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A Study of Autofluorescence and Its Comparative Distribution in the Central Nervous System of Three Genera of Mice

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A STUDY OF AUTOFLUORESCENCE

AND ITS COMPARATIVE DISTRIBUTION

IN THE CENTRAL NERVOUS SYSTEM

OF THREE GENERA OF MICE

BY

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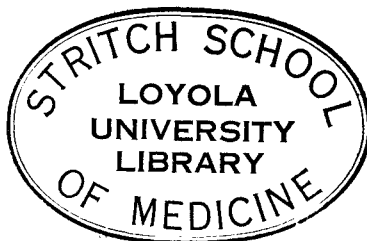
BIOGRAPHY

Thomas J. Sobotka was born in Baltimore, Maryland on August 16, 1942. He was graduated from Loyola High School (Towson, Maryland) in 1960 and, subsequently, from Loyola College (Baltimore, Maryland) in 1964 with the degree of Bachelor of Sciences.

In September of 1964, he began his studies as a Trainee of the National Institutes of Health in the Department of Pharmacology, Stritch School of Medicine.

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INTRODUCTION

One of the most important histological techniques recently developed is the Hillarp-Falck method of localizing catecholamines and related biogenic amines in tissue samples (16). Generally, this technique entails freeze-drying the tissue and exposing it to formaldehyde vapor. This results in the formation of two specific compounds which will fluoresce either green or yellow upon exposure to Ultra Violet light (UV light) and which represent catecholamines and 5-hydroxytryptamine, respectively (17). However, one of the complications of this technique involves distinguishing between the chemically induced fluorescence, i.e., monoamine fluorescence (especially the yellow fluorescence representing 5-hydroxytryptamine) and an inherent fluorescence (17), called autofluorescence (AF) (36), which is also primarily yellow and found in a vast majority of tissues of most common laboratory animals (19, 25, 35, 36, 41, 45) and tunicates (28) as well as man (5, 24, 31, 40). These tissues include lymphocytic tissue (28, 30, 35, 36, 41), kidney (36, 41), skeletal muscle (2, 13), uterus (18, 19, 32, 36), nervous tissue (5, 22, 24, 25, 31, 36, 40, 41, 45) and many others (36, 41).

To resolve this problem of distinguishing AF from the induced monoamine fluorescence, pharmacological, chemical, and physical criteria have been developed (6, 15, 17). However, it

should be recognized that the application of these criteria might be considerably enhanced by a previous knowledge of the general deposition of the AF including its location, intensity, and constancy in different animals and related genera. Therefore, it will be one of the objects of this paper to clarify the deposition of the AF in the Central Nervous System (CNS) of mice.

The AF of the CNS, as well as that of the other tissues of the body, has been found to be restricted primarily to cytoplasmic granules. The nucleus was observed to be devoid of such granules (13, 25, 35, 36). Extracellular AF granules have also been found, e.g., in the cerebellum (40). These AF granules were histochemically identified in neuronal tissue as lipofuscin pigments (also called senility or wear-and-tear pigment) and their non-pigmented precursor substances (7, 28, 31). However, Sainte-Marie (35, 36) in his investigation of a wide assortment of tissues has found no specific correlation between AF and lipofuscin pigmentation. Similarly, Fuxe and Nilsson (19) found that the AF granules of the mouse uterine epithelium are not naturally colored. Therefore, it seems that the AF granules of the body can perhaps be found at different stages of development leading to the formation of pigmented lipofuscin granules. In support of this, Pearse (34) stated that the early precursors of lipofuscin, though autofluorescent, have relatively little or no pigmentation as opposed to the well-developed, yellow-brown lipofuscin pigment.

To further delineate the chemical make-up of AF granules, various physical and histochemical analyses have been employed. The following histochemical tests are some of the known positive reactions shown by AF granules: Carbol Fuchsin (a cationic dye indicating acid-fast material) (7, 13, 24, 28); Luxol Fast Blue (a preferential stain for choline-base phospholipids) (24); Alloxan-Schiff-Luxol Fast Blue (stains protein material) (24, 25), Sudan Black (a lipid-specific stain) (7, 19, 24, 28, 34, 35, 36, 44); Chrome Hematoxylin (stains general neurosecretory substances) (7); Periodic Acid Schiff (stains carbohydrates and/or proteins) (4, 7, 19, 28, 30, 34, 35, 36, 44); Gomori's Method of Aldehyde Fuchsin (this dye has an affinity for proteins and mucopolysaccharides, especially the highly sulfated ones, as well as neurosecretory substances and acid phosphatase) (7, 14, 24, 30); Toluidin Blue (a cationic dye which may stain melanin, lipid pigments and nucleic acids) (35, 36); and Indophenol (stains lipofusin pigment due to the preferential solubility of the non-ionized form of this dye in the pigment deposits) (1). However, all of these reactions are not observed universally in all AF granules and, as a result of extensive work, it is clear that "the more we evaluate the staining and histochemical reactions of these neuronal inclusions and interneuronal material, the more evident it becomes that one set of properties cannot be ascribed to all of them" (24).

Issidorides and Shanklin (24) found that fluorescent brain cells of the same cell group (nucleus) have very similar properties but that fluorescent cells from different nuclei differ in many respects. They concluded that the only two common properties of all AF granules are a white-yellow fluorescence in UV light and a positive reaction to Gomori's method of aldehyde fuchsin. The rest of the staining reactions vary. However, even such a generalization is not necessarily true for some tissues, e.g., in uterine epithelium the AF granules do not stain at all with aldehyde fuchsin (19). Therefore, the position held by Sainte-Marie (35), that the only feature characteristic of the AF granules is the fact that they do autofluoresce in UV light, seems to be the only valid generalization that can be made of these granules. In support of this, Whiteford and Getty (45) have found that fluorescence microscopy can detect granules not observable with routine stains. Like the pigmentation discussed above, the variability in staining can be explained by Pearse's views on the histogenesis of lipofuscin (34). According to this scheme of graded oxidative development of lipofuscin, the basic precursor substance, which may be AF, is some type of lipid, e.g., a triglyceride or a phosphatide. As this lipid precursor commences to undergo oxidation, its histochemical and physical characteristics become progressively altered as is suggested by the fact that its "solubility in fat solvents diminishes as its

ability to dissolve fat stains diminished". Pearse considers the product of early lipid oxidation to be auto-oxidizing lipids. The intermediate stage between auto-oxidizing lipid and lipofuscin consists of a ceroid type of material. As oxidation progressess, pigmentation usually, but not inevitably, increases. Also, autofluorescence intensity increases concomitantly with lipid oxidation. The final product, if oxidation is carried to completion, is therefore, the highly autofluorescent lipofuscin pigment.

D'Angelo et al. (7) were the first researchers to utilize histochemical findings in an attempt to describe the lipofuscin granules in neuronal tissue. They arrived at the conclusion that these AF granules are composed of three components: 1) an acid-fast, Periodic Acid Schiff-positive core, 2) an envelope of a non-acid fast, sudanophilic outer substance which also contained some positive PAS reacting material, and 3) a diffuse ground substance rich in cystine. This work was followed up by Issidorides and Shanklin (24) who characterized the neuronal AF granules as being composed of a complex of lipid (Sudan Black positive) and protein (Alloxan Schiff positive) arranged in successive concentric layers. The protein and an acid-fast material (carbol-fuchsin) constituted the core of the granule with the lipid layer arranged in a radial pattern around this core. They determined this structural relationship by birefringence studies. They also

offered some evidence that phosphatides or cerebrosides are present in the lipid layer of the granule. They arrived at the latter conclusion, since the positive reaction with Sudan Black on formalin-fixed sections signifies "formal-fixed" lipid which is usually phosphatide or cerebroside. The presence of a phospholipid component was also found in the rat uterus AF granules by Fuxe and Nilsson (19) who concluded that the positive PAS and Performic Acid Schiff (PFAS) reactions point to the presence of unsaturated lipids, e.g., phospholipids. Koenig (26) also gave supporting evidence for the presence of a phospholipid component bound to protein in AF granules of the brain when he found that these granules can be removed from fresh, but not fixed, tissues by a chloroform:methanol (2:1) solution. The third constituent of the granule mass found by Issidorides and Shanklin (24) was the same type of ground substances as found by D'Angelo (7). In both cases it was found to be stained by aldehyde fuchsin (Gomori's stain).

Due to the diversity of the histochemical reactions of the AF granules of diverse tissues, the above description of inner protein and outer lipid layers should be confined to granules from the specific structures where they were found, namely the thalamus (7) and the inferior olivary nucleus (24). Only further work of the kind described above will indicate whether the other AF granules of the body possess this same structured arrangement

of protein and lipids.

Other aspects of these AF granules which are of interest are their origin and their function. There are three schools of thought as to the origin of these granules: 1) that they originate from mitochondria (11, 23), 2) that they come from the Golgi apparatus (3, 21), and 3) that they are altered lysosomes (14).

The original proponent of the mitochondrial origin of AF granules was Hess (23). From electron microscopic studies he suggested that the lipofuscin pigment of adult and senile ganglion cells arose from swollen, vacuolated mitochondria. The characteristic ultrastructure of the "old age pigment", as determined by Duncan (11), seemed to support Hess's position. Duncan found small particles of high electron density (osmiophilic), most of which were laminated with a "series of alternate dense and light bands in parallel array". He also suggested that some proteinaceous and/or lipid materials might be implicated in these particles. These bodies were further shown to have a double membrane similar to the mitochondria and in some micrographs they were seen attached to mitochondria with no visible intervening membrane.

On the other hand, Gatenby (21), using phase contrast light-microscopy, has demonstrated a very close relationship between these "senility pigments" and the canals of the Golgi apparatus. Bondareff (3) found pigment-associated vacuoles,

which Hess had identified as swollen mitochondria. However,
Bondareff denied this identification on the basis of the following
information: 1) that the pigment-associated vacuole is bound by
only one membrane and not two, characteristic of mitochondria,
2) that the internal structure of the vacuoles in no way resem-
bles that of mitochondria, and 3) that the single limiting mem-
brane around the vacuoles is usually intact between the vacuole
and the pigment. Due to the association of small vesicles and
membranes (characteristic of the Golgi complex) with the peri-
phery of the pigment-associated vacuoles, Bondareff agreed with
Gatenby's contention of a Golgi complex point of origin for the
"senile pigment".

In studying hepatocellular fine structure, Essner and
Novikoff (14) noticed the deposition of lipofuscin granules in
specific cellular organelles. The fine structure and the
presence of acid phosphatase activity (Gomori's aldehyde fuchsin)
identified the organelles as lysosomes (8) and offered suggestive
evidence for Essner's theory of the lysosomal origin of the lipo-
fuscin granules. Similar findings were also reported by Ehrlich
(12) and Novikoff (33) for the liver. Koenig (25) also found
acid phosphatase activity in AF granules in neuronal, glial, and
skeletal muscle lysosomes. However, he also found AF granules
which were not contained in lysosomes and which did not possess
acid phosphatase activity (26). Metcalf (30) supported Koenig's

findings by obtaining similar results in thymic cells. Also,
Fuxe and Nilsson (19) found no positive reaction for acid phos-
phatase in any of the AF granules of the uterine epithelium.
Such evidence might seem to dispute Essner's theory. However,
since there is some contention as to whether or not the lack of
acid phosphatase activity alone is an adequate parameter to
indicate the absence of lysosomes [Koenig (26) holding the
affirmative and DeDuve (8) the negative view], the lack of acid
phosphatase activity in association with lipofuscin granules does
not necessarily negate Essner's theory of the lysosomal origin of
lipofuscin granules.

Evidently, the resolution of the question of lipofuscin
origin must wait further work. The problem of their func ion
also is not clear. Four primary possibilities have been pre-
sented. According to these theories, the lipofuscin is 1) an
aging pigment, 2) a carrier pigment, 3) a pigment of stress, or
4) a storage pigment.

1) The "aging pigment" theory. This theory considers the
AF granules to be "aging pigments" (also known as wear-and-tear
pigment and senility pigment) (9, 20). The formulation of this
theory was based on the finding that the lipofuscin pigments are
not found in young animals but that they appear later in life and
then irreversibly increase in number with the age of the animal.
This phenomenon has been interpreted to mean that these pigments

are metabolic break-down products which accumulate intracellularly with age. Since Dolley (9) has found that neurons continue to react to the end of their life cycle, these pigments apparently do not interfer with cell function.

2) The "neurosecretory" theory. Shanklin, Issidorides and Nassar (40) suggest that the neuronal pigment granules are carriers of some type of neurosecretory material. They suggest that the cerebellar Purkinje cells produce these lipofuscin granules complexed with some neurosecrtion. They are then passed out of the cell into the interstitial spaces and from there they migrate to nearby blood vessels where the unstainable neurosecretion is released into the blood stream but the lipofuscin granules remain outside the blood vessels. These authors also suggest that this association of lipofuscin granules with neurosecretion might be a widespread phenomenon throughout the central nervous system. However, they also hold that, if this is so, the mechanism of secretion might very possibly vary in different areas of the nervous system, e.g., in the inferior olivary nucleus of the medulla the extracellular lipofuscin granules have been found in great abundance, not near blood vessels but, in synaptic fields, thus indicating another possible type of neurosecretory mechanism (24).

3) The "pigment of stress" theory. In 1960, Sulkin and Srivanij (44) proposed the question of whether the lipofuscin

granules are due to an "intrinsic factor of aging or to some extrinsic environmental factors in which the factor of aging is coincidental". In an attempt to answer this question, they exposed very young animals, who normally have a minimal amount of lipofuscin pigment in their nerve cells, to an altered external environment such as anoxia, vitamin E deficiency, acetanilid and ACTH for prolonged periods of time. In all cases the experimental animals showed an excess of lipofuscin pigment in their nerve cells over the control animals and this pigment production was demonstrated to be a non-reversible phenomenon, i.e., the pigment remained even after the experimental environment was returned to normal. This evidence strongly suggested to them that the production of the so-called "senility pigment" of the nervous tissue was really the result of stressful situations caused by changes in the extrinsic environment such as nutritional deficiency and emotional and physical stress and that this pigment merely accumulates coincidentally with age.

4) The "storage pigment" theory. The most recent theory of function was proposed by Fuxe and Nilsson (18, 19). They noted that a spayed animal had a great deal more AF granules in the uterine epithelium than did non-spayed controls. The fact that this accumulation of AF granules was reversible, as opposed to the non-reversible lipofuscin in nerve cells shown by Sulkin and Srivanij (44), was demonstrated when either an estrogen or

insulin was given to the spayed animals. Both substances caused a decrease in the number of AF granules. On the basis of such experimental evidence and since the AF granules are known to be rich in phospholipids, these investigators proposed that the AF granules in the uterine epithelium seem to constitute a bulk of easily available material usable during cellular activities caused by estrogen and/or insulin, i.e., as cell activity increases, less granules will be seen, since they are being used by the cell; conversely, as cell activity decreases, more granules will be seen, which granules are the storage form. The authors specifically mentioned two possible mechanisms: either the phosphatides of the AF granules are used to facilitate the decomposition of glycerides in the cytoplasm, since such decomposition involves the use of phosphatides, or they are used by the cell for cell membrane synthesis, since estrogen initiates growth of the uterine epithelium.

No new evidence has been presented to substantiate the first theory of "aging pigment". In fact the evidence obtained which led to the formulation of the last two theories mentioned seems to make the "senility pigment" theory no longer justified (24). Additional evidence against this theory is the fact that many investigators, including myself (confer Results), have found that AF does indeed occur in young animals (5, 12, 35, 36) although not in the same quantities as in older animals.

Issidorides and Shanklin, on the other hand, gained some support for their theory of a neurosecretory function from the work of Gatenby (21) and Bondareff (3) both of whom found a very close relationship between the lipofuscin granules and the Golgi apparatus, which cell organelles are known to involve synthesis of secretory products (46). Further confirmation of their theory is offered by the work of Schadewald (37) who investigated lipofuscin in the cerebral cortex. He found granules that were formed in nerve cells, extruded into the interstitum and then lodged on the blood vessels. Also, suggestive evidence in support of this theory was found during the course of the present research. As will be explained later in this paper, high amounts of AF granules were located around parts of the border of the ventricles in the brains of mice in those areas known to be rich in blood vessels. However, this theory seems to be contradicted by the evidence obtained for the accumulation and non-reversibility of the lipofuscin pigment caused by stressful situations (44). Also, if the theory of Shanklin and Issidorides is true for nervous tissue lipofuscin granules (on which type of lipofuscin their work and theory is based), there still remains the question of function of such granules in non-nervous tissue, such as muscle, uterus, lymphatic tissue, etc.

The theory that lipofuscin is an indicator of environmental stress may gain some support from the work of Einarson (13) who

found that a vitamin E deficiency causes a marked increase in
nervous and muscle cell content of AF granules. This AF was also
shown to be non-reversible.

The fact that there is a direct relationship between the
rate of growth of an animal and the amount of lipofuscin granules
(10) in a normal environment is good supporting evidence for the
theory that lipofuscin is intimately related to growth (35).
Here, the effects of stress do not contradict the validity of
this theory, since these two theories seem to deal with two
different types of lipofuscin granules. On the one hand, the
"stress pigment" is non-reversible and apparently very stable (44)
and on the other hand, the "storage pigment" is reversible and
apparently more labile (18).

The data accumulated thusfar on lipofuscin granules seems
to prevent the application of only one of the above four theories
to all the lipofuscin granules of the body. Possibly each organ
of the body has a specialized task for its own special type of
lipofuscin. Such a function might include one of the above
proposed theories, a combination of some or all of them, or even
an entirely unique function.

MATERIALS AND METHODS

A. <u>Comparison Study of AF Location</u>

In the comparison study of occurrence and relative amounts of autofluorescent granules (AF granules), three genera of mice were used - <u>Mus musculus SC-1</u>, <u>Peromyscus maniculatus Bairdii</u> and <u>Perognathus sp</u>. (one mouse of each genus mentioned was used to compile Table 1). The mice were stunned by a blow on the head and decapitated. The brains were immediately removed and quick-frozen in an International-Harris Cryostat. Cross-sections, 10-20 micra thick, were made of the brains with the cryostat. The sections were placed on cold, precleaned, microscope slides and fixed thereon by gently warming the slide with the investigator's hand.

Alternate sections were placed on separate slides: one, for inspection under Ultra Violet light (UV light) and the other for staining with thionine. Since fluorescence only occurred when the tissue sections were thoroughly dry, the slides to be used for UV inspection were dried over phosphorous pentoxide for approximately 1/2 to 1 hour in an enclosed container. They were then mounted in non-fluorescent liquid paraffin and a cover-slip applied. These sections were subsequently examined under UV light using a high pressure mercury vapor bulb (as the UV light source) with a step-up transformer. Three filters were simultaneously used: 1) a clear filter of heat absorbing glass (with

water), positioned immediately in front of the mercury vapor lamp, 2) a CG554 blue excitation filter, which has a maximum transmittance at 420 millimicra, positioned between the heat absorbing filter and the microscope mirror (a mercury mirror), and 3) a barrier filter, placed between the ocular and objective lenses of the microscope, which has a cutoff point at 520 millimicra, thereby, preventing light in the UV range, but not in the visible range (above 520 millimicra), from coming through. The mercury-covered, single surface reflecting mirror, mentioned above, was used to facilitate UV light deflection onto the microscope stage. (All of this UV apparatus was supplied by Cooke, Troughton and Simms, Highland Park, Illinois.)

In these studies, the AF was not found to deteriorate for at least several weeks in mounted slides. Such slides were either examined immediately or several days later with UV light.

Throughout this study, AF intensity was subjectively evaluated as follows. The most intense AF observed (olfactory bulbs of SC-1) was used as the reference intensity. The degree of intensity of other AF regions was determined by comparing the AF of these regions with the maximum AF intensity of the olfactory bulbs of SC-1. Each day, before recording any AF intensity, the investigator allowed his vision to adapt to the dark for about 15 minutes and then referred frequently to the reference areas as new material was evaluated. The reproducibility of such sub-

jective determinations of AF intensity by other investigators is entirely possible in light of the fact that the relationship between s∩nsory evaluations (e.g., vision) of the energies of the environment (e.g., light intensity) is relatively constant from one individual to another (43).

The alternate sections that were stained were used to identify the fluorescing areas. These sections were air dried for 5 minutes; they were then run through the following staining procedure which is a modified form of the "thionine stain for frozen sections" found in "Staining Procedures" (42). After air drying for 5 minutes to assure fixation of the frozen sections to the slide, they were emersed in 0.5% thionine (C.C.) in 20% alcohol, washed in tap water, dehydrated in an alcohol series, counter-stained with eosin Y in 95% alcohol, then further dehydrated in xylene and mounted with Permount.

B. Drug Study

Adult male Mus musculus CF-1 mice were used for the reserpine study. A depleting dose of reserpine (Sandril), 25 mg/kg (29), was injected intraperitoneally (i.p.). Four hours later the mice were sacrificed, their brains removed and frozen immediately as described above. Sections of olfactory bulb, midbrain, cerebellum, pons and medulla were made. These regions were used for this as well as for all subsequent studies due to their relative constancy of a high amount of AF between individual mice.

Alternate sections were made and stained as described in the procedure above.

Similarly, the pargyline: DL-3, 4-dihydroxyphenylalanine (DOPA) study was carried out using the adult male Mus musculus CF-1 mice. A dose of 100 mg/kg pargyline was injected i.p.; 18 hours later, 300 mg/kg DOPA was given i.p., both being effective doses (6, 29). Approximately one hour later, after definite sympathetic signs had occurred, such as piloerection and pseudo-exophthalmus, the animal was sacrificed, its brain removed, frozen and handled as described above.

C. Age Study

An age study was carried out with Mus musculus CF-1 mice at ages of 1 day, 9 days, and adults. All brains were handled as described in Section B.

D. Comparative Study of AF Lability in UV Light and Chloroform: Methanol (2:1)

Several physical properties of the AF granules were studied using sections of olfactory bulb, midbrain, cerebellum, and pons of the adult male Mus musculus SC-1 mouse. The effect of prolonged exposure to UV light on the AF granules was determined by leaving each section on the microscope in the path of the UV light for 5, 15, 30 and 60 minute periods. The percent decrease in AF intensity was subjectively evaluated in the following

manner.

As described above, the intense AF of the olfactory bulbs of SC-1 mice was designated as 100% intensity. The intensities of the rest of the fluorescing brain areas were similarly expressed as percent (%) with reference to the intense AF of the olfactory bulbs of SC-1 mice. Also, the investigator allowed his eyes to adapt to the dark before making any determination of AF intensity. Since these readings of depletion are subjectively evaluated, the amount of depletion is approximated.

The solubility properties of these AF granules were examined by emersing the selected sections in a chloroform:methanol (2:1) solution which, according to Koenig (25), is the only lipid solvent in which this AF material is soluble and in acetone, a less potent lipid solvent, for 5, 15 and 30 minutes. Again, percent decrease in AF intensity was subjectively evaluated as described above.

The photomicrographs were taken with Dupont high speed rapid reversal 35 mm film with exposure times of 1-5 seconds. The sections photographed were magnified 40 times on the microscope and then enlargements were made from the 35 mm slides.

RESULTS

A. General Location, Description and Relative Intensity of Autofluorescence

In all three genera of mice studied, namely Mus musculus SC-1 and CF-1, Peromyscus maniculatus Bairdii and Perognathus sp., autofluorescence (AF) appeared as a yellow-green color. This AF was granular (plates 1 and 2) and localized in the cytoplasm of the neuron cell bodies and sometimes in some non-nervous cell bodies. No AF granules were observed to occur within the nucleus of any of these cells. The AF granules ranged in size from 2 micra to less than a fraction of a micron. In most cases, these granules surrounded the nucleus and filled the cytoplasmic area. But occasionally, the granules were found on only one side of the nucleus. Due to a low resolution, it was not possible to discern whether or not these AF granules occurred extracellularly in these genera of mice.

Fluorescence was most obvious in large neurons, especially of the midbrain, pons, medulla, cerebellar Purkinje cells, and spinal cord of all genera, and uniquely in the olfactory bulbs of Mus musculus SC-1 and CF-1 but not in Perognathus or Peromyscus. AF was present to a variable extent in other brain regions. Also, all of the cells of the brain did not contain such AF granules, but those cells that did fluoresce varied in their intensity from very high to faint. Another observation was that the AF cells

generally appeared in groups of various densities ranging from very high to very low (plate 3). Such AF groups were usually found to occur bilaterally. However, isolated fluorescing cells were also found scattered throughout the brain and appeared to correspond to scattered neurons and neuroglia. This was especially noted throughout the cortex.

Generally, such areas as fiber tracts and fiber bundles were devoid of any specific granular AF, e.g., anterior commissure and corpus callosum of all genera. However, in certain instances, e.g., the internal capsule and fiber tracts associated with the trapezoid nucleus of SC-1, such AF was seen, but these AF cells which were about 8 micra in size appeared in a beaded fashion and were presumably supporting cells.

A background, non-granular, diffuse AF was present throughout the brain. Its intensity ranged from low to very low.

B. Comparative Distribution and Intensity of AF in Three Genera of Mice

Table 1 represents those areas of the mouse brain in which AF cells were seen. Since a definitive atlas of mouse brain is not available, regions of mouse brain were compared to analogous regions of the rat brain for identification. ("The Rat Brain", a stereotaxic atlas (27) as well as "Craigie's Neuroanatomy of the Rat" (48) were used as the comparative texts.)

The density of the groups of AF cells in each area was sub-

jectively evaluated as very low, low, medium, or high. Similarly,
the average relative intensity of each AF cell group was desig-
nated as faint, low, medium, or high.

For the most part, all three genera of mice varied from
each other with respect to the location, average cell size, cell
density of fluorescing regions and intensity of specific regional
AF, for example, the capsula interna of Mus musculus SC-1
contained an aggregate of fluorescing cells with an average AF
cell size of 6 micra, a cell density of low and an AF intensity
of medium; the same structure in Peromyscus maniculatus Bairdii
had an aggregate of AF cells of an average cell size of 20 micra
with a cell density of medium - high and an AF intensity of med-
ium, while Perognathus sp. had only a general occurrence of AF
cells at the site of its capsula interna. Table 1 shows many
more such variabilities between the three genera of mice studied.
However, these mice did resemble one another in several ways:
1) almost all of the large neuronal cell bodies of the brain
stem, especially of the pons and medulla, contained at least a
moderate amount of visible AF granules; 2) all of the cerebellar
Purkinje cells contained some degree of AF; 3) the cells of
cerebellar nuclei usually fluoresced; 4) the border of the IV'th
ventricle contained medium - high AF in all mice. The rest of
the brain areas contained variable and inconsistent amounts of
AF granules and AF cells, as mentioned above.

These same variations and similarities characterized a
comparison of two mice from the same strain of Peromyscus
maniculatus Bairdii.

With one notable exception, the above similarities and
variations also characterized a comparison of two mice from the
SC-1 strain of Mus musculus as well as the comparative location
c brain AF cells between two strains of Mus musculus, namely,
SC-1 and CF-1. Unique to these two strains of Mus musculus were
highly fluorescent cells in the olfactory lamina glomerulosa and
tractus olfactorius lateralis, dorsalis and ventralis. They
appeared repeatedly in all of the mice of both strains. However,
the intensity of fluorescence of these areas in the CF-1 strain
was reduced in comparison to that of the SC-1 strain.

Variability of occurrence of this AF not only occurred
between animals but in some instances it was further noted that
the same structure of the same mouse varied in AF cell density
and intensity from one section to the next, e.g., in Peromyscus
maniculatus Bairdii the AF of an anterior section of the cortex
pyriformis was described as follows: approximately 12 micra aver-
age AF cell size, low - medium density and faint - low intensity.
But a posterior section (sections were about 20 micra apart) of
the same structure appeared: approximately 15 micra average AF
cell size, medium - high density and medium - high intensity.

While the average intensity of the AF was generally in the

low to medium range, it is noteworthy that in several areas the AF intensity was of a high to very high nature: 1) the lamina glomerulosa; tractus olfactorius lateralis, dorsalis, and ventralis; a region lateral to the claustrum and the border of the aqueduct of Sylvius and the IV'th ventricle all of <u>Mus musculus SC-1</u>; 2) the nucleus parafascicularis of <u>Peromyscus maniculatus Bairdii</u>; and 3) the nucleus ruber of <u>Perognathus</u> (Table 1).

C. <u>Effects of Reserpine on AF</u>

In order to test the possibility that this AF material of the mouse brain might be one of the adrenergic compounds found in brain tissue, namely norepinephrine, dihydroxyphenylethanolamine, or serotonin, <u>CF-1</u> mice were treated i.p. with a depleting dose of reserpine, 25 mg/kg (6, 29). The AF intensity of the olfactory bulbs, midbrain, cerebellum, pons and medulla of these mice showed no observable difference from that of the control mice, thereby indicating the AF material to be something other than a reserpine-depletable compound.

D. <u>Effects of DOPA on AF in Pargyline Pretreated Mice</u>

To further test the possibility that the AF may be an adrenergic transmitter or a related substance, the amount of adrenergic substances in the brain was increased by pharmacological means. A monoamine oxidase inhibitor (MAOI), pargyline (100 mg/kg), was given i.p.; 18 hours later, 300 mg/kg of DOPA

was administered i.p. It has been reported that a MAOI in combination with DOPA will significantly increase the amount of norepinephrine and dopamine in the brain (6). The AF intensity of the same regions examined in Section C, above, of the CF-1 mouse did not noticeably differ from the control. Since an increase in AF intensity would have been expected after treatment with a MAOI and DOPA, if the AF material was one of the adrenergic substances, and since no such increase in AF intensity was seen, this evidence further implies that the AF material is something other than an adrenergic substance.

E. Age Study

In the one day old CF-1 mice, definite, typical AF cells were found in the midbrain - pons region at the level of the cerebellum. They occurred in a group of AF cells half way between the ventral surface of the cerebellum and the ventral edge of the brain, lateral to either side of the midline of the section. The AF cells ranged in size from 5-8 micra; their intensity of AF was very low to low; and the cell density was low. By staining these cells with thionine, it was possible to identify them as nerve cells as opposed to connective tissue, etc.

Even though the rest of the areas of the neonate brains investigated showed large neurons, stained with thionine, no specific fluorescence was present in any area other than described above.

The nine day old <u>CF-1</u> mice demonstrated no AF cells in any of the regions investigated.

The adult <u>CF-1</u> mice showed the typical AF pattern of the adult. The specific AF cells were seen in areas studied, but not all of the neuronal cells were seen to fluoresce, as also observed above in Section A.

F. <u>Comparison of AF Properties in Three Brain Regions of SC-1</u>
<u>Strain of Mus musculus</u>

1. <u>Effect of Prolonged Exposure to UV Light (see Graph 1)</u>

AF cells of olfactory bulb of <u>SC-1</u> strain of <u>Mus</u> <u>musculus</u> with an initial intensity of 75% (high - very high) were reduced to a 55% intensity after exposure to UV for 5 minutes; after 15 minutes exposure, they were reduced to 30% intensity and no further decrease in intensity was noted even after 60 minutes exposure.

The AF Purkinje cells had an original intensity of 25% (low - medium). After only 5 minutes exposure to UV light, they were reduced to a 10% intensity at which point no appreciable loss occurred thereafter.

The AF brain stem cells with an initial 50% intensity were reduced to a 25% intensity after the first 5 minutes exposure to UV and then to about a 15% intensity after 15 minutes exposure. Again, no further loss of intensity was noted.

Even after prolonged exposure to UV light, all of the cells still retained variable amounts of AF but always less than control slides. The percent of intensity remaining seemed to be directly proportional to the initial intensity of the cells, i.e., the greater the initial intensity, the greater the residual intensity.

Further, none of the cells appeared to regain their original intensity, even after several hours.

Examination of Graph 1 indicates that the rate of loss of intensity seems to be about the same for all three regions.

2. Effect of Chloroform:Methanol (2:1) (see Graph 1)

The effect of exposing selected sections of the SC-1 mouse brain to a solution of chloroform:methanol (2:1) was the same as prolonged exposure to UV light, i.e., a marked decrease in AF intensity.

However, while all three regions showed about the same rate of loss of intensity in the UV exposed sections, there was some variability between these same brain regions in the rate of decrease of AF intensity in the chloroform:methanol (2:) treated sections.

After 5 minutes exposure, the Purkinje cell AF (initial intensity of 25%) was decreased to 10% and, after 15 minutes, its intensity was about 5% at which level it remained

relatively constant even after 30 minutes. The intensity of the brain stem cells (initial intensity of 50%) dropped to 30% after 5 minutes exposure to chloroform:methanol and then to 15% after 15 minutes exposure at which level they too remained constant even after 45 minutes exposure.

The AF olfactory cells with an initial 75% intensity decreased to a 50% intensity after 5 minutes exposure, 50% after 15 minutes, a 40% intensity after 30 minutes exposure and a 20% intensity after 60 minutes exposure to chloroform:methanol (2:1).

Those AF olfactory cells with an initial 100% intensity showed a quite different rate of loss of intensity. After 5 minutes exposure, their intensity dropped to 90% and then to 80% after 15 minues exposure at which level these cells showed no further decrease in intensity after 30 or 45 minutes exposure to the chloroform:methanol (2:1) solution. But then, after 60 minutes exposure, their intensity dropped to 50% and then to a 30% intensity after a 90 minute exposure to the chloroform:methanol solution.

In all of the above sections treated with chloroform: methanol (2:1), the AF intensity was not regained.

Emersion of brain sections in xylene, a weaker lipid solvent than chloroform:methanol, did not appear to decrease the intensity of the AF cells but denaturation of the tissue by xylene caused a general, non-cellular fluorescence in excess of

controls. This tended to interfere with an exact localization of the natural AF cells present. Such extensive interference did not occur with the chloroform:methanol solution.

DISCUSSION

The variability of AF intensity in these three genera of mice occurs primarily in the "upper" regions of the brain, i.e., telencephalon and diencephalon, while the "lower" regions, i.e., midbrain, pons, medulla and cerebellum of the three genera of mice studied, as well as the olfactory bulb of the SC-1 and CF-1 strains of Mus musculus only, contain an AF which is relatively constant from one animal to the next. Neither the AF variability nor constancy of these regions are unique to brain tissue. Nilsson (32) reported a significant variation in the AF of the uterine epithelium of an inbred strain of adult mice. AF has also been found to be consistently present in other tissues of many animals once these animals have reached maturity (36). The fact that single structures of the "upper" brain regions of the same mouse vary in their AF intensity from one section to the next might imply that this "upper" region AF is in a constant state of metabolic flux. Such fluctuation may also be the basis for the AF variability demonstrated between different animals (32). Also, since there is not such demonstrable irregularity between subsequent sections of the "lower" brain regions or of the olfactory bulbs of the SC-1 or CF-1, such homogeneity of AF occurrence could possibly be explained in a number of ways: 1) there may be a lack of use of this AF, 2) there may be a delicately balanced mechanism between synthesis and use of this

AF material, which maintains a constant supply of this AF here, or 3) both of these may occur (in support of the latter possibility there was found in this research a "stabile" as well as "labile" store of AF in the "lower" brain regions). At any rate, the necessity for monitoring AF by stringent controls can be appreciated when brain amines are histochemically studied by the Hillarp-Falck fluorescence technique. From the present work, I have come to the conclusion that the best approach to distinguishing the AF from the induced fluorescence of the biogenic amines, both fluorescing with a similar color, is to photograph the same brain sections under UV light before and after formaldehyde treatment, a crucial step in the Hillarp-Falck technique. Since the AF does not require any pretreatment, a comparison of the two sets of photographs would enable one to visible distinguish between the AF and the induced fluorescence of the biogenic amines. Another approach, though less desirable, would be to divide the brain in half using one side for formaldehyde treatment and the other half as the AF control, since AF groups of cells are usually, but not always, bilateral.

In all of the regions mentioned above there was present a range of AF intensity. Data reported in this paper implies the presence of two types of AF material in each fluorescent cell examined in SC-1 mice. One type of AF material will lose its fluorescent intensity upon prolonged exposure to either UV light

or a chloroform:methanol (2:1) solution. The other type of AF material is not susceptible to either UV light or chloroform: methanol (Graph 1). Based on such susceptibility, the former AF material shall be termed "labile" and the latter type "stabile". Since the intensity of the "stabile" AF was evaluated to be only 6 to 20%, this type of AF did not significantly influence the overall intensity of each AF cell. Therefore, the range of AF intensities seen throughout the brain was most likely due to some variation of the "labile" AF material. This variation of "labile" AF material could possibly take the form of a qualitative variation, a variability of the physical state of this "labile" AF material (e.g., types of membranous binding, aggregation of the AF granules or density of each AF granule) or even a combination of these possibilities.

The fact that UV light caused a decrease in intensity at about the same rate in all three types of cells examined, namely, olfactory bulb, brain stem and Purkinje cells, might imply that this "labile" AF material is qualitatively similar in all three regions. But since different rates of decrease in intensity were found with chloroform:methanol exposure, this might imply the presence of different physical states of this "labile" AF material. While the type of physical states cannot be discerned from this data, it can be implied (Graph 1) that the olfactory bulb AF material is less easily extracted by a strong lipid

solvent than is the AF material of the brain stem which in turn is less easily extracted than Purkinje cell AF material.

Aside from the possibility that the "labile" AF material of SC-1 mice is a qualitatively similar material found in a variety of physical states, other plausible explanations could be arrived at, e.g., that this material is qualitatively similar but in different concentrations, concentration being directly related to initial intensity. Another possibility is that this "labile" AF is due to qualitatively different materials.

However, based on the fact that the only type of material found to give the type of AF discussed in this paper involves lipids and lipid metabolites (34), and since it has been found that the size, shape and distribution of the AF granules vary widely in different cell groups (nuclei) (24), it seems reasonable to conclude that this "labile" AF material represents a qualitatively similar material (lipid) found in a variety of physical states.

The fact that a "stabile" AF remained in the "lower" brain regions, and possibly in olfactory bulbs of SC-1, even after UV or chloroform:methanol treatment, indicates that this "stabile" AF is due either to a material entirely different from that of "labile" AF or to a material similar to the "labile" AF material but in a much more tightly bound form. The latter suggestion seems more reasonable since, as mentioned above, the only type

of material found to give the type of AF discussed in this paper involves lipids and lipid metabolites (34). Sainte-Marie (36) agrees with this possibility and further suggests that such lack of effect of a lipid solvent is most probably due to a tight binding of the AF material to some proteins or carbohydrates.

At any rate, the above report shows that in the brain we are dealing with at least two general forms of AF lipid material, first, a lipid which is tightly bound to proteins or carbohydrates ("stabile" AF) and second, a lipid which is in a more accessible state ("labile" AF). The latter type of lipid seems to be the predominant form present. The introduction of at least two AF materials which are present in the same cells and which differ from each other both qualitatively and quantitatively suggests the possibility of more than one function for these neuronal AF materials in the same cell. Also, the diversity of histochemical reactions possessed by some, but not all, AF granules (19, 24, 35) further suggests numerous functional differences between groups of AF cells.

The lack of any effect on AF intensity of reserpine, pargyline (a monoamine oxidase inhibitor) and DOPA agrees with the findings of Falck and Owman (15, 17), who also found the AF to be unaffected by these and similar drugs in nervous tissue. While there was no overt change in total intensity, the possibility still exists that a small unobservable change might have occurred

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as a result of the drugs administered. Either a more sensitive method of quantitating the AF material or intensity must be developed or a chronic study with these drugs must be carried out to affirm this possibility. At any rate, in comparison to the relatively immediate effects of these drugs on norepinephrine, dopamine and serotonin level in the brain (6, 29), it seems unlikely that the AF granules represent any of these proposed transmitters. In fact, Shanklin, et al. (40) concluded that the AF granules do not represent a transmitter but that they are involved as carriers in some more general neurosecretory phenomenon. The finding of a large amount of AF granules accumulated around the IV'th ventricle and the aqueduct of Sylvius in all the mice investigated, but none inside the ventricle or blood vessels themselves, may be interpreted as supporting this carrier theory. However, further evidence is needed to determine the truth of this theory.

Evidence found in this study seems to oppose the theory that all of the AF material is due to aging. If these granules were really associated with age, they would be expected to appear randomly throughout the body but in one day old mice they were found in one discrete group of cells, bilaterally, and not scattered throughout the brain. This, together with other evidence presented in the Introduction, makes the "aging theory" highly unlikely.

A possible reason for the presence of a "labile" and

"stabile" AF may be found by considering the two most recent theories of AF function, i.e., the irreversible "pigment of stress" (44 and Introduction) and the reversible "storage granules" (18, 19, 32 and Introduction). The "stabile" AF may represent the type of pigment material due to stress and the "labile" AF may be the depot, usable material. The term "irreversible" was defined as meaning that, once it is formed, it is there to stay (44), while "reversible" implies a material that is used up and replenished (18). The fact that "labile" and "stabile" AF were both found in the so called "constantly occurring" AF regions does not negate the idea of the "labile" AF material being reversible, i.e., used and replenished. As a depot, the stored material should always be present in sufficient or accessible quantities. Such a condition would give the appearance of a constantly occurring amount of AF material but in reality would be in a dynamic state.

Further research along this line should include a study of whether such "labile" and "stabile" AF also occur in genera of mice other than Mus musculus SC-1; also, whether these two types of AF occur in the "upper" regions of the Mus musculus SC-1 brain and in what proportions. Supporting evidence for this theory of two distinct types of AF material could be obtained by inducing an increase in the reversible type AF in the brain in the same manner as did Fuxe and Nilsson (18, 19), then exposing this brain

to UV light or a lipid solvent and determining whether or not the amount of "stabile" AF has been altered. It could also be very interesting to determine whether the "stress" referred to by Sulkin and Srivanij (44) is concerned only with a chemical type of stress or whether it also includes a behaviorally triggered stress. This would be tested by exposing a group of young experimental animals to behaviorally stressful situations, e.g., unsolvable problems, isolation, aggressive episodes, etc. and then comparing their neuronal content of AF with controls.

The only possible correlation between the AF and behavior of the three genera of mice studied involves the phenomenon of aggression. The Mus musculus SC-1 and CF-1 strains of mice, distinguished here from the other two genera of mice by their high intensity olfactory bulb AF, were found to initiate a high number of indiscriminate attacks against other animals, while the other two genera of mice, Peromyscus maniculatus Bairdii and Perognathus sp., initiated a low and moderate number of attacks, respectively (39). If smell, or the lack of it, is involved in such aggression, this would add to the likelyhood of the "labile" AF playing an integral part in cell activity, since the olfactory bulbs of the SC-1 and CF-1 mice were found to have a very high amount of such AF material.

Before summarizing, it should be pointed out that there is a possible source of error due to the excitation filter used.

The filter, CG 554, has a wide band of transmission with a peak transmission at 400 millimicra. Many substances are known to be excited within this band, e.g., vitamin A, carotenes, prophyrin pigments and many others.

Even though the fluorescent colors of most of these substances may not be the same as that of the proposed AF lipofuscin (yellow), it may be assumed that their fluorescence influences the intensity of the AF studied here.

In summary, profuse information accumulated over the years has merely brought to light the heretofore unrealized complexity of this AF material. It has been implicated in stress, growth, neurosecretion and many other phenomena.

The fact that this AF material has been shown here to be variable in some brain areas, while relatively constant in other areas, may prove to be useful in distinguishing inherent AF cells from formaldehyde induced fluorescent cells of the Hillarp-Falck technique. Also, the finding of AF cells in very young animals seems to confirm previous findings which negate the theory of "aging" as a possible explanation of the presence of all AF material. Based on the findings presented here of two types of AF material, a "stabile" and "labile" form, present in the same cell as well as the previous histochemical (Introduction) and drug studies (18, 44), the best available explanation of function of the AF material seems to involve a stress as well as a storage

phenomenon.   Perhaps, future research will more accurately reveal what function or functions this unique material possesses.

TABLE 1

COMPARATIVE DISTRIBUTION AND INTENSITY
OF AF IN THREE GENERA OF MICE

Telencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity
Anterior commissure		none			none			none	
Capsula interna	6 μ (beaded)	low	med	20 μ	med-high	med		general	
Claustrum	15 μ	med	low-med	9 μ	med	med	12 μ	low	med
Corpus callosum		none			none			none	
Cortex pyriformis	10 μ	low	low	12 μ	low-med	faint-low	15 μ	low	faint-low
Globus pallidus		general		20 μ	med-high	med	15 μ	low-med	low
Lamina glomerulosa	11 μ	high-very high	very high		general		10 μ	very low	low-med
Lamina gran-ularis interna		general		9 μ	very low	faint		general	

TABLE 1 (continued)

Telencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Lamina plexiformis internalamina cellulatrum mitralium	9 μ	med	faint		general		20 μ	low	low
Nucleus accumbens		general		9 μ	low	faintlow		general	
Nucleus amygdaloideus lateralis	20 μ	med	med		*			*	
Nucleus interstitialis striae terminalis	10 μ	med	lowmed	20 μ	medhigh	med	15 μ	lowmed	lowmed
Nucleus olfactorius anterior pars externa and pars lateralis		general		10 μ	med	med		general	
Nucleus olfactorius anterior pars medialis and pars dorsalis		general		9 μ	lowmed	faint	12 μ	medhigh	low
Nucleus olfactorius anterior pars posterior		general		9 μ	low	faintlow	15 μ	med	lowmed

TABLE 1 (continued)

Telencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity
Nucleus septi lateralis		general		20 μ	med	med-high		general	
Nucleus septi medialis		general		7 μ	low-med	low		general	
Nucleus tractus diagonalis	10 μ	high	low		*		20 μ	med-high	low-med
Nucleus tractus olfactorius lateralis	15 μ	low	low		general		15 μ	med	low-med
Polus frontalis		general		8 μ	very low	faint-low		general	
Sulcus Rhinalis		general		10 μ	low-med	low-med		general	
Tractus olfactorius lateralis, dorsalis and ventralis	12 μ	high	high		general			general	
Ventral segment of caudatus putamen		*			*		15 μ	low-med	low

TABLE 1 (continued)

Telencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity
Region of anterior amygdala		*		15 μ	med-high	med-high		*	
Region lateral to claustrum	20 μ	med	high	general			general		
Region ventro-lateral to anterior commissure at level of nucleus tractus diagonalis	general			20 μ	low	med	12 μ	low	low
Cortical cells (general)	8 μ	med	low-med	8 μ	low	low-med	10 μ	med	low
A zone of cortex	15 μ	high	med		*		20 μ	med	med

[approximately 250 micra deep and approximately 200-300 micra from cortical surface at level of cortex pyriformis and nucleus tractus diagonalis (this zone extends approximately 700 micra in an anterior - posterior direction)].

Telencephalon	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity
Along border of ventricles I, II and III.	15 μ	med	med-high	20 μ	med	med-high	12 μ	low	med-high

TABLE 1 (continued)

Diencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Dentate Gyrus	5 μ	high	med	15 μ	med-high	med-high	12 μ	med-high	low-med
Fasciculus medialis prosencephali	10 μ	med	low	15 μ	med	med-high	20 μ	med-high	med-high
Fissura choroidea	15 μ	low	med		none			none	
Forel's Field H1 and H2		general		12 μ	med-high	med		general	
Lateral geniculate		*			*		20 μ	low-med	med
Nucleus anterior dorsalis thalami	10 μ	med	med-high		general			general	
Nucleus anterior and nucleus lateralis hypothalami	12 μ	med	low-med	20 μ	med-high	med	15 μ	med	med
Nucleus anterior vantralis thalami	10 μ	med	med		general		10 μ	low-med	low-med

44

TABLE 1 (continued)

Diencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity	Average AF Cell Size	Den-sity	Inten-sity
Nucleus para-fascicularis		general		12 μ	low	very high	20 μ	med-high	med
Nucleus paratenelius	7 μ	low	low		general		15 μ	low-med	med
Nucleus pre-mamillaris ventralis		general			general		15 μ	low-med	med
Nucleus preopticus		general		12 μ	med	low-med	15 μ	low-med	low-med
Nucleus preopticus magnacellaris		general		15 μ	med	med-high		general	
Nucleus reti-cularis thalami		general			general		12 μ	med-high	med
Nucleus supra-chiasmaticus	7 μ	med	med		general			general	
Nucleus ventralis thalami and associated fiber tracts	6 μ (beaded)	low	med	15 μ	low-med	low-med	15 μ	low-med	low-med

TABLE 1 (continued)

Diencephalon	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Tractus cortico habenularis lateralis	8 μ	med	med		general			general	
Tractus cortico hypothalamicus pars lateralis		general			general		20 μ	med-high	med-high
Zona incerta		general		12 μ	med-high	med	20 μ	low-med	med
Area ventral to commissura fornicus ventralis and dorsal to tractus opticus		general			general		12 μ	high	med-high
Region of nucleus lateralis hypothalamis	12 μ	med	low-med	15 μ	med	med-high	15 μ	med	med-high
Region of nucleus medialis thalami		general			general		10 μ	low-med	low-med

TABLE 1 (continued)

Mesencephalon (midbrain) and Myelencephalon (pons and medulla)	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Corpus and nucleus trapezoideum	(beaded)	med	med-high		general			general	
Leminiscus lateralis and tractus cortico-spinalis	(beaded)	low	low-med		general			general	
Nucleus Darkschewitsch	20 μ	med-high	med	20 μ	low-med	low-med	20 μ	high	med-high
Nucleus dorsalis raphes	15 μ	med	low		general			general	
Nucleus Edinger-Westphalia		general			*		12 μ	very high	med-high
Nucleus inter-peduncularis		general			*		12 μ	very high	med-high
Nucleus inter-stitialis		general		20 μ	low-med	low-med	20 μ	high	med-high
Nucleus linearis pars rostralis		general			*			*	

TABLE 1 (continued)

Mesencephalon (midbrain) and Myelencephalon (pons and medulla)	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Nucleus principalis and oculomotoris	15 μ	med	med		*		12 μ	very high	med-high
Nucleus ruber	25 μ	med	med	20 μ	med	med	25 μ	high	very high
Substantia grisea centralis	15 μ	low	low		general			general	
Substantia nigra pars lateralis		general		20 μ	med	med-high		general	
Superior nucleus olivaris	20 μ	low	med		general			general	
Border of aqueduct of Sylvius and IV ventricle	15 μ	low-med	high	15 μ	low-med	med-high	15 μ	low-med	med-high
Brain stem generally (specific areas indistinguishable)	20 μ	med-high	med-high	20 μ	med-high	med	25 μ	med-high	med-high

TABLE 1 (continued)

Cerebellum	Mus musculus SC-1			Peromyscus maniculatus Bairdii			Perognathus sp.		
	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity	Average AF Cell Size	Density	Intensity
Cerebellar nuclei	15 μ	med-high	med-high	15 μ	med-high	med-high	15 μ	med-high	med-high
Purkinje cell layer	15 μ	high	low	20 μ	high	med	20 μ	high	med-high

*   Structure could not be located.

General - No specific grouping of AF cells was noted.

PLATE 1   Olfactory Bulb

The granular appearance of the autofluorescence
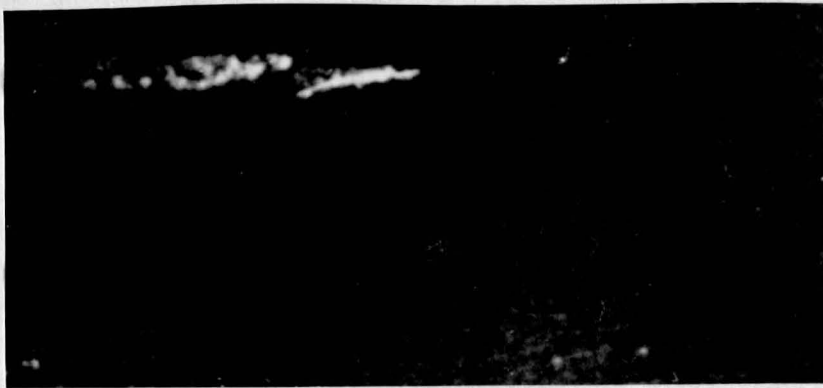can be seen above.   X40 and enlarged.

PLATE 2 Olfactory Bulb

The above enlargement was made in an attempt
to better illustrate the granular appearance
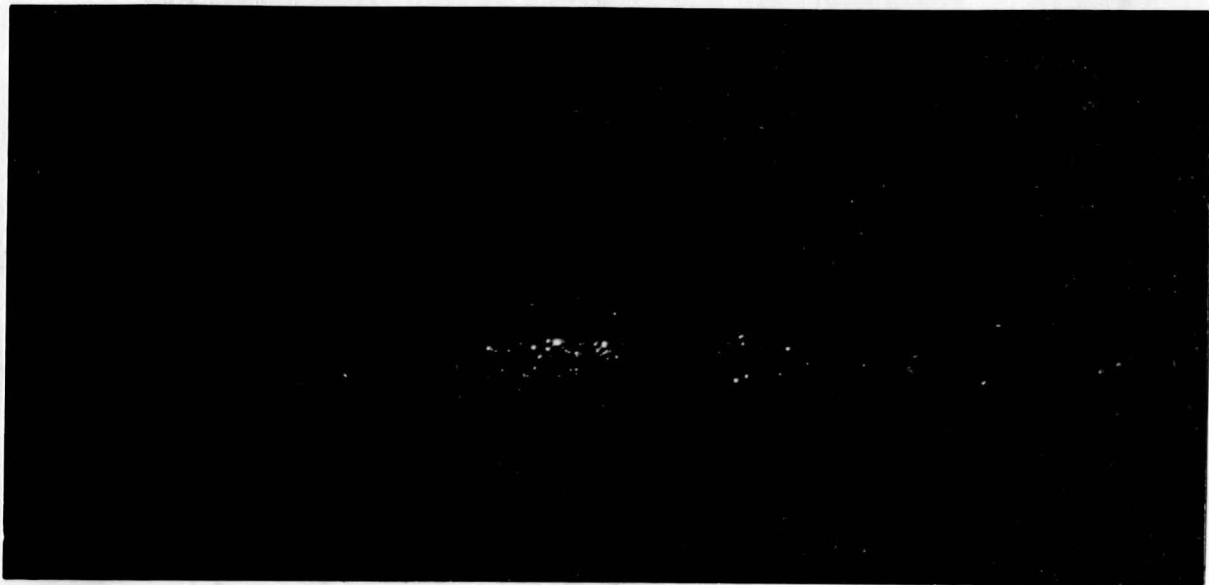of the autofluorescence. X40 and enlarged.

PLATE 3   Diencephalon

The central mass of autofluorescent cells exemplifies a medium-high intensity and a low density. Several artifacts were penciled out. The remainder of the photomicrograph illustrates a general type of faint autofluorescence. X40 and enlarged.

COMPARISON OF AUTOFLUORESCENCE PROPERTIES
IN THREE BRAIN REGIONS
OF MUS MUSCULUS SC-I
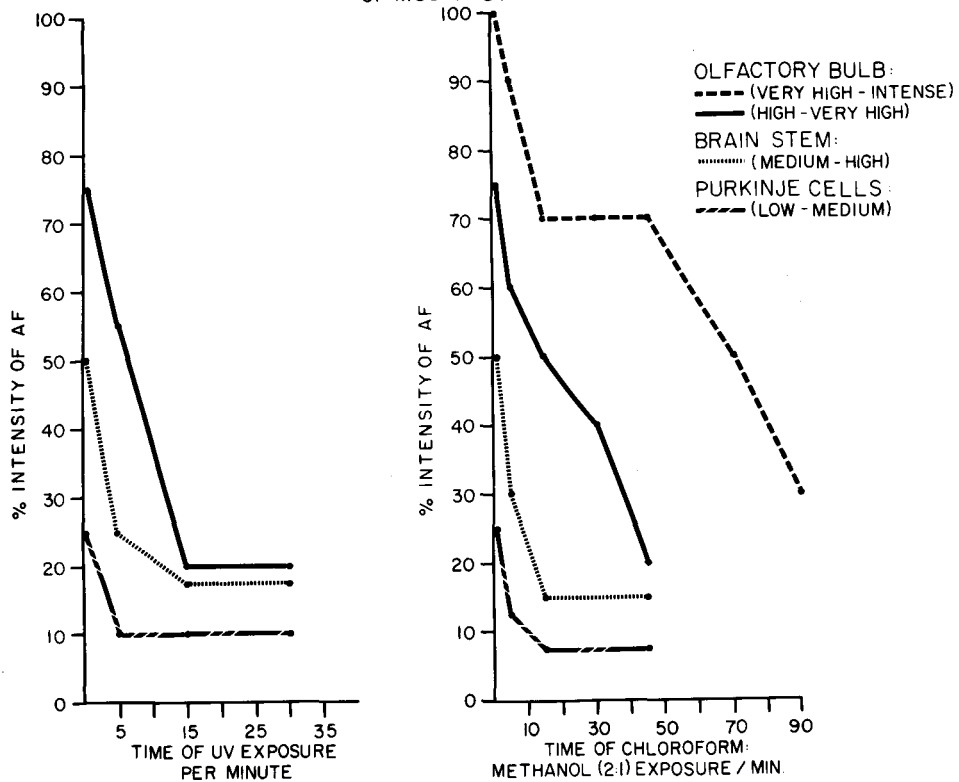
GRAPH 1

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APPROVAL SHEET

The dissertation submitted by Thomas J. Sobotka has been
read and approved by three members of the faculty of the Graduate
School.

The final copies have been examined by the director of the
dissertation and the signature which appears below verifies the
fact that any necessary changes have been incorporated and that
the dissertation is now given final approval with reference to
content, form and mechanical accuracy.

The dissertation is, therefore, accepted in partial ful-
fillment of the requirements for the Degree of Master of Sciences.

May 19, 1967
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Date

Charles F. Scudder
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Signature of Advisor