland of the Galago that was sacrificed two days after the thymidine injection was estimated to contain 730 nuclei. Among them four were labelled. One was luminal and three basal. The mammary duct had 136 nuclei, among them two luminal and 17 basal nuclei were radioactive.

350 nuclei were estimated to be present in the 1.25 mm. lactiferous duct epithelium of the mammary gland of the Galago that was sacrificed four days after the injection. None were radioactive. 1.30 mm. of mammary duct epithelium estimated to contain 442 nuclei, three basal nuclei only were radioactive.

The section of the gland from the Galago that was sacrificed 8 days after the injection had only 104 lactiferous duct epithelium nuclei, but no mammary ducts (Tables 16 and 17).

Table 15

Computation of estimated cell populations based on counting nuclei in grids with an area of 0.0132 mm^2 per grid

Female Genital Organs

Organ	Cell population	Time interval	# of nuclei	# of nuclei per grid	Total area (mm^2)	Estimated cell population
Ovary	corpus luteum	2 hrs.	70	56	0.910	3920
		1 day	--	--	-----	-----
		2 days	80	88	1.04	7040
		4 days	95	76	1.24	7220
		8 days	--	--	-----	-----
Fallopian tube	muscular wall	2 hrs.	10	34	0.132	340
		1 day	10	31	0.132	310
		2 days	10	53	0.132	530
		4 days	10	43	0.132	430
		8 days	10	27	0.132	270
Uterine cornua	stroma	2 hrs.	11	80	0.143	880
		1 day	15	64	0.195	960
		2 days	15	81	0.195	1215
		4 days	5	56	0.065	280
		8 days	10	76	0.132	760

Table 16

Frequency of labeled nuclei in various cell populations of the female genital organs. Turnover time is based on an extrapoltaion of labeling index at 2 hours and 1 day

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Ovary	Follicle I (Diameter 0.01-0.20mm)					
	Granulosa	2 hrs.	10	300	3.3	5
		1 day	17	200	8.5	3.9
		2 days	2	200	1.0	
		4 days	22	300	7.3	
		8 days	0	300	0	
	Thecas (int.& ext.)	2 hrs.	9	200	4.5	3.7
		2 days	3	100	3.0	
	Theca interna	1 day	5	200	2.5	13.3
		4 days	10	300	3.3	
		8 days	0	300	0	
	Theca externa	1 day	6	200	3.0	11.1
		4 days	10	300	3.3	
		8 days	0	300	0	
	Follicle II (Diameter 0.21-0.30mm)					
	Granulosa	2 hrs.	6	100	6.0	2.7
		1 day	28	200	14.0	2.3
		2 days	5	100	5.0	
		4 days	7	100	7.0	
		8 days	0	100	0	
	Thecas (int.& ext.)	2 hrs.	3	100	3.0	5.5
		2 days	6	100	6.0	
	Theca interna	1 day	10	300	3.3	10.1
		4 days	4	100	4.0	
		8 days	0	100	0	

Table 16 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
	Theca externa	1 day	7	200	3.5	9.5
		4 days	3	100	3.0	
		8 days	0	100	0	
	Follicle III (Diameter 0.31-0.50 mm)					
	Granulosa	2 hrs.	14	200	7.0	2.3
		2 days	6	300	2.0	
		8 days	2	300	0.66	
	Thecas (int.& ext.)	2 hrs.	3	100	3.0	5.5
		2 days	9	300	3.0	
		8 days	1	300	0.33	
	Follicle IV (Diameter 0.51 mm)					
	Granulosa	2 hrs.	18	100	18.0	0.92 (22 hrs.)
	Thecas (int.& ext.)	2 hrs.	4	100	4.0	4.1
	Corpus luteum	2 hrs.	16	3920	0.40	41.6
		2 days	13	7040	0.18	
		4 days	11	7220	0.15	
	Stroma	2 hrs.	6	100	6.0	2.7
		1 day	5	200	2.5	13.3
		2 days	0	100	0	
		4 days	2	200	1.0	
		8 days	0	100	0	
	Germinal epith.	2 hrs.	2	100	2.0	8.3
		1 day	3	200	1.5	22.2
		2 days	0	100	0	
		4 days	0	200	0	
		8 days	0	100	0	

Table 16 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
	Primary follicles (ova)	2 hrs.	0	89	0	∞
		1 day	0	86	0	
		2 days	0	104	0	
		4 days	0	54	0	
		8 days	0	43	0	
Fallopian tube	luminal epith.	2 hrs.	5	146	3.42	4.8
		1 day	3	212	1.41	23.6
		2 days	0	113	1.41	
		4 days	0	292	0	
		8 days	0	100	0	
	muscular wall	2 hrs.	0	340	0	∞
		1 day	0	310	0	
		2 days	0	530	0	
		4 days	0	430	0	
		8 days	0	270	0	
Uterine cornu	surface epith.	2 hrs.	11	156	7.05	2.3
		1 day	7	100	7.0	4.7
		2 days	0	476	0	
		4 days	2	144	1.38	
		8 days	0	216	0	
	stroma	2 hrs.	10	880	1.13	14.8
		1 day	5	960	0.52	64.1
		2 days	2	1215	0.16	
		4 days	11	280	3.92	
		8 days	4	760	0.52	
	glandular epith.	2 hrs.	13	139	9.35	1.7
		1 day	14	298	4.69	7.1
		2 days	5	246	2.03	
		4 days	5	376	1.32	
		8 days	9	416	2.16	
Vagina	basal	2 hrs.	7	100	7.0	2.3
		1 day	6	100	6.0	2.7
		2 days	2	100	2.0	8.3
		4 days	3	100	3.0	5.5
		8 days	0	100	0	

Table 16 (cont'd.)

Organ	Cell population	Time interval	Radioactive nuclei	Total nuclei	% of radioactive nuclei	Turnover time (days)
Vagina	spinous	2 hrs.	1	100	1.0	16.6
		1 day	3	100	3.0	5.5
		2 days	5	100	5.0	3.3
		4 days	14	100	14.0	1.1
		8 days	2	100	2.0	8.3
	surface	2 hrs.	0	100	0	
		1 day	2	100	2.0	8.3
		2 days	2	100	2.0	8.3
		4 days	5	100	5.0	3.3
		8 days	1	100	1.0	16.6
Mammary gland	lactiferous duct	2 hrs.	7	376	1.86	8.9
		2 days	4	730	0.54	
		4 days	0	350	0	
		8 days	0	104	0	
	mammary duct	2 hrs.	31	1018	3.04	5.4
		2 days	19	136	13.97	
		4 days	3	442	0.67	

Table 17

The average grain count per nucleus in various cell populations of the Galago.
(Based on ten radioactive nuclei, unless otherwise indicated.)

			Time interval				
Tissue	Cell population	Subunit	2 hours	1 day	2 days	4 days	8 days
Brain	Subependymal		11.3	11.4	6.4	5.9	6.7
Adrenal	Glomerulosa	round nucleus	41.8	27.2	19.6	11.0	19.9
		elongated nucleus	18.1	16.7	12.1	9.2	-(1)
	Fasiculata	round nucleus	32.1	26.3	17.4	8.3(2)	16.1(2)
		elongated nucleus	21.5	18.0	-(1)	-(1)	-(1)
	Reticularis	round nucleus	35.9	30.6	17.6(2)	9.0(2)	-(1)
		elongated nucleus	-(1)	17.2(2)	-(1)	-(1)	-(1)
	Medulla		22.7	18.1(2)	12.6(2)	10.5(2)	-(1)
Tongue	Basal		41.3	24.4	17.7	15.3	7.3(2)
	Spinous		28.3	21.7	18.1	16.8	10.6
	Surface		-(1)	-(1)	7.2(2)	10.2	5.6(2)
Stomach	Fundus	surface epith.	28.3	25.7	17.1	9.0	6.4
		Isthmus	25.0	19.9	18.6	13.3	-(1)
	Pylorus	surface epith.	*(3)	21.5	13.1	9.7	-(1)
			*(3)	16.9	13.9	10.2	-(1)

Table 17 (cont'd.)

Tissue	Cell population	Subunit	Time interval 2 hours	1 day	2 days	4 days	8 days
Duodenum	Crypt		35.0	23.0	19.7	10.0	6.3
	villus core		22.6	16.0	10.8	8.8	8.4
Jejunum	Crypt		28.8	17.1	10.0	9.1	6.0
	villus core		29.5	15.5	12.5	9.6	7.3
Ileum	Crypt		27.1	18.0	13.2	6.2	5.7
	villus core		19.0	16.4	11.3	6.7	6.0
Colon	Crypt		34.5	18.9	8.4	8.0	6.0
	villus core		32.3	16.7	9.5	7.1	6.3
Liver	Parenchymal		67.2	33.1	22.6	15.0	-(1)
	Endothelial		42.0	22.0	10.2	9.3	-(1)
Marrow block	Megakaryocytes		*(3)	11.0(2)	9.8(2)	-(1)	-(1)
	other cells		*(3)	12.2	6.6	6.3	-(1)
Spleen	Lymph nodule		44.2	20.8	21.1	8.0	6.6
	Nuclear aggregation		36.7	22.9	14.9	8.5	5.8
	Open area		25.3	18.3	12.9	7.9	-(1)
Tonsils	Germinal		*(3)	10.2	7.2	6.2	-(1)
	center		*(3)	17.9	10.6	6.3	-(1)
Thymus	medulla		14.9	9.3	7.0	6.5	5.8
	cortex		8.1	7.1	6.6	7.1	6.0

Table 17 (cont'd.)

Tissue	Cell population	Subunit	Time interval				
			2 hours	1 day	2 days	4 days	8 days
Mesenteric lymph node	cortex		19.1	19.2	20.2	10.9	7.0
	subcapsular sinus		19.6	14.9	12.9	8.4	6.3
	medullary cords		17.6	14.2	12.3	9.5	-(1)
	medullary sinus		17.3	14.8	12.7	9.5	-(1)
Kidney	Glomerular tuft		20.0	15.5(2)	13.7(2)	8.3(2)	-(1)
	Bowman's parietal layer		10.9	9.3(2)	-(1)	6.0(2)	-(1)
	Proximal convoluted		16.3	18.1(2)	-(1)	-(1)	-(1)
	Distal convoluted		26.9	15.7(2)	14.0(2)	7.8(2)	-(1)
Urinary bladder	basal		22.0(2)	-(1)	-(1)	17.9	-(1)
	middle		-(1)	-(1)	17.5(2)	12.8	-(1)
	surface		-(1)	-(1)	8.0(2)	-(1)	-(1)
Ovary	corpus luteum		33.5	*(3)	18.3	10.5	*(3)
	Granulosa		32.7	17.5	14.7	10.3	7.0
	Theca interna		28.6	11.6	14.3	9.3	10.3(2)
	Theca externa		24.5	15.1	17.9	10.3	5.5(2)
Uterus	surface epithelium of endometrium		24.9	13.8	-(1)	7.5	-(1)
	Glandular epithelium		16.7	12.5	13.6	6.5(2)	6.8
Vagina	basal		20.5	21.3	21.8(2)	16.8	----
	spinous		13.6	10.4	21.8	10.2	6.0(2)
	surface		13.0(2)	7.5(2)	6.8(2)	8.2	5.0(2)

Table 17 (cont'd.)

Tissue	Cell population	Subunit	Time interval 2 hours	1 day	2 days	4 days	8 days
Mammary gland	lactiferous duct epith.		20.3	*(3)	7.7(2)	-(1)	-(1)
	mammary duct epith.		21.7	*(3)	13.6	8.6	*(3)

Explanation of symbols:

-(1) : No radioactive nuclei available for counting.

(2) : Less than ten radioactive nuclei available for grain count. See tables on radioactive cell frequency for number of radioactive nuclei counted.

*(3) : Tissue unavailable.

DISCUSSION

The explanation of the different modes of growth in the various cell systems is a matter of speculation. Obviously they represent adaptations which ensures maximal functional efficiency.

When cells double their DNA prior to mitosis, they take up various DNA precursors. If labeled precursors are injected into animals, they are incorporated in dividing cells, which are thus tagged and may be traced in radioautographs (Messier and Leblond, 1960).

Radioactive thymidine was used in this project to survey the sites of cell proliferation and to look for signs of migration in a variety of cell populations of the Galago. The turnover rate is equal to the number of cells entering or leaving a compartment per number of cells in the compartment per unit time (Lipkin _et al_, 1963).

Growth expectations were established by building a linear regression model of weights of Galagos (Table 1), sacrificed at various time intervals after radioactive thymidine injections. The weights of the Galagos were measured over a period of 15 months. This period was divided into two intervals of 7 months and 8 months, starting from the first day of measurement.

The formula of a straight line y=ax+b was used to find the theoretical weight values over the two time intervals, for a Galago that was sacrificed two hours after the injection of labelled thymidine.

For the 7 months interval, the variable (a) was calculated to be 0.6 grams increase in body weight per day. For the 8 months interval, the increase was 0.18 grams per day. The curve plateaus between 250-2500 days. This period was considered the turnover time due to growth only.

The calculated weight values were used instead of the observed in calculating the linear increment in weight per unit time, because the actual weights vary from time to time depending on the food and water intake, water and electrolyte balance, excretion and the environmental conditions.

Cells that were in the S phase when the tracer was available incorporated it into newly synthesized DNA (Bertalanffy, 1964). If the duration of the S phase is known, the generation time can be calculated from the labeling index of the cell population before the labelled cells enter mitosis. The labeling index represents the percentage of cells present at any moment in the S phase. The generation time can be calculated by dividing the duration of the synthesis phase by the labeling index.

In this study, the turnover rate was determined by considering the duration of the S phase to be 4 hours (Bertalanffy and Leblond, 1953, Adrian, 1965).

Cell population turnover rates were categorized as follows:

1) Fast, with a turnover rate of less than five days.
2) Moderate, with a turnover rate between 5-20 days.
3) Slow, with a turnover rate between 20-50 days.
4) Sporadic replacement (or slow) with a turnover rate of more than 50 days. In all these, replacement due to growth was also included.
5) When the turnover rate exceeded 150 days it was considered due to growth alone.
6) No growth by mitosis.

Calculation for turnover time was based on the percentage of labelled cells in tissues of Galagos sacrificed two hours and one day after the injection of labelled thymidine. Theoretically they should be equal because we are considering the duration of the S phase to be 4 hours and that one cell division takes place per day. When they are different, this could be due to migration of radioactive cells from the tissue. In cases where the observed number of radioactive cells was small, random sampling differences could lead to large discrepancies in calculated turnover time. In such cases, counts from all 5 animals should be pooled to derive a better estimate of cell turnover.

In the case of stratified squamous epithelium, the turnover time for the various time intervals after injection

was calculated on the same basis because after stem cell division, one of the daughter cells remains in the basal layer and the other moves to the spinous layer. As such it would remain on a 1:1 basis.

To consider an example, the skin (Table 11) showed a range for the most rapid turnover time of two days in the basal layer, two hours after the injection, to 3.3 days in the spinous layer, one day after the injection, to 4.1 days in the surface layer, four days after the injection; thus, placing the skin in the tissue category with the fast turnover time.

By scanning the tables (4, 6, 8, 11, 12, 14, 16) in the "Results" section and considering the turnover time of the various tissues it is obvious that the neuron population of the central nervous system does not show any growth by mitosis (Table 4). The subependymal layer showed a fast turnover time. Although the calculated turnover time of the choroid plexus fits in the sporadic replacement category, this conclusion is based on one radioactive nucleus within a population of 2568 cells which makes the reliability of this figure questionable.

The lymphoid nuclei around the spinal cord belong to the fast turnover population while the satellite cells of the spinal ganglion show moderate turnover time.

In the endocrine system, the anterior lobe of the pituitary shows a slow turnover time, while in the posterior lobe cell proliferation is due to growth only.

The follicular cells of the thyroid show a turnover of cells that is due to growth only while in the non-follicular population there is sporadic replacement or regeneration.

The pancreas, both its endocrine and exocrine portions, are replaced sporadically or by regeneration.

The adrenal gland shows a moderate turnover rate in the glomerulosa region and a slow rate in the fasiculata and reticularis regions. The turnover rate of the medulla calculated from the radioactive index of the animal that was sacrificed two hours after the injection puts it in the moderate turnover category, while that calculated from the animal that was sacrificed one day after the injection showed it to be due to sporadic replacement. Judging from the rest of the figures at the various time intervals after the injection, it is more reliable to consider it to belong to the sporadic replacement category.

In the digestive system, the tongue epithelium is similar to skin and belongs to the fast turnover system.

The alveolar cells of the sublingual gland may constitute slow turnover system, while those of the submandibular and parotid glands belong to the sporadic replacement category.

The surface epithelium of the fundus of the stomach shows a moderate turnover rate while that of the pylorus a low rate. The cells of the isthmus, small and large intestine revealed a fast turnover rate. The parencymal cells of the liver showed a fast turnover rate, based on the radioactive index of the animal that was sacrificed two hours after the injection, but considering the radioactive indeces at the other time intervals it is more reliable to place it in the moderate category. Also the endothelial cells had a moderate turnover rate.

All the lymphoid organs fit in the fast turnover rate category. The difference in numbers between the two hour interval and the one day interval is due to migration of cells from the organ.

Cardiac and skeletal muscle do not show any growth by mitosis.

The parenchymal cells of the lung show a moderate turnover time, but the bronchial epithelium turnover figure is not sufficiently reliable to make a judgement since only one nucleus per 1,000 was labelled in this population.

The megakaryocytes and the other cells of the bone marrow have a fast turnover rate.

The urinary system showed that the cells of the glomerular tuft and the distal convoluted tubules have a moderate turnover time, while the proximal convoluted tubule and the parietal layer of Bowman's capsule have fast turnover times. The collecting tubules of the medulla have a slow turnover rate, while the medullary ducts and Henle's loop show sporadic replacement due to mechanical pressure or toxic materials that cause the death of the cells. The ureter had a slow turnover rate in the basal layer, but this was based on one radioactive nucleus among 512 cells which might be due to mechanical injury. In the urinary bladder, there is no growth by mitosis except in the basal layer of the portion of the thick epithelium where there is a moderate rate of turnover based on one labeled nucleus per 100 cells which might be due also to mechanical injury.

The basal layer of the urethra revealed a fast turnover time, while the middle layer showed sporadic replacement and the surface layer does not have any growth by mitosis. In the female genital system, the granulosa cells have a fast turnover time and so do the thecas and the stroma of the ovary. The corpus luteum had a slow turnover rate, while the germinal epithelium a moderate turnover rate. The ova of the primary follicles does not reveal any growth by mitosis.

The luminal epithelium of the fallopian tube fits in the category of fast turnover rate population and so does the surface epithelium of the uterine cornu. The stroma of the uterus shows a moderate turnover rate, while the glandular epithelium is fast.

The vagina is similar to the skin and the tongue in having a fast turnover rate. The lactiferous and mammary duct epithelia of the mammary gland have a moderate turnover time.

A comparison can be made of the turnover time of some cell populations of the Galago (Table 18) with those of the rat, mouse and human (Table 19) that are available in the literature. Turnover time of the epithelial cells of the jejunum, ileum and colon of the Galago appears to be closer to that of the mouse rather than the rat or the human.

The duodenal epithelial cells of the Galago show a faster turnover rate than any of the other three species.

Cardiac muscle cells did not have any label in the Galago, in contrast to the rat as was reported by Messier and Leblond (1960), but in agreement with what Edwards and Klein (1961) reported about the mouse, and what Walker and Adrian (1966) reported in old mice.

Messier and Leblond (1960) included the liver of the rat among the expanding cell populations. The Galago's liver, besides growth, shows a moderate rate of cell turnover and as such does not fit in the expanding cell category.

Smart (1961) reported that no migration of labelled cells from the subependymal layer into the surrounding brain seems to occur in adult animals. Our findings are in disagreement with this statement because a pattern of migration was very pronounced over the various time intervals from the subependymal layer towards the substance of the brain.

Table 18

Distribution of the Galago's cell populations as to their turnover rate

Fast T/O 5 days	Moderate T/O 5-20 days	Slow T/O 20-50 days	Sporadic T/O 50-150 days	Growth only more than 150 days	No growth by mitosis
Subependymal	Satellite cells	Pituitary (ant. lobe)	Choroid plexus	Pituitary (post. lobe)	Neurons
lymphoid nuclei	Adrenal (glomerulosa)				(cerebrum)
(spinal cord) Tongue		Adrenal (Fasicul.) (Reticul.)	Thyroid (non-follicular)	Thyroid (follicular)	Cerebellum Spinal cord
Stomach (isthmus)	Stomach (surface epith. fundus)	Stomach (surface epith.)	Pancreas		Cardiac muscle
Duodenum	(pyloric glands)	pylorus	Adrenal (medulla)		Skeletal muscle Smooth muscle
Jejunum Ileum Colon	Liver	Sublingual	submandibular Parotid		
Skin lymphoid organs	Lung	ureter			
Bone marrow Kidney (parietal layer Bowman) (proximal convol.)	kidney (glomerular tuft) (distal convol.) (collecting tubul.)	corpus luteum	Kidney (med. ducts) (Henle's loops)		

Table 18 (cont'd.)

Fast T/O 5 days	Moderate T/O 5-20 days	Slow T/O 20-50 days	Sporadic T/O 50-150 days	Growth only more than 150 days	No growth by mitosis
Urethra					
ovary (Granulosa)	ovary (Germinal epith.)				
(Thecas)					
(Stroma)					
Fallopian tube					
uterine cornu (surface epith)	uterine cornu (Stroma)				
(Glandular epith.)					
Vagina	mammary gland				

Table 19

Turnover time of various cell populations of the mouse, rat and human

Cell population	Species	T/O time days	Reference
Adrenal (zona glomerulosa)	mouse	30-90	Walker & Rennels, 1961
(zona fasiculata)		373	
(zona reticularis)		386	
Lung (Bronchial epith.)	mouse	59	Koburg, 1962
		21	Spencer & Shorter, 1962
ureter (Transitional epith.)	mouse	112	Blenkinsopp, 1969
Tongue (superior surface)	mouse	4	Oehlert & Buchner, 1961
		5	Creamer *et al*, 1961
		6.9	Koburg, 1962
(Inferior surface)		8.4	Koburg, 1962
Stomach surface epithelium (Fundus)	rat	3	Stevens & Leblond, 1953
	mouse	4	Oehlert & Buchner, 1961
	mouse	5	Creamer *et al*, 1961
	human	4-6	McDonald *et al*, 1964
Duodenum epithelial cells	rat	1.5	Leblond & Stevens, 1948
	mouse	2-3	Creamer *et al*, 1961
		2	Oehlert & Bucher, 1961
		1.7-2.2	Fry *et al*, 1962
	human	5-6	MacDonald *et al*, 1964

Table 19 (cont'd.)

Cell population	Species	T/O time	Reference
Jejunum epithelial cells	mouse	3	Leblond & Messier, 1958
		1.1	Quastler & Sherman, 1959
		1.8-2.2	Fry et al, 1962
		1.9	Steel, 1963
Ileum epithelial cells	mouse	1	Creamer et al, 1961
		1.4-1.8	Fry et al, 1962
	rat	1.35	Leblond & Stevens, 1948
Colon epithelial cells	rat	3	Messier, 1960
	mouse	0.7	Lipkin & Quastler, 1962
	human	4-6	Cole & McKalen, 1963

SUMMARY AND CONCLUSIONS

1) The gross anatomy of the adrenals, and the urogenital tract of the female Galago senegalensis was described.

2) Tritium labeled thymidine was injected to five Galagos, which were sacrificed 2 hours, 1, 2, 4 and 8 days after the injection.

3) A radioautographic investigation of 36 different tissues was carried out and the radioactive index and turnover time were determined.

4) The significance of the results were discussed, on the basis of classifying the various tissues into various categories depending on their turnover rate.

5) Turnover rates were categorized into fast, moderate, slow, sporadic replacement, growth only and no growth by mitosis depending on the number of days of turnover.

6) The growth factor is included in the turnover time of the fast, moderate and slow turnover times.

7) Sporadic replacement, or regeneration, apparently is due to replacement of dead cells in certain areas of the tissue, or due to injury, mechanical or otherwise.

8) A comparison was made between the turnover time of some tissues of the Galago with those available in the literature of the rat, mouse and man.

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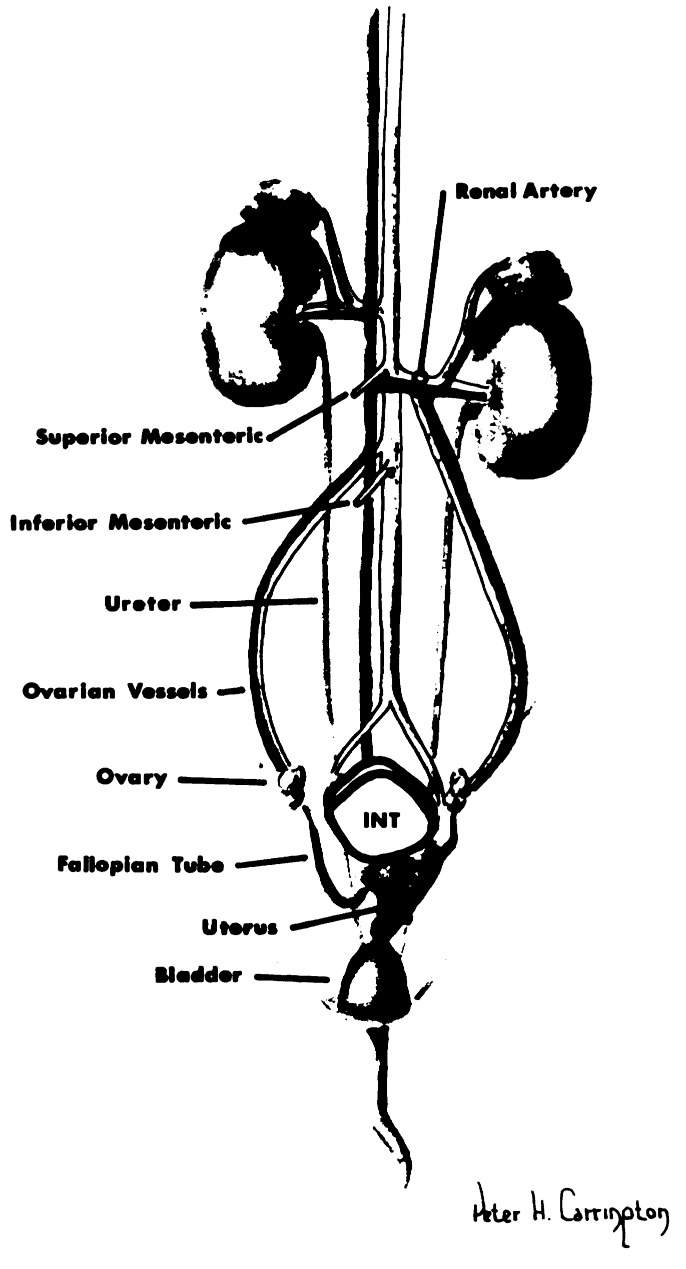
APPENDIX

Figure 1.--Female Galago senegalensis



Peter H. Carrington

Figure 2.--A shematic drawing of the adrenal glands and the urogenital system of the female Galago

Figure 3.--A photograph illustrating the relations of the left adrenal gland and the urogenital organs to the major blood vessels in the area

Figure 4.--A cross section of the adrenal gland of the Galago

Figure 5.--A general picture of the abdominal cavity of the Galago showing the position of the kidneys with respect to the other organs

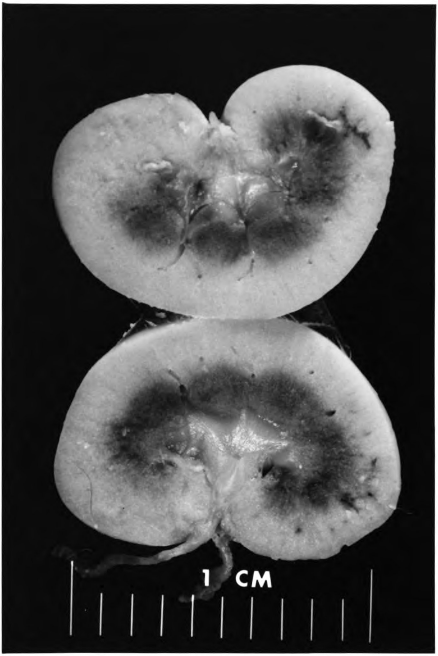


Figure 6.--A cross section of the kidney of the Galago

Figure 7.--A photograph of the urinary bladder and the urethra of the Galago

Figure 8.--A transilluminated photograph of the isolated urinary bladder and urethra of the Galago

Figure 9.--A general picture of the abdominal cavity of the Galago to illustrate the position of the genital organs with respect to the rectum

Figure 10.--A transilluminated photograph of the isolated right and left ovaries of the Galago

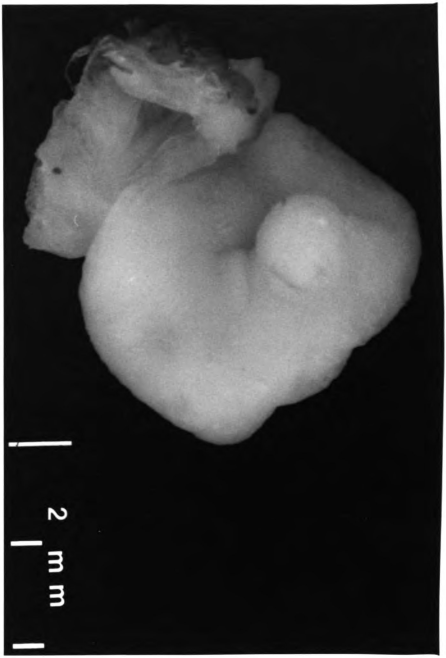


Figure 11.--An enlarged transilluminated photograph of the isolated left ovary of the Galago

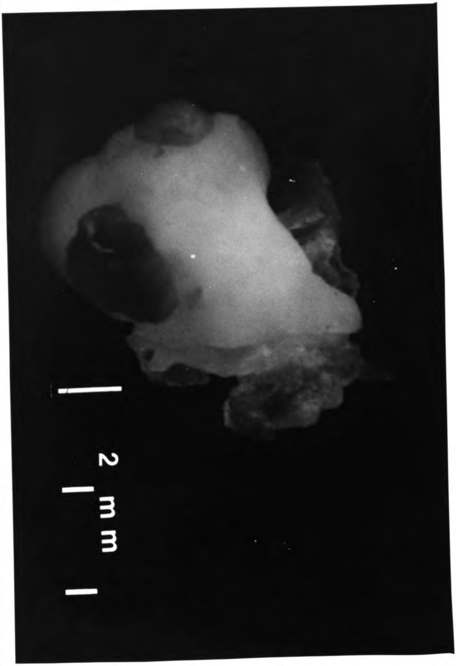


Figure 12.--An enlarged transilluminated photograph of the isolated right ovary showing two corpora lutea bodies bulging as dark structures on the surface

Figure 13.--A photograph of the isolated right and left ovaries. The left ovary shows two corpora lutea bodies

Figure 14.--A transilluminated picture of the isolated genital organs of the female Galago (anterior surface)

Figure 15.--A photograph of the uterus and vagina of the Galago, dissected and held up to show its posterior surface and its relation to the rectum

Figure 16.--A photograph of the ovaries and uterus insitu. The bladder was held down between the pubic bones to illustrate its relation to the uterus

Figure 17.--An overall picture of the dissected vaginal canal *in situ*. The bladder was removed.

Figure 18.--A photograph of the isolated genital organs of the female Galago (posterior surface)

Figure 19.--A photograph of the clitoris and vulva of the female Galago