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Comparative morphology of the adrenal glands of wild and domesticated mice and the effect of captivity on adrenal structure.

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Dissertation

COMPARATIVE MORPHOLOGY OF THE ADRENAL GLANDS
OF WILD AND DOMESTICATED MICE AND THE EFFECT
OF CAPTIVITY ON ADRENAL STRUCTURE

By

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I. INTRODUCTION

Anyone who is acquainted with the familiar scene of wild mice or or rats scampering about either house or field, always on the alert, as well as with the lethargic movements of the seemingly unconcerned laboratory rodent, must have wondered if these behavioral differences were related to more deeply rooted structural differences. The wild rodent is faster moving, more fearful and more nervous than its domesticated laboratory counterpart.

It is quite obvious that the behavioral characteristics of these wild animals are adaptive in that such traits enable them to live in environments in which physical strength, endurance and alertness are at a premium. Wild animals have to work hard and, sometimes, even fight for food, shelter and mates. They have to defend themselves against attack from enemies of all kinds as well as protect themselves from the elements. They must be on the alert and ready to flee whenever it is necessary.

This is in great contrast to the common rodents used in most of our experimental investigations. In the laboratory environment such problems as the above are at a minimum. Food, shelter, temperature, protection from predators, and even the choice of a mate are all problems for the experimenter, not the rodent. It is apparent that the wild environment involves more stress producing situations than are found in the laboratory, and since an animal's ability to withstand such sudden and prolonged stresses is related to adrenal function, it would be only logical to suspect adrenal differences between wild and
domesticated animals.

Previous investigations by Watson (1907a), Donaldson (1926) and later by Richter (1946) and Rogers and Richter (1948) have shown that the adrenal glands are much smaller in the domesticated than in the wild Norway rat. Richter (1946), while concerned with the validity of endocrine research on the domesticated Norway rat, proposed that the domesticated rat may be "nothing more than an artificial creation of the laboratory experimenter, produced by a long period of selective breeding under unnatural conditions." He proposed that in view of the known differences it might be profitable to use wild rats in some of our experimental work.

In order to determine whether such variations in adrenal structure were peculiar to rats, or represented a generalization in nature, it was decided to investigate the differences between the wild and domesticated state in another species. For this purpose a comparison of the adrenal structure of wild and domesticated mice was undertaken. In addition it was decided to determine what effect a change in environment might have on wild mice confined in captivity and on their first generation offspring.
II. REVIEW OF THE LITERATURE

A. HISTIOGENESIS OF THE MOUSE ADRENAL

1. Prenatal development of the mouse adrenal

The earliest adrenal anlage have been observed in the twelve day embryo (Inaba, 1891; Fusari, 1893; Soulie, 1903; Waring, 1935). At this stage Waring describes serial transverse sections showing a group of cells immediately above the peritoneum in the region where the nephric tubules pass toward the Wolffian duct. The cortical anlage is situated in the angle of the mesentery between the aorta and the germinal ridge. Waring (1935) and Inaba (1891) observed that on the thirteenth or fourteenth day of foetal life a definite sympathetic mass was closely applied to the cortical anlage.

By the fourteenth day the cortical anlage is clearly separated from the general mesenchyme by a well marked mesodermal sheath (Soulie, 1903; Waring, 1935). Inaba (1891) says that separation is complete at thirteen days and describes a type of infiltration in which fibers enter the cortical anlage from the sympathetic mass and run deeply into it, ending in small sympathetic cells. From the fourteenth to the sixteenth day of intrauterine life, there seems to be a gradual concentration of the scattered sympathetic cells into a more compact mass in the center of the gland. Immigration of the sympathetic cells continues accompanied by a multiplication of the medullary cells in situ (Inaba, 1891; Waring, 1935). Waring describes a change in the cortical cells during the period from sixteen days to
birth when they assume features characteristic of the future fasciculata. Waring also described an interlocking zone beginning at sixteen days, in which some of the inner cortical cells retain their dark eosinophilic appearance and seem to intermingle with and surround the sympatheo-chromaffin cells. McPhail and Read (1942) could not confirm Waring's observation on the appearance of the interlocking or X-zone in the sixteen day embryo. They first noticed such cells at the day of birth.

Both Waring (1935) and Inaba (1891) observed that at birth the medulla had cortical cells intermixed throughout its substance, with some of the cortical strands extending across the central mass.

2. The mouse adrenal from birth to early maturity

Towards the end of the first week after birth there is a noticeable increase in the proportion of the medulla to the cortex, and the capsule surrounding the gland becomes more fibrous. There is also a gradual increase in the interlocking zone to a width of two or three cells and a disappearance of the cortical strands traversing the medulla (Waring, 1935). Whereas Waring had observed interlocking or X-zone cells in the sixteen day embryo, Masui and Tamura (1926) and Howard (1939) failed to find an X-zone until from five to fifteen days after birth. McPhail and Read (1942) placed the beginning of the X-zone at birth when a few scattered cells appeared. At eighteen days the average male had an interlocking zone eight cells wide while that of the female averaged ten cells (Waring, 1935). Howard-Miller (1927) and Masui and Tamura (1926) detected the beginning of a sex difference in the mouse adrenal at twenty-one to twenty-five days.
Waring (1935) noticed that the individual cells of the fasciculata elongated in a radial direction and at twenty-five days he found a reticular arrangement of the cells at the inner ends of the fascicular columns, where they contacted the interlocking zone. Waring considered this the beginning of the reticular zone of the adult cortex, apart from the interlocking zone which was seen at the same time. McPhail and Read (1942) observed no such zone separating the interlocking zone from the fasciculata. With the exception of Waring, most writers use the terms reticularis, X-zone, interlocking zone and inner cortical zone interchangeably for the part of the cortex seen between the fasciculata and medulla during this early phase of adrenal development.

Waring (1935) described the differentiation of the fascicular cords by the end of the first week. At twenty-one days the glomerulosa conformed to the arched arrangement of cells typical of the adult. Bartels (1941) observed that the glomerulosa comprised one-half of the cortex by the end of the first week, and gradually decreased until at three weeks it consisted of a single row of groups of four to eight cells. Both Inaba (1891) and Waring (1935) noticed a gradual enlargement of the medullary nuclei until at the eighth day they appeared slightly larger than those of the cortex. Inaba (1891) observed that for a month after birth the cortical nuclei gradually decreased in size, while at the same time the medullary nuclei enlarged slightly.

3. Sex differences in the mouse adrenal

Sex differences in the mouse adrenal were first described by Ma-sui and Tamura (1926) and were later studied histologically and experi-
mentally by Howard-Miller (1927) and Deanesly (1928). They have since been the subject of a great number of publications. Deanesly (1928) stated that no other species so far studied has shown a similar histological distinction between male and female adrenal glands; however, Ingle (1942) said that in most, if not all, species of mammals the adrenal cortex of the female is much larger than that of the male. The hamster is an exception in that the adrenals of males are larger than those of non-hibernating females (cf. Jones, 1935).

Most of the sex differences observed in the adrenals of mice involves the X-zone or interlocking zone. This zone is of special interest since it is histologically comparable to a zone in the human adrenal cortex which develops before birth and degenerates and disappears during the first year of life (Elliot and Armour, 1911; Howard-Miller, 1927; Keene and Hsuer, 1929; Deanesly, 1928; Howard, 1930; Waring, 1926; and Hickman and Overman, 1939).

The interlocking zone of the male attains its maximum size on the twenty-first day and remains constant until the thirtieth day. In the female this zone continues to enlarge (Waring, 1926; Bartels, 1941; Gersh and Grollman, 1939; Masui and Tamura, 1926). Deanesly (1928) observed that at three weeks neither the male nor the female adrenal cortex had developed adult characteristics. During the next two weeks, however, she described both as having reached their adult size, and in the male a partially degenerated cortex and a band of fibrous reticular tissue around the medulla was evident. Howard-Miller (1927) noticed that the X-zone of the male ceased to grow at four weeks and disappeared
with a variation of ten days. Both Howard-Miller (1927) and Deanesly (1926) correlated the disappearance of the X-zone with the time of appearance of sexual maturity of the male and agreed with Masui and Tamura (1926) and Preston (1928) that the X-zone disappeared at about thirty-seven to forty days. At this time a connective tissue layer which stains blue with Mallory triple stain appeared around the male medulla. By the sixth week, without exception, degeneration of the inner cortical zone of the male was complete (Bartels, 1941; Waring, 1938; Deanesly, 1928; Masui and Tamura, 1926). This is the final developmental stage and the mature condition of the male.

In the unmated female the X-zone reaches the peak of its development by the fifth to sixth week (Bartels, 1941; Gersh and Grollman, 1939; Masui and Tamura, 1926). Howard-Miller (1927) reported that the structure which is attained by the male before forty days is not attained by the female until from eighty to two hundred days and that at the height of its development in both sexes the X-zone constitutes twelve to fifty-five per cent of the cortex. Bartels (1941) described degeneration of the X-zone of the virgin female beginning in one-half of his specimens at fifty days and in all cases by ninety-three days. Whitehead (1951) placed the time of disappearance of the X-zone of the female from eighty to two hundred days and said that after degeneration the male and female adrenal were indistinguishable. Waring (1938), Whitehead (1933) and Jones (1965) described a connective tissue membrane replacing the X-zone of the female; whereas, Masui and Tamura (1926), Deanesly (1928) and Howard-Miller (1927) did not observe such
a membrane in the female.

Deanesly (1928) reported that degeneration involved only the inner
layer of the X-zone, the outer portion giving rise to the reticular
layer. Howard-Miller (1927) and Waring (1935) recognized the reticu-
laris after degeneration of the X-zone but considered it the inner ends
of the fasciculate columns.

The adrenal of the adult female varies considerably with the re-
productive state of the mouse. It is well known that during pregnancy
degeneration of the inner cortex occurs and proceeds until all the
cells of the X-zone have disappeared (Masui and Tamura, 1926; Waring,
1935; Starkey and Schmidt, 1938; McPhail and Read, 1942). Howard-Miller
(1927) found that when pregnancy occurred in an individual which had
previously lost the X-zone, no further reduction occurred. Following
pregnancy and the ensuing premature degeneration of the X-zone, Masui
and Tamura (1926) and McPhail and Read (1942) observed a regeneration
of this zone. Although Deanesly (1928) noticed a regeneration of the
X-zone of unmated females, she recorded no such secondary X-zone in
the pregnant female. McPhail and Read (1942) found that preventing
lactation by destroying the young at birth would precipitate a regenera-
tion of the X-zone; however, if the young were permitted to nurse no
such regeneration occurred.

Although considerable variation in size occurred in the female
adrenal during various stages of the estrous cycle, Howard-Miller (1927)
and Deanesly (1928) concluded that the specific changes were in no way
correlated with the estrous changes. However, Masui and Tamura (1926)
related the alternation of degeneration and regeneration of the zona reticularis to the cyclic changes of the ovary.

B. CYTOLOGY AND HISTOCHEMISTRY OF THE MOUSE ADRENAL

1. General cell characteristics

In the zona glomerulosa the nuclei are round or slightly spher-ical in shape and closely gathered together, making it difficult to make out the cell membrane. The cells of this zone are smaller than those of the fasciculata (Masui and Tamura, 1936; Waring, 1935), and the cytoplasm is moderately eosinophilic and somewhat basophilic, with few vacuoles (Nicander, 1952). The cells of the transitional zone be-tween the glomerulosa and fasciculata are smaller than in either adja-cent zone and have more flattened nuclei (Miller, 1950; Nicander, 1952).

The cells of the zona fasciculata are larger than those of the glomerulosa. They have approximately the same width throughout the zone but vary in length. The fascicular cells next to the glomerulosa are the shortest and those nearest the center are the longest (Waring, 1935). The fasciculata has larger and paler nuclei than the glomeru-losa. The outer and inner zones of the fasciculata can be distinguished since the latter has fewer and smaller vacuoles and a more eosinophilic cytoplasm (Nicander, 1952).

The zona reticularis consists of dark staining cells with darker and smaller nuclei than the fasciculata (Deanesly, 1928). Some of these vertically flattened reticular cells contain large vacuoles, pigmented walls and pigment granules (Nicander, 1952).

When the X-zone is present in the mouse adrenal it is usually
characterized by strongly eosinophilic, non-vacuolated cytoplasm, and spherical basophilic nuclei (Jones, 1948 and 1955). As has been previously mentioned the cells of the X-zone degenerate soon after the mouse becomes mature. Howard-Miller (1927) describes the cellular changes involved in degeneration. The cell contents appear to be filled with fine globules and groups of three to five cells begin to fuse. Meanwhile the nuclei become very large and contain clumps of densely stained chromatin, or small nuclei may appear with irregular jagged boundaries, and in various stages of fragmentation. She describes this process as continuing until the whole area of the X-zone becomes a mass of large, clear, homogeneous vacoules, and many of the nuclei disappear. If the nuclei remain they are pressed to the side of the cells and are distorted in shape.

Deanally (1928) describes the same type of degeneration as Howard-Miller, but adds that in most glands another type of degeneration occurs in which the cells of the X-zone seem to disappear gradually, beginning either at the outside of the zone or next to the medulla.

The cells of the medulla are clearly distinguished from those of the cortex by the strongly basophilic cytoplasm and nuclei (Waring, 1935). Both Waring (1935) and Imba (1891) state that the medullary nuclei are larger, and the cytoplasm is more vacculated than that of the cortex.

2. Lipids of the mouse adrenal

Meeg et al (1954) is the only source of observations on the lipid characteristics of the adrenals of very young mice. Using Sudan black stain, she described the presence of lipid droplets in all zones of the two day old mouse adrenal; however, the X-zone took a very faint
stain. By the fourth day all zones showed increased sudanophilia, except for the outer fasciculata which was prominently sudanophilic at two days. By nine days stainability declined, particularly in the X-zone and inner fasciculata. By twelve days all zones stained lightly and from fourteen to sixteen days sudanophilia increased in the glomerulosa and outer fasciculata, with the X-zone remaining pale. By twenty days the glomerulosa and outer fasciculata settled down to a moderate and unchanging sudanophilia, but the inner fasciculata became still more intensely fatty during the fifth and sixth weeks.

Whitehead (1933 c) reported that lipids were most abundant in the male and female while the X-zone was present. He noticed that when the X-zone disappeared the amount of cortical lipid decreased. Whitehead (1933 a) described the X-zone as "lipid rich" in contrast to the "pale" X-zone of Moog et al (1954).

Miller (1950) described the lipids of the adult mouse adrenal. The lipid droplets of the glomerulosa were smaller, more uniform in size and more densely colored by Sudan stains than those of the fascicular zone. There was a progressive increase in lipid concentration toward the deeper part of the glomerulosa. In the transitional zone between the glomerulosa and fasciculata Miller observed considerable lipid although the zone as a whole appeared somewhat sudanophobic. This situation resulted from the fact that the nuclei of the transitional zone constituted a major portion of the cell volume.

Nancander (1952) observed that the amount of lipid droplets decreased by degrees toward the inner fasciculata, and adjacent to the reticularis, wherever lipid droplets were seen, they were small and
situated around the nucleus. Both Nicander (1952) and Miller (1950) described the reticularis as a zone with few or no lipid droplets, although the presence of pigmented granules in the reticularis often gave the appearance of sudenophilia. Nicander (1952) agreed with the observation of Moeg (1954) that the X-zone showed a weak and diffuse sudanophilia.

3. Other histochemical observations on the mouse adrenal

According to Nicander (1952) cholesterol and phospholipids were present in each of the lipid rich zones of the mouse adrenal. The phospholipids were not present in the large droplets, but were seen in the small droplets, presumably located in the mitochondria.

Acid phosphatase was found to be abundant in the zona glomerulosa and fasciculata, and to be sparsely distributed in the zona reticularis and the X-zone. Alkaline phosphatase (which is not detectable in small quantities) was not observed in the adrenal of the female mouse, but was noticed in the fasciculata and reticularis of the male (Nicander, 1942).

4. Mitochondria of the mouse adrenal

All three forms of mitochondria — granules, rods and filaments — were seen in the glomerulosa, although the granular form predominated (Miller, 1950). Those cells with the most mitochondria were located just beneath the capsule. Miller noticed that glomerulosa cells which were rich in mitochondria were poor in lipids, and believed that the inner lipid filled cells of this zone derived their lipids from the mitochondrial cells. In the transitional zone the mitochondria were fewer in number than in the glomerulosa.
Miller (1960) observed that the mitochondria of the fasciculata were not as clearly defined as in the glomerulosa since the cytoplasm had an affinity for either acid or basic fuchsin rather than for the counterstains of the mitochondria technique. The fascicular mitochondria were almost entirely granular with an occasional rod-shaped form. Small accumulations of mitochondria were seen adjacent to the nucleus. According to Miller (1960) the mitochondrial stain colored intensely certain large, spherical structures, the size of lipid droplets. These structures were called liposomes by Hoerr (1936) who considered them as lipids "rendered insoluble by chromatin."

Miller (1960) observed that granular mitochondria were conspicuous in the zona reticularis of the mouse, since they were not dispersed by lipids and since they contrasted sharply with the ground cytoplasm which had a pronounced affinity for the counterstains of the mitochondrial techniques.

5. Golgi apparatus of the mouse adrenal

Miller (1960) was successful in impregnating the Golgi apparatus with osmic acid in only a few preparations. He described the Golgi apparatus as a conical mass of strands appearing against the nuclear membrane. A more extensive Golgi apparatus was seen in cells which had many mitochondria than in those with few.

6. RELATIONSHIP BETWEEN ADRENA L MORPHOLOGY AND ADRENA L FUNCTION

If a comparison of the adrenals of different groups of mice, or of adrenal reactions to different experimental situations, is to be meaningful, some attempt should be made to relate the various histological,
cytological and histochemical findings to the functional state of the
gland. That inferences made from this type of data are difficult to
validate is easily seen when such a prominent worker in the field as
George Sayers (1950) states "there is no entirely satisfactory method
for measuring the secretory activity of the adrenal cortex." No matter
how tenuous each proposed index of cortical secretion might be, an
attempt to correlate a number of such indices might contribute to our
understanding of the possible functional state of the adrenal glands
of the groups under study.

1. Gross anatomical status as an index of adrenal function

Many workers have held that, following stress producing proce-
dures, there is an increase in the size of the adrenal cortex (Selye,
1936 and 1937; Emery et al, 1940; Bernstein, 1941; Mulinos et al,
1942; Deane and Shaw, 1947). Tepperman et al (1943) has written an
extensive review of experiments in which adrenal enlargement was asso-
ciated with exposure to stressing stimuli. In such a situation the
adrenal is supposed to be secreting at a rapid rate. However, Tepper-
man warns that unless determinations of water, protein and fat content
are made, we cannot be sure that the increased adrenal weight is in-
dicative of an increase in protoplasm. Ingle (1938) stated that new
protoplasrn is laid down at an early stage following stimulation, since
the increase in weight of the adrenal which follows twelve hours of
muscular activity cannot be accounted for entirely by accumulation of
water. Oakberg (1951) warned that weights of organs cannot be con-
sidered as reliable estimates of physiological activity, since hyper-
trophy of a gland may indicate either hyper- or hypofunction. The
necessity for interpreting gross anatomical data in the light of other
more analytical evidence is clearly seen.

2. Cytological indices of adrenal function

The size of the nucleolus and the RNA system is directly concerned
with secretion (Caspersson, 1950; Lesher, 1951; Miller, 1954). Cas-
person described an increase in nucleolar mass associated with cyto-
plasmic synthesis and Lesher observed a close correlation between
cytoplasmic basophilia and nucleolar size. However, if the nucleoli
are vacuolated the cell is considered to be in a state of decreased
functional activity (Donahue and Parke, 1936).

Miller (1950), in comparing mitochondria in normal and hypertro-
phied adrenals of mice, described a proliferation of mitochondria in
both the glomerulosa and fasciculata. The mitochondria were larger
than normal and so crowded that it was difficult to distinguish indi-
vidual ones. In the glomerulosa and fasciculata of the adrenals of
hypophysectomized mice, fewer and smaller mitochondria were noticed.
From this Miller concluded that numerous mitochondria were character-
istic of actively secreting cells. This conclusion agrees with that
of Deane and Shaw (1947) and Knigge (1954). Deane and McKibbin (1946)
reported a similar increase in the mitochondria of the fasciculata
following deficiency of pantothenic acid or thiamin, and Deane and
Greep (1946) described a reduction in size of mitochondria of the fasci-
culata following hypophysectomy. Since nutritional deficiencies are
characterized by an increase in the activity of the adrenal cortex and
hypophysectomy by a decrease, the above experiments are considered as
further evidence of the relationship of mitochondria to secretion.

The form of the Golgi network is considered to be related to the degree of secretory activity of the adrenal cortical cells, since it is compact during inactivity and diffuse and ramifying during marked activity (Bourne, 1934; Greep and Deane, 1949 b). This was further supported by the fact that after hypophysectomy degenerative changes were seen in the Golgi apparatus, whereas, following adrenocorticotropin hormone injection the Golgi apparatus hypertrophied and extended around the nucleus (Reese and Moon, 1938; Miller and Riddle, 1939). A somewhat conflicting point of view was expressed by Knigge (1954) who reported that following acute starvation in the hamster, the Golgi apparatus became granular and dispersed in the glomerulosa, and vesicular and reduced in the fasciculata. Since hypertrophied mitochondria were seen in both the glomerulosa and fasciculata concurrent with the above mentioned contrasting forms of the Golgi network, it is difficult to interpret any one form of the Golgi apparatus with a state of functional activity.

3. Histochemical indices of adrenal function

Many attempts have been made to locate some of the complex chemical compounds of the adrenal cortex in tissue sections. Harrow (1950) included triglycerides, phosphatides, free fatty acids, cholesterol, cholesterol esters, and steroids among the cortical lipids. It is the view of many histochemists that a knowledge of the distribution of these substances will help us understand the functional nature of the cortex.
Histochemical techniques have a distinct advantage over chemical analyses in their ability to locate chemical substances in histological sections.

One of the most common means of localizing lipids in the adrenal cortex involves the use of the Sudan dyes which stain lipids by means of their oil solubility. Kaufmann and Lehmann (1928) observed that the Sudan dyes stained free fatty acids, triglycerides of fatty acids, cholesterol esters, and free and combined lecithin. Among the Sudan dyes, Sudan IV, Sudan Orange, and Oil Red O color lipid red (Lison, 1956), whereas Sudan Black B, first introduced into biological technique by Lison and Dagnelie (1935) and recently modified by Baker (1944), produces an intense blue-black coloration. Gatenby and Moussa (1949) observed that the Sudan Black B reaction was more energetic than that of the others.

Most workers have interpreted a decrease in sudanophilia with a state of increased activity of the adrenal cortex (Miller and Riddle, 1939; Doane and Dalton, 1941; Selye and Stone, 1950; Miller, 1950; Baker, 1952; Mcg., 1954). The decrease in sudanophilia accompanying increased activity was associated with the presence of fine lipid droplets in the cortex, whereas the presence of coarse lipid droplets during inactivity produced an increased sudanophilic reaction (Dalton et al., 1944; Sarason, 1945; Selye, 1957). Since mitochondria and lipids were inversely related to each other in the actively secreting state, the former abundant and latter depleted. Miller (1950) suggested that mitochondria were intimately involved in the formation of lipid and dis-
appeared as visible entities in the course of this formation.

Using osmium tetroxide as a lipid indicator, Flexner and Groll-
man (1939) interpreted the decrease in lipids of the cells of the glo-
merulosa following administration of an excess of adrenal cortical ex-
tact as a decrease in functional activity. If, however, one accepts
the functional autonomy concept of Deane and Greep (1946) regarding
glomerular function, this response may be independent of an opposite
response in the fasciculata.

A further understanding of the relationship between adrenal func-
tion and structure can be obtained if one studies the functional and
structural changes which occur during adrenocortical regeneration. Kom-
rad and Wyman (1950) reported that adrenal transplants were capable of
providing resistance to histamine and cold stress as early as the seventh
to tenth day of regeneration. In a study of the cytological changes dur-
ing adrenal regeneration, Brenner, Patt and Wyman (1953) observed that
the cortical cells were deficient of lipids during the period from seven
to ten days, whereas both before and after this period the cortical
cells were considerably more sudanophilic. This is a confirmation of
the previously mentioned conclusion that a decrease in sudanophilia is
characteristic of an increase in functional activity of the adrenal
cortex. However, it must be kept in mind that an adrenal gland might
be able to secrete at such a rate that it can meet the needs of the
organism and still store some secretions.

Several advocates of the various "ketosteroid stains", which are
employed as indicators of cortical activity, object to the sole use of
Sudan dyes for this purpose, because of their lack of specificity and
failure of complete agreement with the results of these new stains
(Greep and Deane, 1947 and 1949 b; Dempsey and Bassett, 1943; Dempsey
and Wislocki, 1946; Dempsey, 1948; Bennett, 1940; Popjak, 1949). They
prefer to rely upon a "battery of tests" which are supposed to localize
steroids which possess active carbonyl groups. According to Dempsey
(1948) no substance, other than those which belong to the ketosteroid
group, is presently known which exhibits all five of the following
reactions: 1) acetone solubility, 2) reactivity with carbonyl rea-
gents (phenylhydrazine, Feulgen's leucofuchsin), 3) autofluorescence,
4) birefringence, and 5) reactivity with concentrated sulfuric acid,
as in the Liebermann-Burchard reaction. A modification of the phenyl-
hydrazine reaction known as the Ashbel-Seligman reaction was added to
this battery in 1949 and was reported to be highly specific for ketos-
steroids (Ashbel and Seligman, 1949). Only the adrenal, testis, ovary
and placenta give all of these reactions.

Recently, several independent workers have shown experimentally
that the above reactions are neither singly, nor collectively tests for
ketosteroids. Gomori (1942 and 1952), using fresh adrenals and these
fixed for six hours in formalin, showed that neither preparation would
react visibly with Schiff's reagent or with phenylhydrazine for several
hours. Both techniques prescribe oxidation of the sections before
applying the reagent. After oxidation the acetal linkage breaks and
both reactions become positive. Gomori stated that only a ketone test
which is directly positive without previous oxidative treatment of the
tissues can be accepted as evidence for the presence of ketosteroids.
Claesson and Hillarp (1947) agreed with Gomori's objection to the phenylhydrazine test and added that birefringent substances were not identical with the phenylhydrazones-forming substances. The former could be mobilized from the cells by means of gonadotropins without affecting the phenylhydrazine reaction. Claesson and Hillarp also showed that the fluorescent response did not appear until after the steroid had been treated with sulfuric acid. They concluded that fluorescence originated from vitamin A rather than from ketosteroids, since this response was labile to ultra violet light.

With these objections to the specificity of the "ketosteroid stains" in mind, one can easily understand how Sayers (1950) could say "the battery of specialized staining techniques which has been applied to the adrenal has contributed little more to our knowledge of the chemistry and physiology of the adrenal cortex than has the simple Sudan method."

Furthermore, Feldman (1950), besides agreeing with Gomori (1943) and Claesson and Hillarp (1947) on certain objections to the "ketosteroid tests," has revealed an advantage to the Sudan technique. Whereas the "battery of tests" would not stain fresh adrenal tissue, the Sudan method gave a dark green color to the lipid droplets in fresh adrenals. If the sections were previously treated with acetone the droplets would not become colored.

Thus, it appears that the non-specific Sudan stains are now in the same rank as the similarly non-specific, but more complex, "battery tests." The most that can be said for any of these tests is that they are indicators of relative secretory activity and not of actual hormone content. If Vogt (1947) and Hemphill and Reiss (1947) were correct in
concluding that hormones were released from the adrenal gland almost immediately after they were secreted, and Pincus (1950) was accurate when he demonstrated that adrenal cortical response to ACTH can occur prior to a demonstrable change in morphology, then both biological assay and histochemical methods must be employed with caution.

4. Chemical indices of adrenal function

Cholesterol and ascorbic acid determinations have been used to indicate the relative degree of activity of the adrenal cortex. In general there is a close parallelism between the concentrations of cholesterol, ascorbic acid and lipids in the adrenal (Sayers, 1950). A decrease in the amount of any of these substances is interpreted as indicative of an increase in adrenal activity. However, caution should be used in the interpretation of these tests, since each of these substances varies in its distribution under different circumstances. Following hypophysectomy the concentration of cholesterol in the adrenal remains fixed, or increases (Sayers et al, 1945), whereas the concentration of ascorbic acid decreases (Tyslowitz, 1943). In the rat, immediately after administration of ACTH, or application of stress, the adrenal is more rapidly depleted of ascorbic acid than of cholesterol. During the recovery phase, the pre-stress level of ascorbic acid is attained much more quickly than that of cholesterol (Sayers et al, 1948).

Furthermore, Brown et al (1957) has revealed a discrepancy in reports of lipid content as estimated by stains and by chemical analysis.
Most histochemical methods localize lipid in the cortex only. The use of chemical methods show lipids in the medulla as well. More phospholipids and total fatty acids are found in the cortex and more cholesterol in the medulla.

D. THE ADRENAL GLAND AND STRESS

Nearly three-quarters of a century ago a great French physiologist, Claude Bernard (1887), pointed out, in particular reference to animals, that the multicellular organism actually exists in two environments, the external one of the physical world and the internal one of the fluid medium that surrounds and bathes its cells and tissues. He wrote that "constancy of the internal environment is the condition of a free and independent life." In order for an organism to carry on an existence not immediately governed by conditions in the external world it needs mechanisms which can maintain such constancy. The role of these mechanisms, specially that of the sympathetic-adrenal system, in enabling the organism to live with relative freedom from a constantly changing environment has been clearly presented by Cannon (1939). In the years which followed, the pituitary-adrenocortical system was recognized as playing an even more important role in homeostasis than the sympathetic-adrenal system. The adrenal cortex has been revealed as an organ which endows the organism with the ability to resist not a few, but all types of stress (Sayers, 1950).

1. The "general adaptation syndrome"

A unified theory of stress, "the general adaptation syndrome," was
proposed by Selye (1937 and 1946) and describes a three-stage reaction. The first or "alarm reaction" is the sum of all non-specific systemic phenomena elicited by a sudden exposure to stimuli to which the organism is not adapted (Selye, 1946). The transition between a condition of no alarm response and a fully developed "alarm response" is imperceptible (Selye, 1950). During this phase the adrenal cortex shows marked hyperplasia, with a rapid loss of lipoid granules during the first twenty-four hours, followed by a gradual reappearance of these granules (Selye, 1937; Dosne and Dalton, 1941; Popjak, 1944). The adrenal medulla loses its chromaffin granules and vacuoles appear in the periphery of its cells. If the alarm reaction is very severe the medullary cells may even undergo necrosis (Selye, 1937). The "alarm phase" is also characterized by other systemic responses such as involution of the thymus and lymphatic tissue, lymphopenia, increase in circulating leukocytes and rise in blood sugar (Selye, 1946). During the "alarm reaction" or "shock phase" defense mechanisms are set up, stimulated by cortical and medullary secretions, which bring about the next phase of the syndrome.

During the second phase or "stage of resistance" most of the morphologic and biochemical changes of the "alarm reaction" disappear and the organism is characterized by an increased resistance to that particular agent and a decreased resistance to other types of stress (Selye, 1946). At this time the adrenal is able to meet its secretory needs as well as store some of its secretions. Thus the adrenal cortex is characterized by gross enlargement and an increase in lipids (Selye, 1937; Dosne and Dalton, 1941; Popjak, 1944; Deane and McKibbin, 1946; Deane and Shaw,
1947). Animals in the resistant stage show no signs of medullary damage, and even the chromaffin granules reappear after a certain time (Selye, 1937). The course of "resistance" or adaptation is influenced by the condition of the animal at the time the alarm stimulus is given. Hunger and cold aggravate the effects of the alarming stimuli. Old animals react more readily than do young ones, probably because much of their adaptation energy has already been used to meet the incidental demands of life.

If the organism endures a prolonged or severe stress, one in which adaptation had been developed but could no longer be maintained, it is said to enter the "stage of exhaustion" (Selye, 1937, 1946). In this stage cortical hormone is again needed in excessive amounts and as a result there is a decrease in cortical lipids (Deane and Dalton, 1941; Popjak, 1944; Deane and McKibbin, 1946; Deane and Shaw, 1947). Following continued injections of ACTH and subsequent production of adrenal exhaustion, an accumulation of lipids was observed by Baker (1952). However, Baker was careful to state that the observed lipids were not the type related to secretion. It may be that he was observing lipid droplets characteristic of a degenerative state which follows exhaustion, the fatty metaplasia of the cortex described by Selye and Stone (1950). Sayers (1950) is unconvinced that "exhaustion" or hypercorticism can ever proceed from either cortical or pituitary hyperactivity.

2. Classification of adrenal responses to stress

Sayers (1949, 1950) has categorized the numerous responses of the adrenal cortex to stress into nine definite groups. Five of these
classifications involve responses arising from a reaction of the normal organism to changes in either internal or external environment. The other four groups involve pathological changes in the organism and are not related to this discussion. Interpretation of experimentally produced or naturally occurring stresses depends upon a) the intensity of the stimulation; b) the duration of the stimulation; and c) the time, during or after stimulation, at which the adrenals are analyzed.

The following classification of Sayers (1950) is intended as a guide to interpret functional activity of the adrenal cortex on the basis of change in size, sudanophilic substance, cholesterol, and ascorbic acid content of the gland:

I. Sudden temporary period of stress — in which the stimulus acts for a short period and is followed by a sudden, temporary increased demand for cortical hormone. The sudanophilic substance, cholesterol, and ascorbic acid undergo rapid depletion without any significant increase in size of the gland. As the animal recovers there is a return to normal.

II. Very gradual change in internal or external environment — in which a very gradual increase in the intensity of a stimulus over a prolonged period of time evokes no significant change in histochemical analysis, but does result in a gradual increase in size of the gland as new secretory units develop to meet the increased need.

III. Intense continuous stress ending in death — in which a very intense, continuous stress results in a rapid decline in sudanophilic substances, cholesterol and ascorbic acid, as well as marked hypertrophy and hyperplasia of the cortex. As previously mentioned Sayers is unconvinced that "exhaustion" can ever proceed from either cortical or pituitary hyperactivity.

IV. Recovery from period of severe stress — in which the organism, once the stressing stimulus is withdrawn, finds itself with an enlarged adrenal which is depleted of sudanophilic substance, cholesterol and ascorbic acid.
acid. Following sudden withdrawal of the stimulus there is an accumulation of precursor material so that there is a normal, and later, above normal concentration of these substances.

V. Adaptation to stress -- in which the adrenal responds to a continuous stimulus, first, by the characteristic depletion of histochemical contents, and later, as the organism's internal environment becomes adapted to the change, there is a reduction in the requirement for the cortical hormone and the gland returns to a pre-stress state of functional activity.

VI. ECOLOGICAL FACTORS AND ADRENAL STRUCTURE

That many of the previously discussed forms of stress are at work in the natural environment in which an animal lives, is an easily accepted fact. Furthermore, nature doesn't restrict itself to the isolated stresses which are so widely discussed in experimental work, but provides a complex of stresses which are constantly changing. Some stresses are probably maintained at such a level that an animal becomes adapted to them for a period of time, only to have the environment change and necessitate a re-adaptation (chronic hunger, seasonal temperature changes, crowding). Other stresses never exist long enough for the animal to become adapted to them (predators, fighting, sudden temperature changes, short periods of acute hunger).

Just how the interaction of such stresses affect the adrenal is difficult to ascertain, since we have only a general knowledge of the history of a wild animal prior to capture. A number of experiments have been conducted in which an attempt was made to simulate environmental stresses. A knowledge of how these individual factors affect the
adrenal might help in an interpretation of the possible effects of the wild environment on the adrenal gland.

1. Temperature changes

That the adrenal gland responds to temperature changes had been attested by numerous workers. Following exposure to cold the adrenal gland enlarges and the concentration of precursor materials decreases (Bernstein, 1941; Rabinovici, 1951; Dosne and Dalton, 1941; Levin, 1945). Using the reduction of osmic acid as an indicator of adrenal activity, Flexner and Grollman (1939) came to the opposite conclusion. They found that after one to four hours exposure to cold there was an increase in reducing substances in the cortex; however, after one day exposure a loss in reducing substances was observed. Emery et al (1940) studied the effects of prolonged exposure to cold on the weights of various organs. Following exposures of sixteen hours per day, for various periods of time from fifteen to sixty days, they observed an increase in weight of the adrenals, thyroid, liver, kidney, heart, lungs and intestines, and a decrease in weight of the pituitary, ovaries, uterus, testes, epididymis, spleen and thymus. The adrenals were eight per cent larger in the female and fifteen per cent larger in the male.

Bernstein (1941) compared seasonal variations in the degree of sudanophilia in the adrenals of laboratory rats which were kept in an animal room with a temperature range from 55-75 degrees Fahrenheit). He observed a twenty-five per cent reduction in sudanophilia during the winter as compared with a forty per cent reduction in sudanophilia when the rats were subjected to experimental cold conditions. Bernstein
concluded that the seasonal variations were related to variations in
physical activity, since rats were nocturnal and exhibited more activ-
ity in winter. Rogers and Richter (1948) found that the adrenals of
wild, male Norway rats were fifty percent heavier in winter than in
summer. The seasonal variation in adrenal size in the female was too
slight to be significant, thus indicating a greater sensitivity to tem-
perature change in the male.

Levin (1945) has shown that the adrenals of rats can become adapted
to cold. A reduction in cholesterol was observed following sixteen to
twenty-two hours exposure to cold, whereas after exposure for seventy-
two hours the concentration of cholesterol returned to normal. That
there are species differences in adrenal response to cold was shown by
Deane and Lyman (1954). Whereas, in the rat, the cortex hypertrophied
and lipids decreased, in the hamster, the cortex narrowed and the li-
pids increased.

In experiments in which animals are exposed to heat, one gets the
reverse effects of exposure to cold. The adrenal cortex increases its
sudanophilia (Bernstein, 1941). Geiger (1959) studied the adrenals of
mice subjected to heat and found that whereas most of the cortex widen-
ed, degeneration of the X-zone was accelerated. This effect was more
clearly evidenced in the female than in the male.

2. Nutrition and starvation

There is considerable difference of opinion as to the effect of
starvation on the adrenal gland. In comparing the adrenal gland before
and after fasting, Anderson (1935) and Oleson and Bloor (1941) found
no change in adrenal weight; O'Kuneff (1923) observed no change in
adrenal lipids; and Mouriquand and Lenlier (1927) noticed no change in adrenal cholesterol. In contrast, Sarason (1943) and Mulinos et al. (1942) observed adrenal hypertrophy following acute hunger; however, the former found a decrease in lipid content, and the latter observed an increase in ascorbic acid. Following chronic starvation, Mulinos et al. (1942) observed adrenal atrophy accompanied by a decrease in ascorbic acid; Babinovici (1931) noticed a drastic reduction in "ketosteroids"; and MacLachlan et al. (1941) described a rise in total cholesterol, and a rise followed by a fall in neutral fats. Marrion (1923) found that adrenal hypertrophy following underfeeding of pigeons involved the medulla more than the cortex. After fasting Whitehead (1942) reported an increase in lipids in the inner half of the fasciculata and part of the reticularis as well as a decrease in lipids in the outer fasciculata. In a similar study of fasting in rabbits, Whitehead observed no obvious changes.

In contrast to the opposing results reported above considerably more agreement was seen in several studies concerning the effect of vitamin deficiencies on the adrenal gland. Sure and Theis (1939) and Deane and Shaw (1947) described a reduction in precursor substances of adrenal following thiamin deficiency. The latter group failed to find a similar reduction following riboflavin and pyridoxine deficiencies. Deane and McKibbin (1946) found hypertrophy and exhaustion of the cortex as a result of pantethenic acid deficiency.

Howard (1947) found that a diet high in protein and fat, as compared to one high in carbohydrates, produced a reduction in adrenal weight as well as a decrease in the X-zone of mice. In experiments on
rats Sarason (1948) reported hypertrophy and a decrease in lipid content of the adrenal following a high protein diet, and Blumenfeld (1954) noticed atrophy of both cortex and medulla after a fat-free diet.

Variations in the zona glomerulosa were seen in several experiments in which the sodium and potassium ratios in the diet were altered. Following a potassium-free diet atrophy of the glomerulosa was observed (Nichols, 1948 b; Deane et al., 1948; Deane, 1960). The same workers also reported that after a sodium-free diet the glomerulosa enlarged and its lipid content was reduced. A diet lacking both sodium and potassium produces a moderate enlargement of the glomerulosa but no depletion of precursor substances (Deane, 1950).

3. Fighting and increased activity

Clarke (1953) found that the adrenal glands and spleen increased in weight, and the thymus decreased when strange voles were put in cages with voles chosen for their fighting ability. Various investigators have shown that the adrenal gland responds to sudden increases in muscular exercise by enlargement (Donaldson, 1933; Anderson, 1935; Ingle, 1935) and reduction of cholesterol and lipid content in parts of the cortex (Knouff et al., 1941).

4. Crowding

Christian (1955) determined the effect of crowding on the adrenals of albino mice and a colony of laboratory-raised wild house mice. Crowding resulted in an increase in adrenal weight which was proportional to the population density in each cage. The adrenals of wild house mice attained peak weights at lower population sizes than those of albino mice. The maximum weights for the wild mice was twice that
of the albino mice. In another experiment Christian and Davis (1955) demonstrated a reduction in adrenal weight coincident with a reduction in population size. There was a decline of twenty-six per cent in adrenal weight soon after natural populations of wild Norway rats were reduced in size by periodic trappings. The population size was held down by monthly trappings for a period of seven months, at which time a decline in adrenal weight of twenty-one per cent was found. The response of the male adrenal was ten per cent greater than that of the female. This was consistent with the greater enlargement of the adrenal cortex seen in male mice in increasing populations (Christian, 1955).

Bullock (1952) found that the cortex and medulla increased thirty and eighty per cent, respectively, when adult male mice were subjected to crowded conditions. In this experiment, however, there was considerable fighting, since five female mice were suspended on a grid over each cage of twenty male mice.

F. DIFFERENCES BETWEEN WILD AND DOMESTICATED ANIMALS

1. Structural differences between wild and domesticated rats

As was previously mentioned the adrenal gland of the wild Norway rat is considerably larger than that of the domesticated Norway rat. The extent of these differences is shown in Figure I.

Whereas Rogers and Richter (1948) found that both wild and domesticated rats had similar medullary weights, Donaldson (1928) observed that the medulla was heavier in wild rats. Both agree, however, that the greatest difference was in cortical weight. The fasciculata and reticularis were more extensive in wild rats than in domesticated rats (Rogers and Richter, 1948).
Figure 1. Comparative adrenal weights in wild and albino Norway rats (Hatai, 1914)
Many structural differences between wild and domesticated rats, other than those involving the adrenal gland, have been observed. These are listed in Table I.

2. Functional differences between wild and domesticated rats

The adrenals of the wild Norway rat are greater not only in size but in their content of cholesterol and ascorbic acid (Nichols, 1950) and of the various lipids that stain with Sudan dyes (Woods, 1955; Mossier, 1952). Sudan IV stained sections of the adrenals of domesticated rats show only a sparse deposition of lipid in the fasciculata and even smaller amounts in the glomerulosa and reticularis, as compared with the wild rat.

These two strains react very differently to many stress producing situations. According to Woods (1955), more adrenocorticotropic hormone is necessary to produce a reduction in ascorbic acid in the wild rat than in the albino rat. Woods also found that wild rats could endure noise and fighting without a decrease in ascorbic acid or stainable lipids. Woods (1954) subjected both strains of rats to low temperature conditions for ten days. At the end of this period the adrenal characteristics were the same for both the control and the experimental groups of wild rats. The adrenals of the domesticated rats in the experimental group were hypertrophied following prolonged cold stress.

Wild rats cannot be kept alive after adrenalectomy by use of salt therapy, regardless of the amount (Richter et al., 1950) and do not survive with any consistency even when treated with cortisone or desoxy­corticosterone acetate, or both (Covian, 1949). When both wild and
<table>
<thead>
<tr>
<th>Structural Differences Between Wild and Domesticated Rats</th>
<th>Source:</th>
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<tr>
<td>The wild Norway rat is characterized by: (as compared to the albino rat)</td>
<td></td>
</tr>
<tr>
<td>Larger adrenal glands</td>
<td>Watson, 1907a; Hatai, 1914; Donaldson, 1928; Rogers and Richter, 1948</td>
</tr>
<tr>
<td>More extensive fasciculata and reticularis</td>
<td>Rogers and Richter, 1948</td>
</tr>
<tr>
<td>Smaller pituitary gland</td>
<td>Donaldson and King, 1929; Hatai, 1914; Richter, 1952</td>
</tr>
<tr>
<td>Lighter gonads (when young)</td>
<td>Hatai, 1914; Richter, 1949</td>
</tr>
<tr>
<td>Heavier gonads (when older)</td>
<td></td>
</tr>
<tr>
<td>More and larger fungiform papillae</td>
<td>Fish and Richter, 1946</td>
</tr>
<tr>
<td>Smaller thymus</td>
<td>Richter and Uhlenhuth, 1954</td>
</tr>
<tr>
<td>Fewer Peyer's patches</td>
<td>Richter and Hall, 1947</td>
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<tr>
<td>Larger thyroid gland</td>
<td>Richter, 1949</td>
</tr>
<tr>
<td>Smaller preputial glands, liver, spleen, brain, heart, kidneys, pancreas</td>
<td>Hall, 1948</td>
</tr>
<tr>
<td>Lighter total body weight</td>
<td>Richter, 1949</td>
</tr>
</tbody>
</table>
domesticated rats were given concentrations of salt ranging from two to thirty-five per cent, for a period of 70-80 days, strikingly different adrenal responses were noticed. Even at the lowest concentrations most of the domesticated rats lost all lipids from the glomerulosa; whereas the wild rats did not lose lipid from this layer even when fed the highest concentrations (Richter, 1952).

Following twenty-four hours of starvation or irradiation with 400r the lipid content of the adrenals of domesticated rats was reduced, whereas no change occurred in the adrenal lipids of the wild rat (Richter, 1952). In an attempt to explain these differences Richter postulated that "the wild rat's adrenals can produce hormones as fast as they are being depleted, whereas the domesticated rat's adrenals utilize them faster then they can be produced."

An exception to the previously mentioned failures to evoke an adrenal response in wild rats was reported by Nichols (1948). He used wild rats which had suffered fractures when caught by their legs in spring traps. These rats showed a cholesterol content lower than the albino rat. When wild rats were shot and the adrenals fixed within seconds, the cholesterol content was observed to be twice that of the albino rat (Nichols, 1950).

Wild and domesticated rats exhibit a number of differences which involve reproductive capacities. Domesticated rats mature earlier than do wild rats, as indicated by an earlier opening of the vagina and by reproduction at an earlier age. They mate freely at any time of the year, whereas wild rats mate only during the spring and fall (Richter, 1949, 1952). Many wild rats apparently never become pregnant, since
Davis and Emlen (1948) failed to find placental scars in a large number of captured wild females. In the domesticated rat gross bodily activity has been shown to be dependent on gonadal secretion, since after gonadectomy the rats became very inactive (Wang, 1923). However, in the wild rat gonadectomy has no detectable effect on the level of running activity (Richter and Uhlenhuth, 1954). That this difference was related to the adrenal differences between the two groups was shown by experiments in which domesticated rats were given cortisone immediately after gonadectomy. They remained active after gonadectomy just as the wild rats did without treatment. Wood (1954) found that hypophysectomy, which makes a domesticated rat almost totally inactive, has a much less depressive effect on some wild rats; whereas others, especially very old and heavy rats, do not survive the operation. Richter (1954) postulated that "in the domesticated rat the hypophysis had become enlarged in an effort to correct for the failing secretion of the adrenal glands. Thus we may see here a pattern of less active adrenals, more active gonads, and a more active hypophysis."

A number of other differences between wild and domesticated rats has been reported. According to Richter (1954) wild rats have a higher metabolic rate as evidenced by a higher food and water intake. Wild rats are more suspicious of their food and more difficult to poison than are domesticated rats (Richter, 1953). Wild rats can be induced to fight easily and kill other rats readily, whereas domesticated rats don't fight even if prodded by electric shocks (Richter, 1954). Fasting increased the amount of physical activity in both wild and domesticated rats; however, in the former the increase was four times that seen in the latter.
This difference was seen to be of value in increasing the chances for a hungry animal to find food (Richter and Rice, 1954). Griffiths (1944) found the wild Norway rat to be free of audiogenic seizures. Audiogenic fits can be produced in both wild and domesticated rats if they are fed a magnesium deficient diet. However, albino rats die from the fits whereas the wild rats do not (Griffiths, 1947).

3. **Structural and functional differences in other wild and domesticated animals**

Apart from the previously mentioned comparisons in rats, very little is known concerning the structural and functional differences between wild and domesticated animals. The dog appears to be an animal which could lend itself very readily to such a comparison, since it has existed in both the wild and domesticated state for eighty centuries. The rat has been domesticated for less than a century.

Scott (1954) reviews some of the most obvious results of domestication on the dog. The most obvious effect is the increased variability of the domesticated dog as compared to the wild wolves. Most breeds of dogs are less aggressive than wolves, a condition which might indicate an adrenal difference similar to that seen in the less aggressive domesticated rats. Furthermore, dogs reach sexual maturity at an earlier age and exhibit greater fertility than wolves.

Richter (1962) refers to a study of the rabbit *Oryctolagus cuniculus* by Nachtsheim (1949) as another possible source of information on the problem of domestication. This rabbit is widely used for food and for fur and its ancestors have survived and are still living in the wild state in Europe. Richter states that in many ways the Norway rat and the rabbit have undergone the same changes during the process of becoming
domesticated.

In a study of wild and domestic fowl, Quiring (1951) found that the wild fowl had a higher ratio of both adrenal and thyroid tissue to body weight than the domestic fowl. In a similar comparison of adrenal weight to body weight among Ungulates, Quiring (1951) discovered that the elephant, rhinoceros, zebra and mare had the highest ratio. He postulated that the relatively great size or fast speed of these animals might make demands upon the adrenal gland in connection with setting these great bodies into rapid movement and for the maintenance of muscle, heart and blood vessel tonus.

C. THE EFFECT OF CONFINEMENT, INBREEDING, AND CROSSBREEDING ON WILD ANIMALS

1. Confinement

Watson (1907 b), Rogers and Richter (1948), and Christian and Ratcliffe (1952) found that a reduction occurred in adrenal size in wild animals following confinement in captivity for various lengths of time. Watson reported a reduction in adrenal weight of twenty-eight per cent in rats confined for ten weeks. Watson did not record the sexes, and consequently, since the weight of the adrenals showed nearly fifty-four per cent difference according to sex, the reduction of twenty-eight per cent cannot be accepted without reservation. Although a reduction in adrenal size occurred when wild rats were confined in captivity, Rogers and Richter conceded that the results were not consistent. Furthermore, the fourteen carnivores studied by Christian and Ratcliffe were not randomly selected, but were chosen because they exhibited gross symptoms of
shock. In a study of the early changes in adrenal structure following captivity, Nichols (1950) found an increase in the adrenal-body weight ratio during the first twenty-four hours of captivity. This was followed by a gradual reduction during the remainder of the first ten weeks of confinement, at which time the adrenal-body weight ratio was about that of the wild rat shot while still in its natural environment. These changes are shown in Figure 2.

Thus, if as is the usual practise, animals which have been in traps for several hours are used as an example of the wild state, a reduction in adrenal size following several weeks confinement might in actuality be a return to normal. However, the traps Nichols (1950) used were of the type which held the rats captive by crushing the appendages, a more severe stress than confinement in wire guaze or metal box traps.

Animals which are confined in captivity often exhibit changes in organs other than the adrenal glands. Quay (1958) found that longer periods of captivity were correlated with a slight increase in volume of the anterior hypophysis and pars intermedia in both sexes of deer mice. Confinement in captivity resulted in an increase in thyroid size, underdevelopment of muscles, and a diminution of brain weight in females (Klatt, 1932), a reduction in mating and reproduction of rats (Farris and Yeakel, 1943), and an increase in total body weight in rats (Rogers and Richter, 1948).

2. Inbreeding and crossbreeding

While there is general agreement that the adrenals of wild rats born in captivity undergo a reduction in weight, there is disagreement as to the extent of this reduction. Donaldson (1929), who studied
adrenal weight changes in captive Norway rats for ten generations, found that after an initial drop in adrenal weight in the first generation, there was no further progressive reduction in weight. The adrenal-body weight ratio of wild rats born in captivity showed a deviation from that of the albino rat of thirty-seven per cent in the male and fourteen per cent in the female. Since the adrenal gland of female rats is considerably larger than that of males, this reduction indicates that the female adrenal is more responsive to the effects of captivity than the male. When these slight differences between captivity-born wild rats and albino rats are compared with the two to three hundred per cent difference between wild and domesticated Norway rats, it can be seen that most of the reduction in adrenal size occurs early in the process of domestication.

Rogers and Richter (1948) did not find that inbreeding in captivity produced such sharp reductions in adrenal size of wild rats. They state that

"While there was only a slight reduction in the weight of the adrenals of wild rats born in the laboratory, the F₁ generation of wild-domestic crosses had adrenals that were almost as small as those of the domestic. Thus, while in matings between wild rats many generations are needed to reduce the size of the adrenals down to the level of domestic rats, this result apparently can almost be achieved in crosses between wild and domestic rats in one generation,"

Donaldson (1923) and Freudenberger (1932) agree that the weights of the adrenals in hybrid rats of the F₁ generation showed a sharp reduction from that found in the wild rats. They weighed slightly more, however, than in the albino rat.

Several other organs change in size during inbreeding and cross-breeding. Donaldson (1923) found that gross body weight and the weights of the pituitary, gonads and brain increased when wild rats were bred in
captivity. By the twenty-fifth generation growth acceleration was about equal to that of the albino rat and the body weight of adult rats was twenty per cent heavier than in the first generation adults (King, 1939).

In the Long-Evans rat, a hybrid from a cross between domesticated and wild Norway rats, growth acceleration of the gonads and of total body weight was at first less than, and later, greater than that of the albino rat, whereas that of the female was smaller.

As previously mentioned, organ weights usually show sharp changes in the first generation of crosses between wild and domesticated rats. However, such traits as nervousness and wildness often persist for many generations (King, 1939) and in some cases the behavior seemingly never completely approaches that of the domesticated parent (Freudenberger, 1932). Other studies on mice and rats have shown that the factors for wildness (Dawson, 1932; Yerkes, 1915; Coburn, 1922) and quick reaction time (Vicari, 1929) are dominant over those for tameness and slow reaction time. It thus appears that many structural changes associated with the domestication process (reduction in adrenal weight and increase in pituitary weight, body weight and early development of gonads) are brought about before the above behavioral changes.
III. MATERIALS AND METHODS

A. ANIMALS USED IN THIS STUDY

For the purpose of this investigation 174 mice were studied. Three strains were represented: 83 Peromyscus leucopus novoboracensis (white-footed mice, wood mice); 21 Mus musculus domesticus (house mice); 70 Mus musculus domesticus (Carworth Farms—W—albino mice).

Peromyscus, the most common mammal in North America, was selected as an example of a wild animal living a completely undomesticated existence. It is a highly nervous animal which continues to be wild in captivity (Cahalane, 1947). Ordinarily the female does not travel more than 500 feet away from home and thus males are more easily caught than females (Cahalane, 1947; Scheffer, 1924). The wood mouse probably doesn’t live longer than a maximum of one year in nature (Howard, 1949) and has an average life span of a little over four months (Blair, 1948).

Mus musculus domesticus (house mouse) is the commensal descendant of the Wagner strain of wild Mus musculus which originated in Russian Turkestan (Schwarz and Schwarz, 1943; Cahalane, 1947). Mice of this strain migrated along the caravan routes through Asia Minor, across North Africa, and to Italy and Spain. In the latter two countries they separated into two strains "brevirostris" and "domesticus." The latter race was spread from Italy into the Balkans, Eastern Germany, France, British Isles and Norway. They were brought to America at the time of the Revolution by way of English and French ships, and spread over northern United States, all of Canada and Alaska (Cahalane, 1947).

Here, as well as in the countries of their origin, they maintained their
commensal connections with human habitations, and thus afford an opportunity for the study of a semi-domesticated mouse. House mice and *Peromyscus* are distantly related to each other in that the family *Muridae* (*Mus*) probably arose from the family *Cricetidae* (*Peromyscus*) toward the end of the miocene (Simpson, 1945).

*Mus musculus domesticus* - albino mice of the Carworth Farms Webster (CFW) strain were obtained from Carworth Farms, New City, New York. This strain originated from the stock of the late Dr. Leslie Webster of the Rockefeller Institute who initiated his colony from the "Swiss" colony maintained at the Rockefeller Institute. This stock was brought to the United States from Switzerland by Dr. Clara Lynch. These mice have undergone many generations of inbreeding and have attained a high degree of homozygosity. This group is thus an example of a highly domesticated mouse.

Wild *Peromyscus* and house mice were trapped in Sherman-small mammal live traps (obtained from H. J. Sherman, Gainesville, Florida). These traps do not injure the mice. All traps were set at twilight and collected soon after dawn the following morning. Depending upon when the mice entered the trap, they were killed anytime from two to twelve hours after being caught. All mice were killed either by ether alone, or by subduing them with ether followed by a neck-stroke. In order to avoid the stimulating effect of prolonged exposure to cold on the adrenal glands, trapping was limited to late spring, summer, and early fall. Wild *Peromyscus* were trapped mostly in and around the wooded regions of the Blue Hills Reservation south of Boston. Wild house mice were trapped in homes in and around Metropolitan Boston. Mice which were confined in captivity or born in captivity were kept in wire gauze.
cages, two to four mice in a cage, and supplied with tap water and Purina Laboratory Chow ad libitum.

For the purpose of the present investigation males and females of the above strains of mice were divided into the following six groups:

1. *Peromyscus* wild caught

   Forty-two adult wild mice were killed, as described above, as soon after capture as possible.

2. *Peromyscus* confined in captivity

   Twenty-one wild caught *Peromyscus* were confined in captivity for periods of time ranging from eleven days to eight months.

3. *Peromyscus* born in captivity

   Twenty mice were born in captivity from females which were either pregnant when caught or from females which mated in captivity. One, two, three, and four month-old mice were used. The measurements for one month old mice were not averaged with those for adult mice.

4. House mice wild caught

   Nine house mice were included in this group.

5. House mice confined in captivity

   Twelve house mice were kept in the laboratory for from three to four months before they were sacrificed.

6. Carworth Farms - W Albino mice (CWF)

   Seventy mice of this strain were sacrificed three to four days after their arrival from Carworth Farms or were the first generation offspring from parent animals supplied by this source. Representative mice were killed at one, two,
four and six months of age. Measurements for one month old mice were not averaged with those for adult mice.

B. PREPARATION OF TISSUES

Two staining techniques were utilized in order to understand the structural and functional characteristics of the adrenal glands of the above six groups of mice. In most instances both adrenals were removed, fixed in Bouin's fluid, embedded in paraffin, serially sectioned at ten microns, stained with Harris' haematoxylin and counterstained in eosin. In some animals the left adrenal was prepared according to the preceding method, and the right adrenal was processed in such a way that the lipid nature of the adrenal could be determined. In still other mice both adrenals were prepared for lipid staining.

In order to stain the adrenal lipids the glands were dissected free from the surrounding fat and placed in ten per cent neutral formalin buffered to pH 7.0 with Sorenson's phosphate buffer (Lillie, 1948). The adrenals were fixed for approximately two to three weeks, after which time they were washed for two hours in four changes of distilled water. They were then embedded in twenty-five per cent melted gelatin at thirty-seven degrees centigrade in a calcium chloride desiccator, according to the method recommended by Baker (1944) for obtaining thin frozen sections. Desiccation was stopped when the gelatin became extremely viscous and tended to gel, at which time the gelatin dishes were refrigerated for one to two hours. Small blocks containing the embedded adrenals were cut out and hardened a minimum of twenty-four hours in ten per cent formalin before sectioning.
Prior to sectioning the blocks of gelatin were washed in distilled water for from fifteen to twenty minutes in order to remove the formalin which lowers the freezing point of the gelatin. Frozen sections of five microns thickness were cut, washed in distilled water, mounted on albuminized slides and dried one-half to one hour. The tissues were then processed through fifty per cent alcohol; Mayer's hemalum for one minute; fifty per cent alcohol; seventy per cent alcohol; Sudan Black B saturated in seventy per cent alcohol for four minutes; seventy per cent alcohol for one minute; fifty per cent alcohol; and two rinses in distilled water before mounting in warm Kaiser's glycerine jelly. Mayer's hemalum, when used without an alkaline rinse, stains nuclei pale pink in contrast to the blue-black lipid droplets stained with Sudan Black B.

C. METHODS OF MEASUREMENT

1. Volume determination

The normal mouse adrenal is so small that any method of weight determination would be too liable to error for use in experimental work. In order to compare the size differences in the adrenal glands of the above groups of mice, it was decided to measure the volume according to the method of Dornfield, Slater and Scheffe (1941). Approximately every tenth section of serially sectioned adrenals (ten microns thick) was projected at a magnification of thirty-seven and a half times. The outlines of the cortex and medulla were traced and their magnified areas were measured by means of a polar planimeter. By plotting the areas of the magnified tracings against their serial number
and measuring the area under the curve by use of a planimeter, it was found that the volume of the cortex and medulla could be measured except for constant factors of magnification and thickness. Using coordinate paper ruled twenty lines to the inch, Dornfield et al. let the horizontal units represent the serial number of the sections and the vertical units represent the areas of the magnified tracings in square inches (Figure 3). By means of the curve thus constructed the volume of cortex and medulla can be calculated from the last planimeter reading P by the following formula:

\[
V = \left( \frac{PNY \times 645}{M^2} \right) \left( \frac{t}{1,000} \right)
\]

- \(V\) = volume of the organ in cubic millimeters
- \(P\) = planimeter reading under the curve in square inches
- \(N\) = linear magnification of the projected tracing
- \(M\) = thickness of each section in microns
- \(t\) = number of sections per horizontal inch on the graph
- \(Y\) = square inches per vertical inch on the graph

By this method the volumes of the cortex, medulla, and whole gland were determined for both left and right adrenals. In certain mice which were difficult to obtain, only the left adrenal was measured volumetrically. The right adrenal was reserved for a histochemical study of lipids. In such cases the volume of the left adrenal was multiplied by a constant which represented the difference in volume between the left and right adrenal for the group to which that animal belonged.
Figure 5. Method for determining the volume of the adrenal cortex and medulla. Planimetric measurements of the areas under the curves are used to determine the volume.

(From the method of Dornfield et al, 1941).
2. **Zonal dimensions**

In order to determine which of the histological zones of the adrenal cortex were involved in the volumetric differences among the groups of mice studies, some means of measuring these zones was necessary. For this purpose, the largest slice of each of the serially sectioned adrenals was projected at 150X. Linear measurements of the depth of the glomerulosa, fasciculata, reticularis, and X-zone were made in a region of average cortical thickness for that section. All measurements were reduced by the magnification factor.

3. **Cell measurements**

It is widely known that organ enlargement can be due to more than one factor. Vascular pooling, hypertrophy, and hyperplasia are all capable of increasing the volume of the adrenal gland. In an attempt to determine which of these factors were involved, cell size and cell compactness were measured. Since cell membranes were quite indistinct in most tissues it seemed advisable to measure nuclear dimensions as an index of cell size (cf. Hertwig's nucleoplasmic constant in DeRobertis et al., 1955). As a check on using nuclear diameter as an index of cell size, measurements of the compactness of cells were made by counting the number of nuclei which were seen at a single focal level along measured lengths of epithelial cords. Since both cell size and compactness vary within the same gland, several readings were taken for each section studied. All measurements were made by means of an ocular micrometer.
D. STATISTICAL METHODS

Volumetric determinations of the amount of medullary and cortical tissue per gram of body weight for each group of mice studied were compared for significant differences at the ninety-five per cent probability level. In each case the Snedecor F-test was applied to determine whether the variances of the groups being compared could have come from a single population variance. If the difference in variance was not significant the Student-Fisher t-test was used to ascertain significance of the different means. When the variances were significantly different, statistical confidence was tested by means of the approximate method of Cochran and Cox.
IV. RESULTS

A. VOLUMETRIC MEASUREMENTS OF THE MOUSE ADRENAI

1. Comparison of wild caught Peromyscus and Carworth Farms albino laboratory mice

In order to determine if mice living in a natural wild environment exhibited adrenal differences from the common laboratory mouse, the adrenal volumes of wild caught Peromyscus and Carworth Farms albino mice were compared. The results of this comparison are shown in Table II. It is evident that the adrenals of wild caught Peromyscus males are 32 per cent larger than in CFW mice. This difference is accentuated when cortical and medullary comparisons are made since the former group has a larger cortex (66 per cent larger) and the latter has a larger medulla (188 per cent larger). In as much as wild mice are considerably lighter than laboratory mice, a study of the ratio of adrenal tissue per gram of body weight was made. In this case the whole gland and cortical differences were increased to 97 per cent and 144 per cent respectively, whereas the medullary difference was reduced to 69 per cent.

In the female, however, greater mean volumes for whole gland, cortex and medulla were found in the CFW albino mouse than in wild caught Peromyscus (an increase of 20 per cent, 14 per cent, and 103 per cent respectively). When interpreted in terms of adrenal-body weight ratio this increase was reversed for whole gland and cortex, and reduced for the medulla. The wild caught female Peromyscus had 31 per cent more cortical tissue and 35 per cent less medullary tissue per gram of body.
Table II

Volumetric Comparison of the Adrenals of Adult Peromyscus Wild Caught and C F W Albino Mice

(Percentages refer to differences from wild caught mice)

<table>
<thead>
<tr>
<th></th>
<th>No. Animals</th>
<th>Vol: in cu. mm.</th>
<th>Cu. mm. / gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole gland</td>
<td>Medulla</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>23</td>
<td>2.98</td>
<td>.22</td>
</tr>
<tr>
<td>C F W albino mice</td>
<td>24</td>
<td>2.25</td>
<td>.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(24%)</td>
<td>(168%)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>13</td>
<td>4.05</td>
<td>.31</td>
</tr>
<tr>
<td>C F W albino mice</td>
<td>23</td>
<td>4.87</td>
<td>.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20%)</td>
<td>(103%)</td>
</tr>
</tbody>
</table>
weight than was found in the female laboratory mouse. In both the male and female the differences in adrenal-body weight ratios were statistically significant.

2. Comparison of the adrenals of Peromyscus wild caught, confined in captivity and bred in captivity

In an attempt to understand the effect on adrenal structure of a change from the wild to the laboratory environment, the adrenals of wild caught Peromyscus, Peromyscus which had been confined in captivity for periods of time ranging from eleven days to eight months, and those which were born in captivity were compared.

The total adrenal tissue of wild caught male Peromyscus increased 92 per cent in volume during the first eleven days in captivity with no apparent decrease for eight months. The cortex increased in volume (91 per cent and 68 per cent respectively). Even when interpreted in terms of adrenal-body weight ratio, the increases are statistically significant in whole gland, medulla and cortex (53 per cent, 46 per cent and 62 per cent respectively). A smaller increase occurs in the female under these conditions. The wild female has 36 per cent more adrenal tissue than the male, so an increase in adrenal-body weight ratio of 31 per cent during captivity resulted in a slightly larger adrenal in the captivity raised female than in the male.

When the adrenal of the wild caught male Peromyscus is compared with that of the first generation captivity bred mouse, the latter shows an increase of only 30 per cent in volume and 24 per cent more adrenal tissue per gram of body weight. The first generation of captivity born females
shows only a slight decrease in adrenal volume from that of wild caught mice. The difference between wild caught Peromyscus and Peromyscus born in captivity is significant only at the 36 per cent probability level in males, and is not significant in females. These comparisons are shown in Table III and Figures 4 and 5.

5. Comparison of the adrenals of wild caught house mice, house mice confined in captivity and CFW albino mice

Since house mice and CFW albino mice live in different environments and are members of the same species, Mus musculus, the adrenals of these two forms were compared. For comparative purposes some of the house mice were studied in their wild caught state and others were confined in captivity prior to killing.

In both males and females the volumes of cortex and medulla were greater in house mice confined in captivity than in those caught wild. However, since those mice which were confined in captivity weighed one-third more than wild caught mice, their adrenal-body weight ratio was the reverse of that given above. Male house mice confined in captivity had less medullary and cortical tissue per gram of body weight than did wild caught house mice (25 per cent and 14 per cent, respectively). In the female, there was no significant difference between the adrenal-body weight ratios of wild caught and confined house mice.

Both males and females of CFW albino mice had larger adrenals than did wild caught house mice. However, since laboratory mice weigh two to three times as much as wild house mice, these differences are reversed when they are compared as to adrenal-body weight ratio. The wild male house mouse has 192 per cent more cortical tissue and 32 per cent more
<table>
<thead>
<tr>
<th></th>
<th>No. animals</th>
<th>Vol. in cu. mm.</th>
<th>Cu. mm. / gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole gland</td>
<td>Medulla</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus confined in captivity</td>
<td>9</td>
<td>5.72</td>
<td>.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(92%)</td>
<td>(91%)</td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>23</td>
<td>2.98</td>
<td>.22</td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>5</td>
<td>3.87</td>
<td>.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(30%)</td>
<td>(32%)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus confined in captivity</td>
<td>8</td>
<td>5.83</td>
<td>.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44%)</td>
<td>(45%)</td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>13</td>
<td>4.05</td>
<td>.31</td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>9</td>
<td>3.59</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11%)</td>
<td>(12%)</td>
</tr>
</tbody>
</table>
Explanation of Figure 4

The measurements of adrenal volume and adrenal-body weight ratios represent the total for both adrenals. The six groups of mice compared are:

- PCC - Peromyscus confined in captivity
- PWC - Peromyscus wild caught
- PBC - Peromyscus born in captivity
- HMCC - House mice confined in captivity
- HMWC - House mice wild caught
- CFW - Carworth Farms W albino mice
COMPARISON OF ADRENAL VOLUME AND ADRENAL-BODY WEIGHT RATIO

IN ADULT WILD AND DOMESTICATED MICE

Adrenal Cortex:
- Male
- Female

Adrenal Medulla:
- Male
- Female

Figure 4
<table>
<thead>
<tr>
<th>Mouse</th>
<th>Caudate Wild</th>
<th>Caudate Contained in House Mouse</th>
<th>Periventricular Cortical Area</th>
<th>Caudate Wild</th>
<th>Caudate Contained in House Mouse</th>
<th>Periventricular Cortical Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. The adrenal cortex in adult male.
medullary tissue per gram of body weight than the albino mouse. The wild female house mouse exhibits a similar increase (67 per cent and 59 per cent respectively). These differences are statistically significant.

Since the CFW albino mouse is a highly domesticated form of wild Mus musculus, this is an indication that the adrenal-body weight ratio has undergone a reduction during the process of domestication. These comparisons are shown in Table IV and in Figures 4 and 5.

4. Comparison of the adrenals of wild and domesticated mice at different ages

The adrenal differences between wild and domesticated mice at different ages are shown in Tables V, VI and VII. An attempt was made to estimate the ages of wild caught male Peromyscus according to the method of Cockrum (1954). The six age groups listed might be interpreted roughly as months of age. Because of the smaller sample size wild caught female Peromyscus were not divided into age groups. Age groupings for wild caught Peromyscus were based upon estimations of tooth wear, order of tooth eruption, pelage color and body weight. None of the wild caught mice was young enough to be in age group one. Adrenal volumes for the different age groups of Peromyscus born in captivity and CFW albino mice were also determined.

The most important factor which is seen when mice of different age groups are compared is that the age of maximum adrenal-body weight ratio is different for each group. The age at which there is the maximum amount of adrenal tissue per gram of body weight for male mice is one month in the albino, two months in the captivity bred Peromyscus, and four to five months in wild caught Peromyscus. Since no three month old group
### Table IV

**Volumetric Comparison of the Adrenals of Adults of House Mice Wild Caught, House Mice Confin ed in Captivity, and C F W Albino Mice**

(Percentages refer to differences from wild caught house mice)

<table>
<thead>
<tr>
<th>No. animals</th>
<th>Vol. in cu. mm.</th>
<th>Cu. mm. / gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole gland</td>
<td>Medulla</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mice confined in captivity</td>
<td>5</td>
<td>2.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12%)</td>
</tr>
<tr>
<td>House mice wild caught</td>
<td>5</td>
<td>2.03</td>
</tr>
<tr>
<td>C F W albino mice</td>
<td>24</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11%)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mice confined in captivity</td>
<td>7</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34%)</td>
</tr>
<tr>
<td>House mice wild caught</td>
<td>4</td>
<td>2.41</td>
</tr>
<tr>
<td>C F W albino mice</td>
<td>23</td>
<td>4.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(102%)</td>
</tr>
</tbody>
</table>
## TABLE V

**VOLUMETRIC COMPARISON OF THE ADRENALS OF WILD CAUGHT MALE PEROMYSCUS AT ESTIMATED AGES**

<table>
<thead>
<tr>
<th>Age group - 2</th>
<th>No. Animals</th>
<th>Vol. in cu. mm.</th>
<th>Cu. mm. / gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole gland</td>
<td>Medulla</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2.23</td>
<td>.19</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2.43</td>
<td>.20</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3.68</td>
<td>.21</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3.77</td>
<td>.23</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3.48</td>
<td>.34</td>
</tr>
</tbody>
</table>

*Age groupings for wild caught Peromyscus were based upon estimations of the amount of tooth wear, order of tooth eruption, pelage color and body weight. None of the wild caught mice were in group 1. The group numbers approximate months of age. (Method of Cockrum, 1954)*
Table VI

VOLUMETRIC COMPARISON OF THE ADRENALS OF PEROMYSCUS AT DIFFERENT AGES

<table>
<thead>
<tr>
<th>No. Animals</th>
<th>Vol. in cu. mm.</th>
<th>Cu. mm. / gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole gland</td>
<td>Medulla</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month old</td>
<td>2</td>
<td>2.48</td>
</tr>
<tr>
<td>2 months old</td>
<td>2</td>
<td>5.12</td>
</tr>
<tr>
<td>4 months old</td>
<td>3</td>
<td>3.04</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month old</td>
<td>4</td>
<td>2.44</td>
</tr>
<tr>
<td>2 months old</td>
<td>3</td>
<td>4.22</td>
</tr>
<tr>
<td>3 months old</td>
<td>4</td>
<td>3.25</td>
</tr>
<tr>
<td>4 months old</td>
<td>3</td>
<td>3.33</td>
</tr>
</tbody>
</table>
was studied for male *Peromyscus* born in captivity the maximum ratio might be found in this group. When these results are compared with the histological results of these groups (to be discussed later) it is noticeable that maximum adrenal size is attained at a later age in wild than in the more domesticated mice. In the female, the age at which the maximum adrenal-body weight ratio is attained is later than in the male. This ratio reaches its peak in two months in both the CFW albino mouse and *Peromyscus* born in captivity. In both groups this ratio is maintained near peak level throughout the fourth month.

5. **Comparison of the cortex-medulla ratios of wild and domesticated mice**

As can be seen in Table VII there is a definite distinction between the cortex-medulla ratios of *Peromyscus* and *Mus musculus*. The former has a higher cortex-medulla ratio (with no significant difference between male and female) and the latter has a lower ratio (lower in males than in females). The CFW male, with a cortex-medulla ratio of 2.96 is considerably below the mean ratio for any of the other groups. Female laboratory mice and male and female house mice have cortex-medulla ratios which range from 5 to 6. In both male and female *Peromyscus* the cortex-medulla ratio varies from 11 to 13. The cortex-medulla ratio is not significantly altered if calculated from the adrenal-body weight ratio (rather than from volume differences.)

6. **Variations in size between left and right adrenal**

Only in CFW albino mice was a consistent difference between left and right adrenal observed. In this group the left adrenal averaged 13 percent larger than the right adrenal in both male and female. The difference
TABLE VIII

COMPARISON OF THE CORTEX-MEDULLA RATIOS OF WILD AND DOMESTICATED MICE

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peromyscus confined</td>
<td>12.75</td>
<td>12.38</td>
</tr>
<tr>
<td>in captivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>12.22</td>
<td>12.51</td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>13.09</td>
<td>11.36</td>
</tr>
<tr>
<td>House mice confined in captivity</td>
<td>5.32</td>
<td>8.45</td>
</tr>
<tr>
<td>House mice wild caught</td>
<td>6.43</td>
<td>8.24</td>
</tr>
<tr>
<td>G F W Albino mice</td>
<td>2.96</td>
<td>7.11</td>
</tr>
</tbody>
</table>
between left and right adrenal was the least in wild caught Peromyscus (left adrenal 4 per cent larger than the right). In this group and all other Peromyscus the left adrenal was usually slightly larger than the right, but since many exceptions were found this difference cannot be considered significant.

B. HISTOLOGICAL ANALYSIS OF THE MOUSE ADRENAL

1. Cortical zonation in adult wild and domesticated mice

The general arrangement of the tissues of the adrenal gland of wild mice is similar to the description given previously for the adrenal gland of the laboratory mouse. There are, however, differences in the relative proportions of the histological zones of the cortex in several of the groups studied. These differences are shown for adult mice in Table IX and Figure 6.

The zona glomerulosa is wider in CFW albino mice and house mice than it is in any of the groups of Peromyscus. However, the circumference of the adrenal of Peromyscus exceeds that of other groups. In such cases, a narrow glomerulosa stretched over a large gland might very well contain as much or more glomerular tissue than a wide band surrounding a small adrenal.

The zonal differences which contributed most to the volumetric variations previously discussed were those in the zona fasciculata and zona reticularis.

In all groups of Peromyscus, except the female born in captivity, the zona fasciculata is wider than in either house mice or CFW albino mice. In both the male and female the depths of the fascicular and
**TABLE IX**

COMPARISON OF THE DEPTH OF CORTICAL ZONES IN ADULT WILD AND DOMESTICATED MICE

(Percentages refer to differences from wild caught mice)

<table>
<thead>
<tr>
<th></th>
<th>Zonal depths in males (mm.)</th>
<th>Zonal depths in females (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. animals</td>
<td>Glomer-</td>
</tr>
<tr>
<td>Peromyscus confined in captivity</td>
<td>17</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33%)</td>
</tr>
<tr>
<td>Peromyscus wild caught</td>
<td>36</td>
<td>.03</td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>14</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33%)</td>
</tr>
<tr>
<td>House mice confined in captivity</td>
<td>12</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7%)</td>
</tr>
<tr>
<td>House mice wild caught</td>
<td>9</td>
<td>.04</td>
</tr>
<tr>
<td>C F W albino mice</td>
<td>47</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25%)</td>
</tr>
</tbody>
</table>
DEPTH OF CORTICAL ZONES IN THE ADRENALS
OF ADULT WILD AND DOMESTICATED MICE

Figure 6
reticular zones of *Peromyscus* confined in captivity exceeded those of any other group. Although the fasciculata of captivity bred male *Peromyscus* showed only a slight reduction as compared to the wild form, the fasciculata of the female was considerably reduced. The fasciculata of both groups of house mice was wider than that of either male or female CFW mice.

The zona reticularis of all groups of male *Peromyscus* was wider than in any group of *Mus musculus*. In the female, however, considerable variation was observed in the reticularis and no significant conclusions could be drawn as to its part in adrenal enlargement. The fact that an X-zone was found in several female *Peromyscus* which had been confined in captivity for as long as six months suggests that this zone is either capable of responding to the stress of confinement or that possibly a "secondary X-zone" (as described by Deanesly, 1938, and Masui and Tamura, 1936) had developed.

2. Cortical zonation in captivity bred *Peromyscus* and CFW mice at different ages

Whereas the zona glomerulosa was approximately the same depth in both males and females of *Peromyscus* and CFW at the age of one month (0.05 mm.), this zone diminished in width in older *Peromyscus* (0.02 - 0.025 mm.). In CFW the zona glomerulosa maintained the same width throughout the period studied.

In the male the greatest depth of the zona fasciculata, as well as of the whole cortex, was in the first month in CFW, and the second month in *Peromyscus*. After this the width of the zona fasciculata decreased in both groups. In the female the zona fasciculata reached a peak by the
first month in CFW and the third month in Peromyscus. This width was maintained in both groups throughout the first four to six months. These results are shown in Table X.

In both male and female the X-zone developed to greater proportions in Peromyscus than it did in laboratory mice, and at its peak, the X-zone was three times wider than in the CFW albino mice. In the male this zone reached its maximum depth in both groups at one month. In male CFW the X-zone degenerated quickly for it wasn't noticeable at two months even in a degenerate form. In male Peromyscus most of the viable cells of the X-zone had disappeared by two months, but a relatively wide band showing fatty degeneration was still present. This indicates that the X-zone persists longer in male Peromyscus than in male CFW mice. In both groups the X-zone was replaced by a band of connective tissue separating the cortex and medulla.

Whereas the X-zone persisted longer in male Peromyscus than in CFW albino mice, it degenerated earlier in female Peromyscus both in captivity than in female CFW mice. In both groups the X-zone reached its peak at two months (.32 mm. in Peromyscus and .11 mm. in CFW). In Peromyscus this zone diminished to a depth of .06 mm. by the third month. At this time there were a few viable X-zone cells amidst many degenerating cells. By the fourth month the X-zone of female Peromyscus had disappeared entirely in most animals and was replaced by a band of connective tissue. In CFW females the X-zone persisted at its peak width throughout the fourth month, at which time, however, there were only a few viable cells amidst many degenerate and fat-laden cells. By the sixth month only a few degenerate cells of the X-zone were present in the
TABLE X
COMPARISON OF THE DEPTH OF CORTICAL ZONES IN WILD AND DOMESTICATED MICE AT DIFFERENT AGES

<table>
<thead>
<tr>
<th></th>
<th>Zonal depths in males (mm.)</th>
<th>Zonal depths in females (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glomerulosa</td>
<td>Fasciculata</td>
</tr>
<tr>
<td>Peromyscus born</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in captivity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month old</td>
<td>0.05</td>
<td>0.26</td>
</tr>
<tr>
<td>2 months old</td>
<td>0.02</td>
<td>0.38</td>
</tr>
<tr>
<td>3 months old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 months old</td>
<td>0.02</td>
<td>0.28</td>
</tr>
<tr>
<td>G.F.W. Albino mice:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month old</td>
<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>2 months old</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>4 months old</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>6 months old</td>
<td>0.04</td>
<td>0.09</td>
</tr>
</tbody>
</table>
female CFW, along with a narrow band of connective tissue separating
cortex and medulla. Many of these differences can be seen in Figure 7.

In neither male nor female Peromyscus was a reticular zone observed
during the time that the X-zone was at its maximum diameter. The
zone reticularis was seen only after the X-zone had undergone consider-
able diminution. This can be interpreted as evidence for either the
formation of reticular cells from outer X-zone cells (as was described
by Deanesly, 1928), or a late differentiation of the cells of the inner
ends of the fascicular cords to fill in the area left by the degenera-
ting X-zone. In albino mice a small reticular zone was observed at the
earliest age studied in both males and females. This zone increased in
breadth, however, when the X-zone disappeared.

C. CYTOLOGICAL OBSERVATIONS ON THE MOUSE ADRENAL

1. Cell size

Measurements of nuclear diameter in microns and the number of nuclei
per linear millimeter were used as an index of cell size. These results
are shown in Table XI. All groups of Peromyscus, both male and female
had larger cells in the zona fasciculata than were found in any group of
Mus musculus. With the exception of wild caught female house mice, all
Mus musculus had larger glomerular cells than found in Peromyscus. In
contrast to these differences there was a striking similarity in medullary
cell size in all groups studied.

Differences in variability were observed in several groups of mice.
In CFW albino mice there was almost a complete absence of variation in
cell size. All cells in any one zone were approximately the same size.
Both groups of house mice showed a slight amount of variation, especially
Figure 7. The X-zone of the adrenal cortex of male and female, wild and domesticated mice, X 450.

A. Male Garworth Farms albino mouse one month old. Notice the narrow X-zone composed of cells of approximately the same size.

B. Male Garworth Farms albino mouse two months old. The X-zone has been replaced by a narrow band of connective tissue.

C. Male Peromyscus one month old. Notice the large X-zone cells.

D. Male Peromyscus two months old. A few viable X-zone cells are seen among large fat vacuoles.

E. Female Garworth Farms albino mouse two months old. A wide X-zone is seen in the middle half of the photograph. The cells of the X-zone are no larger than the other cells.

F. Female Peromyscus two months old. A very vascular X-zone fills the entire photograph. The boundaries of this zone are at the upper and lower edges of the photograph.

G. Female Peromyscus wild caught. The X-zone is wide and vascular and fills the entire photograph.

H. Female Peromyscus confined in captivity for six months.

Some of the cells of the X-zone are vacuolated and in the process of degenerating.
<table>
<thead>
<tr>
<th></th>
<th>Diameter of nuclei in microns</th>
<th>Number of cells per linear mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glomer-</td>
<td>Fascic-</td>
</tr>
<tr>
<td></td>
<td>ulosa</td>
<td>ulata</td>
</tr>
<tr>
<td>Peromyscus confined</td>
<td>5.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus wild</td>
<td>4.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>5.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Peromyscus confined</td>
<td>4.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Female</td>
<td>5.1</td>
<td>8.7</td>
</tr>
<tr>
<td>Peromyscus born in captivity</td>
<td>4.5</td>
<td>7.7</td>
</tr>
<tr>
<td>House mice confined</td>
<td>5.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mice wild</td>
<td>5.5</td>
<td>6.1</td>
</tr>
<tr>
<td>Female</td>
<td>5.8</td>
<td>6.4</td>
</tr>
<tr>
<td>C F W Albino mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mice confined</td>
<td>5.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Female</td>
<td>5.1</td>
<td>6.4</td>
</tr>
<tr>
<td>C F W Albino mice</td>
<td>5.5</td>
<td>6.1</td>
</tr>
</tbody>
</table>
in the genus fasciculata. Considerable variation in cell size was seen in all groups of *Peromyscus*. Some cells were three times as large as other cells. Within any one group cell size varied more in female mice than in males.

An increase in cell size in the zona fasciculata was seen when wild male *Peromyscus*, and to a lesser extent when wild male and female house mice were confined in captivity. In female *Peromyscus* confinement produced a slight decrease in cell size. In both males and females the cells of the zona fasciculata were smaller in captivity bred *Peromyscus* than in either the wild caught or wild confined groups. There was no consistent relationship between reticular cell size and the various conditions of captivity and wildness.

The cells of the X-zones were considerably larger in *Peromyscus* than in CFW albino mice for each age group compared. The average nuclear diameter in microns for X-zone cells of one month old males was 9.3 in *Peromyscus* and 6.1 in CFW. For one month old females the mean diameter was 10.3 and 6.8, respectively.

Since volume differences and somal dimensions of most of the groups compared exceeded the differences in cell size for these groups, it is evident that both hypertrophy and hyperplasia are involved when enlargement occurs.

2. Lipid distribution

The results of Sudan Black B staining are shown in Table XII. The adrenal cortex of each group of *Mus musculus* was considerably more sudanophilic than the cortex of any group of *Peromyscus*. In the former group almost the entire fasciculata was blackened by the presence of
### TABLE XII
COMPARATIVE SUDANOPHILIA OF THE HISTOLOGICAL ZONES OF THE ADRENAL CORTEX OF WILD AND DOMESTICATED MICE

<table>
<thead>
<tr>
<th></th>
<th>Glomerulosa</th>
<th>Outer fasciculata</th>
<th>Inner fasciculata</th>
<th>Reticularis</th>
<th>X-Zone</th>
<th>% Cortex XX or XXX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peromyscus confined in captivity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>X</td>
<td>XX</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>25%</td>
</tr>
<tr>
<td>Female</td>
<td>XX</td>
<td>XXX</td>
<td>0</td>
<td>0</td>
<td>Æ</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Peromyscus wild caught:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>XX</td>
<td>XX</td>
<td>Æ</td>
<td>Æ</td>
<td>—</td>
<td>33%</td>
</tr>
<tr>
<td>Female</td>
<td>X</td>
<td>XX</td>
<td>0</td>
<td>0</td>
<td>Æ</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Peromyscus born in captivity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>XX</td>
<td>XXX</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>50%</td>
</tr>
<tr>
<td>Female</td>
<td>XX</td>
<td>XXX</td>
<td>0</td>
<td>0</td>
<td>Æ</td>
<td>33%</td>
</tr>
<tr>
<td><strong>House mouse wild caught:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>XXX</td>
<td>XXX</td>
<td>XX</td>
<td>Æ</td>
<td>—</td>
<td>75%</td>
</tr>
<tr>
<td>Female</td>
<td>XX</td>
<td>XX</td>
<td>X</td>
<td>0</td>
<td>—</td>
<td>67%</td>
</tr>
<tr>
<td><strong>C F W Albino mouse:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>X</td>
<td>XXX</td>
<td>XXX</td>
<td>XX</td>
<td>—</td>
<td>90%</td>
</tr>
<tr>
<td>Female</td>
<td>X</td>
<td>XXX</td>
<td>XXX</td>
<td>0</td>
<td>X</td>
<td>75%</td>
</tr>
</tbody>
</table>

**X - XXX** Relative concentration of lipid droplets

0 Absence of lipid droplets

Æ No visible droplets, cytoplasm distinctly gray
lipid droplets, whereas in the latter the inner half or two-thirds of
the fasciculata was devoid of lipid droplets. Furthermore, there were
differences in droplet size in several groups. In general lipid drop-
lets were the largest in CFW albinos, medium-sized in house mice, and
the smallest in Peromyscus. In any one adrenal gland there was a ten-
dency for the lipid droplets to decrease in size from the glomerulosa to
the inner fasciculata. When lipid droplets were present in the reticu-
laris they were larger and more irregularly shaped than in the other
zones. In most instances the reticularis and X-zone were devoid of
lipids. (Figures 8 and 9).

In all groups studied (with the exception of Peromyscus confined
in captivity) the adrenal cortex of males was more sudanophilic than
that of females. The adrenal cortex of Peromyscus confined in captivity
was less sudanophilic than that of males born in captivity. There was
seemingly no difference in the lipid reaction when the three groups of
female Peromyscus were compared.

The adrenal cortex of wild caught house mice was intermediate be-
tween Peromyscus and CFW albino mice in its reaction to Sudan Black B.
If a decrease in sudanophilia is interpreted as indicative of increased
activity of the gland, then wild mice either have more active adrenals
than domesticated mice, or their glands became more active in the few
hours they were in the traps before being killed.

3. Other cytological observations

The size of the nucleolus was observed to be closely related to the
dimensions of the cell. Peromyscus, which had larger cells than Mus mus-
culus, likewise had larger nucleoli. It was also noticed that the chro-
matin network was more dense in the adrenals of Peromyscus than in Mus
Figure 9. Lipid concentration in the adrenal glands of wild and
domesticated mice and the albino rat. X 100, Sudan Black B.

A. Peromyscus. Only the outer one-third of the cortex is
sudanophilic. The rest is free of visible lipids.

B. House mouse. Except for a small portion of the inner
fasciculata and reticularis, all of the cortex is sudano-
philic.

C. Carworth Farms - W albino mouse. The entire cortex gives
a strong sudanophilic reaction.

D. Carworth Farms - W albino mouse with X-zone. Notice that
the cells of the X-zone are lipid free in contrast to the
rest of the cortex.

E. Albino Wistar rat. Except for a narrow sudanophobic
zone, the glomerulosa and outer fasciculata exhibit high
concentrations of lipid droplets. Sudanophilia is reduced
in the inner fasciculata and reticularis.
Figure 9. Relative size of lipid droplets in the adrenal glands of wild and domesticated mice. X 1200, Sudan Black B.

A. Peromyscus. Notice the highly concentrated, fine lipid droplets surrounding the unstained nuclei.

B. House mouse. Notice the lipid droplets which are considerably larger than those of Peromyscus.

C. Gerworth Farms – W albino mouse. The lipid droplets are slightly larger than those of the house mouse.
musculus. This network was more noticeable in the adrenals of females than of males. Although no mitotic counts were made, this difference might be an indication of mere mitotic activity in the wild mouse than in the domesticated mouse.

Observations were made on the staining affinity of the cytoplasm of the adrenal cells. With the exception of mice in the older age groups, the cytoplasm of fascicular cells of Peromyscus was more basophilic than that of the laboratory mouse. No such distinction could be made between the staining affinities of the cytoplasm of cells in other zones or in other groups.
V DISCUSSION

A. VOLUMETRIC DIFFERENCES IN THE ADRENALS OF WILD AND DOMESTICATED ANIMALS

The greater ratio of adrenal tissue to body weight that is characteristic of wild caught Peromyscus and house mice as compared with CFW domesticated mice, is in agreement with the results of other wild-domestic comparisons. In several other ways, however, the results reported for Peromyscus and house mice are at variance with those of similar studies of other animals. Even though the adrenal glands of wild caught female Peromyscus and house mice are considerably larger than those of males, they are smaller than the adrenals of the female CFW mouse. Only when adrenal-body weight ratios are compared does either group of female mice exceed the ratio of the domesticated female CFW. Rogers and Richter (1948) found that in the Norway rat the difference between adrenal weights in wild and domesticated forms was greater in the female than in the male.

The fact that the adrenal size of females as compared with males is only 36 per cent greater in wild caught Peromyscus and from 35 to 75 per cent greater in wild Norway rats, might be related to the prominence of an X-zone in the adrenals of mice, and its absence in rats. As was previously mentioned this zone often constitutes more than half the width of the cortex in female mice, and is more prominent in female Peromyscus than in female albino mice. During early pregnancy the X-zone undergoes rapid degeneration in the mouse so that at the middle of the gestation
period only a trace remains (Masui and Tamura, 1926; Tamura, 1926; Deanes-
ly, 1928). Since most of the wild caught female Peromyscus were in a
state of early pregnancy when caught, the reduction in X-zone probably re-
duced the average volume for the group. None of the CFW females used in
this comparison was pregnant, and thus the adrenal X-zone, not being inhi-
bited., maintained its enlarged state. In late pregnancy and post partum
in mice the fasciculata hypertrophies, but mice in this state seldom
wander far enough away from the nest to be trapped.

Whereas both wild and domesticated Norway rats are similar in medul-
inary size (Rogers and Richter, 1946), wild Peromyscus and the house mouse
have a smaller medulla than does the laboratory mouse. When medullary
measurements are expressed as adrenal-body weight ratios, a higher ratio
is seen for the medulla of the house mouse than for the CFW albino mouse.
Thus, there is no consistent relationship between the medulla-body weight
ratio and the condition of wildness. These variations which are seen
appear to be species differences more than differences due to environmen-
tal stress.

The adrenal cortex-body weight ratio is the only measurement which
consistently shows that there is relatively more adrenal tissue in the
wild form. Since wild animals are considerably lighter than the domesti-
cated form of the same species, the use of such a ratio is probably the
only valid means of comparison.

Inasmuch as the X-zone develops to a greater extent in both male and
female Peromyscus than it does in CFW albino mice and persists longer in
male Peromyscus than in male albino mice, there is a possibility that this
difference is an adaptive reaction. However, in view of the fact that little is known regarding the function of the X-zone in mice, no definite conclusions can be drawn from the differences seen in this zone in wild and domesticated mice.

B. EVIDENCE OF SECRETORY DIFFERENCES IN THE ADRENALS OF WILD AND DOMESTICATED ANIMALS

Quite different results follow the use of lipid stains in studying the adrenals of wild and domesticated rats and mice. The use of Sudan Black B as a lipid indicator reveals a strongly sudanophilic cortex in CFW albino mice (almost the whole cortex was darkened by the stain), a moderately sudanophilic cortex in house mice (approximately two-thirds of the cortex darkened), and a slightly sudanophilic cortex in Peromyscus (from one-fourth to one-third of the cortex darkened). Using Sudan IV Mosier (1952) and Woods (1953) found only a sparse deposition of lipids in the fasciculata and even smaller amounts in the glomerulosa and reticularis of albino rats as compared with a greater sudanophilia in wild rats.

In a separate investigation I have studied Sudan Black B preparations of the adrenals of domesticated male Wistar rats (Figure 8). I would not say that there was only "a sparse distribution of lipids" in their adrenals, but would classify their adrenals as more sudanophilic than those of Peromyscus, but slightly less than those of CFW albino mice. Since neither Mosier (1952) nor Woods (1953) published photographs showing the lipid nature of the adrenals of wild and domesticated rats, I cannot say whether the differences in our observations are the result of studying different strains of albino rats, or represent differences in the use
of the word "sparse."

The interpretation of Mosier (1952) and Woods (1953) that the increased sudanophilic reaction seen in wild rats is indicative of a high degree of activity, is contrary to the usual interpretation of lipid stains (Sayers, 1950; Deane and Dalton, 1941). The hypothesis of increased secretory activity in the adrenal cortex of wild rats is strengthened, however, when the reactions of their cortices to various experimental stresses are considered. Following exposure to noise, fighting, low temperature (Woods, 1953, 1954), starvation, irradiation and high salt diet (Richter, 1952) the adrenal lipids of the wild rat were not significantly altered from their normal state. In the albino rat, however, lipid decreases were associated with each of these stresses. This, combined with the fact that more ACTH is necessary to produce a reduction in ascorbic acid in wild than in albino rats (Woods, 1953), tends to show that the wild rat's adrenal can produce hormones as fast as they are being depleted under these experimental conditions.

The failure to evoke adrenal responses in wild rats, however, is not without exception. Nichols (1948, 1950) showed that the adrenals of wild rats hypertrophy and undergo a reduction in cholesterol during the first week following the stress of capture and confinement. After the first week their adrenals returned to a state similar to that of the wild caught rat, a reaction similar to Sayers' (1950) type V adaptation to stress. In Peromyscus the stress of confinement led to a slight decrease in sudanophilia in the male and marked hypertrophy in both male and female. This condition was maintained throughout the entire time of confinement. Taken by itself this would indicate that during confinement the adrenals of Peromyscus are more active than is
the case in the rat. This might be interpreted as evidence that the internal environment of Peromyscus is less able to adapt to the stress of confinement. This reaction doesn't conform to any of the responses to stress described by Sayers (1950). In Peromyscus confinement appears to be a sudden, but continuous stress to which the adrenal responds by permanent enlargement without a return to normal. Since no other experimental stresses were studied in Peromyscus it would be premature to generalize further on this subject.

The observations on mice and rats reveal cellular differences in the histological zones of the adrenal cortex in these two groups. The cells of the zona fasciculata are larger and less sudanophilic in Peromyscus than in most house mice. The opposite condition is seen in glomerular cells. If increased cell size and decreased sudanophilia are characteristic of an increased secretory state, it would appear that Peromyscus has a more active fasciculata and Mus musculus, a more active glomerulosa. Since the fasciculata of albino mice is generally more sudanophilic than this same in wild mice, and the glomerulosa, less sudanophilic, it seems that wildness favors a more active fasciculata and domestication, a more active glomerulosa. These observations in mice are not the same as those in rats. Woods (1953) and Mosier (1952) found that both the glomerulosa and the fasciculata were less sudanophilic in the domesticated rat than in the wild rat. The difference was more obvious in the glomerulosa, since these lipid reactions in rats were related to the ability to resist experimental stresses known to affect both the glomerulosa and fasciculata, Richter (1954) interpreted this staining response as evidence indicating that the adrenal
secretions necessary both for salt metabolism and for reaction to stress are more effective in the wild than in the domesticated rat.

Christian (1955) found that when both albino mice and a strain of laboratory raised wild house mice were subjected to the stress of crowding, the latter attained peak adrenal weights at lower population sizes, and exhibited an increase in adrenal weight twice that of the albino mouse. This experiment suggests an increased sensitivity of the laboratory raised wild house mouse to stress, which is opposite to the results reported for wild rats. It thus appears that there are species differences in the sensitivity of wild animals to stress.

G. THE EFFECT OF CONFINEMENT IN CAPTIVITY ON ADRENAL STRUCTURE

The increase in adrenal size which accompanies confinement in captivity in both male and female *Peromyscus* is likewise seen in both male and female house mice. However, when measured in terms of adrenal-body weight ratio, the confinement effect is still statistically significant in *Peromyscus*, whereas it is reversed in house mice. Although this reduction in adrenal-body weight ratio in house mice confined in captivity is slight and not statistically significant, it is in agreement with a similar, and also slight, reduction in wild rats confined in captivity (Watson, 1907 b; Rogers and Richter, 1948; Christian and Ratcliffe, 1952).

The only other indication of an increase in adrenal size associated with confinement is the work of Nachols (1950) on rats. In this instance he observed a 48 per cent increase in adrenal-body weight ratio in the first twenty-four hours after captivity, followed by a return to 20 per cent above the wild caught level in the first week, and return to the
wild caught level by the seventh week (Figure 2). As previously mentioned these rats were caught in spring traps which held them by their extremities, producing fractures in many. Whatever the effect of confinement might be in this early period would be camouflaged by the traumatic response to the method of trapping. Nichols (1950) used wild rats shot in their natural environment as his control, and Rogers and Richter (1948) used rats which had been in traps for from six to twelve hours before being removed. The slight reduction in adrenal weight during confinement reported by the latter group could be a return to the wild level from an early hypertrophied state used as a control. Since the adrenal-body weight ratio for confined rats in Nichols' experiment returned to the normal level for wild rats, this example of adrenal hypertrophy during confinement is still not comparable to the sustained hypertrophy seen in Peromyscus. Nevertheless, a study of the effects in Peromyscus of the first few hours of confinement would clear up this point. Such a study is being planned.

That adrenal enlargement during confinement is not just an increase associated with increased weight or age is shown by the fact that the extent of hypertrophy in mice confined for only two weeks was the same as in those confined for eight months. Furthermore, the adrenals of Peromyscus born in captivity decrease in volume with age, after reaching their maximum volume at two months. Thus, the effect of confinement is opposite to that of ageing. The uniqueness of the response of Peromyscus to confinement is probably a reflection of its nervous temperament and points to the fact that there are species differences in response to stress in wild animals.

The presence of an X-zone in female Peromyscus which had been con-
fined in captivity for as long as six months suggests that this zone might contribute in part to the response to stress (fascicular and reticular zones also increase). In contrast to the mitotically inactive "juvenile cortex" of rats, the X-zone of mice is mitotically active (Whitehead, 1933 a; McPhail and Read, 1942; Mitchell, 1949). However, the adrenals of male Peromyscus do not have an X-zone and yet they undergo an even greater enlargement during confinement than those of the female. In the male increases in the fascicular and reticular zones account for the increase during confinement.

The medulla of both male and female Peromyscus appears to respond to the stress of confinement by increasing in volume, whereas that of Mus musculus does not. In view of the fact that medullary cell size is practically the same in all mice studies, alternations in mitotic rate must account for the differences seen.

D. THE EFFECT OF BREEDING IN CAPTIVITY
ON ADRENAL STRUCTURE

There is general agreement that the adrenals of wild rats born in captivity undergo a reduction in weight. Donaldson (1929) found that after a sizable initial drop in weight in the first generation, there was no further progressive reduction in weight. The first generation reduction resulted in an adrenal weight in the female only 14 per cent heavier, and in the male, 37 per cent heavier than in the albino rat (as compared with the two to three hundred per cent difference between wild and domesticated Norway rats). In contrast to this Rogers and Richter (1948) found that inbreeding in captivity produced only slight reductions in adrenal size and stated that many generations were re-
quired for more noticeable reductions. They did not, however, report the data from which these conclusions were drawn.

The first generation offspring of wild caught Peromyscus had an adrenal-body weight ratio 24 per cent higher than the parent group for males, and 8 per cent smaller for females. This represents considerably less adrenal tissue than found in the enlarged adrenals of the confined parent, but does not indicate any significant difference in adrenal-body weight ratio from the wild caught state. These results are in contrast to those of Donaldson (1929) but similar to those of Rogers and Richter (1943). It is obvious that adrenal measurements on succeeding generations of Peromyscus born in captivity will have to be made in order to ascertain what further changes, if any, will occur.

E. SIGNIFICANCE OF THE DIFFERENCES BETWEEN

WILD AND DOMESTICATED ANIMALS

Since most of the observed differences between wild and domesticated animals are due to variations in the cortex, one would expect that cortical secretions are important in enabling wild animals to adapt to the stresses of their environment. That the adrenal does aid an animal in resisting such stresses is supported by the fact that whenever both adrenalectomized and normal animals are subjected to stress producing situations, the former are less able to withstand the conditions of the stress (Wyman and tum Suden, 1929). However, since Konrad and Wyman (1950) have shown that even small masses of adrenal transplants can provide resistance to several common stresses, one might ask why large masses of adrenal tissue are necessary in wild animals? It is probable
that during chronic exposure to stress (as presumably exists in the wild environment), enlarged adrenals, with their additional cells, can meet the needs of the animals with less likelihood of exhaustion. With fewer cells each cell would have to maintain a capacity work load over a long period of time, and thus face the possibility of exhaustion. It has been shown in lipid stained preparations of the adrenals, that there are differences in the way wild rats and mice adapt to stress. The wild rat has a greater adrenal-body weight ratio than does wild Peromyscus. The wild rat is thus able to meet the needs of the environment and even to store precursor materials. This may account for the increased sudanophilia of the adrenal of the wild rat over that of Peromyscus. The adrenals of wild Peromyscus appear to meet environmental requirements with less enlargement, a more rapid rate of secretion, and less storage of precursor materials.

Richter (1952, 1954) has attempted to study the patterns of the process of domestication in wild Norway rats. After reviewing the available data on this subject, he postulated that during the process of domestication the adrenals became less important to the rat, whereas the gonads and the pituitary became more important. According to Richter, selection in the artificial environment of the laboratory favors those animals which are tame, gentle and fertile. Thus the tamer rats will be more likely to propagate, and thus the characteristics associated with tameness would be selected for, e.g., smaller adrenals and more active gonads. Richter postulated that in the domesticated rat the pituitary became enlarged in an effort to correct for the failing adrenal glands. As an alternative explanation Richter suggested that a reduced pituitary in
wild animals might also mean that the target organs have greater autonomy and need less stimulation from the pituitary.

In the present investigation gonadal and pituitary differences were not studied. However, Quay (1956) found that during confinement in captivity the pituitary of Peromyscus increased in size. I found that under similar circumstances the adrenals also enlarge in Peromyscus. None of those who studied the effects of confinement on rats included the pituitary in their measurements; however, Donaldson (1929) and Richter (1953, 1954) found an increase in pituitary size when wild rats were bred in captivity. Since adrenal and pituitary size were inversely related in rats, and the rat adrenal gave an opposite response to the stress of confinement than did Peromyscus, there is no reason to expect conformity between rats and mice in pituitary response to confinement stress. Since both the adrenal and the pituitary enlarged in Peromyscus during the period of confinement, it appears that there is no increase in the functional autonomy of this target gland in wild Peromyscus, as postulated by Richter (1952, 1954) for wild rats.

Since many pregnant females were found among the wild caught Peromyscus, it cannot be said that wild mice do not breed readily in nature. This is opposite to the condition found in rats which, according to Davis and Emlen (1946), have a very low reproductive rate in nature. However, after wild Peromyscus were confined in captivity there was almost a complete cessation of reproductive activity. In all cases in which Peromyscus were born in captivity they were the offspring of parents which were pregnant when caught. In a few instances when males were in the same cage with wild caught pregnant females, a second or third litter would appear, then breeding ceased. Several male and female captivity-bred
Peromyscus have been put together in an attempt to breed a second generation in captivity. Up to the present time none of these mice has reproduced. However, Dice (1947) has had considerable success in breeding Peromyscus in his laboratory at the University of Michigan. Further work will have to be done before one can intelligently describe the pattern of response which characterize wild mice during the process of domestication. I intend to pursue this type of investigation by continuing to study the effects of domestication on Peromyscus and the house mouse.

Since many of the observations on Peromyscus and house mice have shown definite species differences in adrenal characteristics, other endocrine glands should be examined to determine if similar species differences occur.

Richter (1952, 1954) has attempted to apply the data from his study of rats to the process of domestication in man. He assumes that primitive man had an endocrine system analogous to that of wild rats, and that modern man, who controls his environment with the aim of removing stresses, has an endocrine system analogous to the laboratory rat. Snyder (1954) comments:

"Can man properly be said to be domesticated, or even to have been subjected to the process of domestication? To reclaim from the wild state, to civize or domesticate, logically implies a reclamer -- a domesticator. Man himself plays this role in the usual examples of reclamation of animals and plants; can he then wear two hats in regard to his own species? Can he be both domesticator and domesticates?"

Snyder believes that at first natural selection probably favored strength, aggressiveness and freedom from physical and mental defect. However, according to Snyder (1954):

"... man possessed unique specializations which enabled
him to rearrange his environment in such a way as to pro-
vide even more efficiently the essentials and even the
comforts of life, and to minimize the imminent dangers,
thus allowing the high attainments in art, religion, state-
craft, law and science — in short, culture. Concurrently
the selective advantages of sheer strength and aggressiveness
probably declined."

Snyder believes that the one strong selective pressure to which mankind
has been continuously subjected during his period of domestication is
selection for educability. It thus appears that modern man is confront-
ed with more psychic stresses than physical stresses. This necessitates
a study of the effects of such psychic stresses on man’s endocrine sys-
tem, before it can be said that modern man is in the same stress-free
state as the laboratory rat.

It is this writer’s opinion that before such generalizations are
made more work should be done in studying the effects of domestication
in several species of wild animals.
VI SUMMARY

Volumetric measurements as well as histological and cytological observations were made on the adrenal glands of males and females of *Peromyscus leucopus* novieboracensis (wild caught, confined in captivity, and born in captivity), and *Mus musculus domesticus* (wild caught and confined house mice, and Carworth Farms W albino mice).

All groups of wild mice had higher adrenal cortex-body weight ratios than did the domesticated albino mice. In all groups but captivity bred *Peromyscus* this ratio was higher in females than in males. The medulla-body weight ratio was higher in *Mus musculus* than in *Peromyscus*.

Confinement in captivity resulted in an increase in both cortex- and medulla-body weight ratios in *Peromyscus* during the first eleven days of captivity with no apparent decrease for eight months. A decrease in these ratios was found to occur in house mice when confined in captivity.

The first generation of captivity bred male *Peromyscus* showed a slight increase in adrenal cortex-body weight ratio as compared to the wild caught form. In the female there was a slight decrease in this ratio during confinement. In neither case was the difference statistically significant.

The age at which there is a maximum adrenal-body weight ratio in male mice is one month in CFW albino mice, two months in captivity bred *Peromyscus*, and approximately four months in wild caught *Peromyscus*. In the female the maximum adrenal-body weight ratio occurs at two months in both the albino mice and *Peromyscus* and is maintained at a near maximum level through the fourth month.
The zona glomerulosa is wider in CFW albino mice and house mice than it is in any of the groups of Peromyscus. In all groups of Peromyscus, except the female born in captivity, the zona fasciculata is wider than in either house mice or CFW albino mice. The zona reticularis of all groups of male Peromyscus was wider than in any group of Mus musculus. In the female, however, the zona reticularis varied so much that there was no consistent relationship between this zone and the conditions of the environment.

In both males and females the X-zone developed to greater proportions in Peromyscus than in the albino mice, and, at its peak, the X-zone was three times wider than in CFW albino mice. An X-zone was observed for as long as six months in many female Peromyscus confined in captivity. In both Peromyscus and Mus musculus the X-zone was replaced by a connective tissue band separating the cortex and the medulla.

All groups of Peromyscus had larger cells in the zona fasciculata than were found in any group of Mus musculus. With the exception of wild caught female house mice, all Mus musculus had larger glomerular cells. There was a striking similarity in medullary cell size in all mice studied. A greater variation in cell size was found in wild mice than in laboratory mice.

The use of Sudan Black B as a lipid indicator revealed a greater concentration of lipid droplets in the adrenal cortex of albino mice than in house mice. Peromyscus had fewer and finer lipid droplets than any group of Mus musculus. If the amount of sudanophilia bears an inverse relationship to adrenal activity, then the adrenals of wild mice are more active.
than those of albino mice.

The difference in adrenal size between wild and domesticated rats is greater than the difference between Peromyscus and CFW albino mice. Moreover the adrenals of wild rats respond to prolonged confinement by a reduction in size (Rogers and Richter, 1949) ; fail to give responses to other forms of stress (Woods, 1955, 1954; Richter, 1952); exhibit marked decreases in volume (Donaldson, 1929) or slight decreases (Rogers and Richter, 1948) in the first generation born in captivity; and are more sudanophilic than the adrenals of domesticated rats. It is, therefore, apparent that there are species differences in the way wild rats and Peromyscus respond to changes in the environment. Similar species differences are apparent when the responses of Peromyscus and house mice are compared.

Any attempt to discover patterns in the domestication process in mice must await the study of future generations of laboratory bred wild mice.
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COMPARATIVE MORPHOLOGY OF THE ADRENAL GLANDS
OF WILD AND DOMESTICATED MICE AND THE EFFECT
OF CAPTIVITY ON ADRENAL STRUCTURE

Abstract of a Dissertation

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

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VIII ABSTRACT

Several previous investigators have shown that the adrenal glands are much larger in wild Norway rats than in domesticated Norway rats. In order to determine whether such variations are peculiar to rats, or represent a generalization in nature, the adrenals were examined in other species of rodents. For this purpose the adrenals of white-footed mice (Peromyscus leucopus noveboracensis), house mice (Mus musculus domesticus), and Garworth Farms - W albino mice (Mus musculus domesticus) were compared. Furthermore, an attempt was made to determine the effect on adrenal structure when wild mice were confined in captivity or bred in captivity.

Experimental procedures

All wild mice were caught in live traps either in homes or in and around the wooded areas of Metropolitan Boston. These traps were set in the evening and picked up the following morning.

Volumetric determinations were made by projecting every tenth section of serially sectioned mouse adrenals and tracing the outline of cortex and medulla of left and right glands. The areas of the magnified tracings were measured with a polar planimeter and plotted on graph paper, from which volume determinations were then made. The depth of each histological zone of the adrenal cortex was measured linearly on the largest slice of each gland. A comparison of cell size was made by means of ocular measurements of nuclear diameter and
tissue compactness. A lipid stain, Sudan Black B, was used as an indicator of the functional state of the adrenals of most groups of mice studied.

Observations

Inasmuch as wild mice are considerably lighter in weight than domesticated laboratory mice, adrenal-body weight ratios were used as a basis of comparison of the different groups of mice.

All groups of wild mice had higher adrenal cortex-body weight ratios than did CFW laboratory mice. These ratios, expressed in cu. mm. of adrenal tissue per gram of body weight, varied from .140 - .255 in wild males, and from .194 - .278 in wild females. In CFW albino mice the adrenal cortex-body weight ratio was .064 in males and .168 in females. The medulla-bodyweight ratios were higher in all groups of Mus musculus than in Peromyscus, regardless of the condition of the environment. These ratios varied from .023 - .032 in Mus musculus, and from .017 - .023 in Peromyscus.

When wild caught Peromyscus were confined in captivity, both cortex- and medulla-body weight ratios showed marked increases during the first eleven days of captivity with no apparent decrease for eight months (in males, 62 per cent and 46 per cent, respectively; in females, 31 per cent and 35 per cent, respectively). When wild caught house mice were confined in captivity the ratios of cortex and medulla to body weight were reduced (in males, 54 per cent and 16 per cent, respectively; in females, 4 per cent and 10 per cent, respectively). Apparently con-
finement acts as a stress in Peromyscus and not in the house mouse, indicating that there are species differences in the way wild animals respond to stress.

The first generation offspring of male Peromyscus born in captivity showed no statistically significant difference in the adrenal cortex-body weight ratio over the wild caught form. Thus breeding in captivity apparently results in a return of the adrenal to the wild state as compared to the much enlarged adrenal of the confined parent. A study of the effects of further inbreeding of wild mice in captivity will be necessary in order to determine if any additional changes in adrenal size will result from a shift from the wild environment to the laboratory environment.

The rate of adrenal development was different in several groups of mice studied. In males the age of maximum adrenal-body weight ratio is one month in albino mice, two months in captivity bred Peromyscus, and approximately four months in wild caught Peromyscus. In females the maximum adrenal-body weight ratio occurs at two months in albino mice and Peromyscus. The adrenal-body weight ratio is maintained close to this maximum level through the fourth month in females.

Histological differences were observed in most groups of wild and domesticated mice. A wider zona glomerulosa was seen in CFW albino mice and house mice than in any of the groups of Peromyscus. Since the zona fasciculata is the most extensive zone in the adrenal cortex, variations in this zone account for most of the differences between wild and domesticated mice. In all groups of Peromyscus, except the
female born in captivity, the zona fasciculata was wider than in either
house mice or CFW albino mice. The zona reticularis of all groups of male
Peromyscus was wider than in any group of Mus musculus. In the female,
however, the reticularis varied so much that there was no consistent
relationship between this zone and the conditions of the environment.
In both male and female the X-zone developed to greater proportions in
Peromyscus than it did in laboratory mice, and at its peak, the X-zone
was three times wider than in CFW albino mice. Furthermore, an X-zone
was observed for as long as six months in many female Peromyscus which
had been confined in captivity. Since the X-zone of the mouse adrenal is
mitotically active it is possible that this zone responds to environ-
mental stresses. However, since little is known regarding the function
of this zone, no definite conclusions can be drawn from these changes.
In all mice a band of connective tissue replaced the degenerating X-zone.

Cytological differences between the adrenals of wild and domesticated
mice were also observed. In all groups of Peromyscus the cells of the
zone fasciculata were larger than in any group of Mus musculus. With
the exception of wild caught female house mice, all Mus musculus had
larger glomerular cells than found in Peromyscus. The cells of the adrenal
medulla exhibited a striking uniformity in size in all groups of mice.
Considerably more variation in cortical cell size was seen in Peromyscus
than in house mice, and the latter showed more variation than did CFW
albino mice. The cells of CFW albino mice were slightly more sudano-
philic than those of house mice, and the cells of the latter were con-
siderably more sudanophilic than those of Peromyscus. An increase in
the number and size of cells, and a decrease in sudanophilia are generally accepted as indices of increased activity of adrenal tissue within a given species. It might be concluded, therefore, that the adrenals of Peromyscus are more active than those of House mice, and the adrenals of the latter are more active than those of CFW albino mice. According to the same criterion, the glomerulosa is probably more active in Mus musculus, and the fasciculata, in Peromyscus.

Since there are many variations in the ways Peromyscus, house mice (as shown in this investigation), and wild Norway rats (as shown by Rogers and Richter, 1948) respond to the factors present in the wild and laboratory environment, any attempt to discover the general patterns in the domestication process must await more detailed study in mice as well as in other animal species.

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